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Role of the Karyopherin Pathway in Human Immunodeficiency Virus Type 1 Nuclear Import

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The interaction of the human immunodeficiency virus type 1 (HIV-1) nucleoprotein complex with the cell nuclear import machinery is necessary for viral replication in macrophages and for the establishment of infection in quiescent T lymphocytes. The karyophilic properties of two viral proteins, matrix (MA) and Vpr, are keys to this process. Here, we show that an early step of HIV-1 nuclear import is the recognition of the MA nuclear localization signal (NLS) by Rch1, a member of the karyopherin-α family. Furthermore, we demonstrate that an N-terminally truncated form of Rch1 which binds MA but fails to localize to the nucleus efficiently blocks MA- but not Vpr-mediated HIV-1 nuclear import. Correspondingly, NLS peptide inhibits the nuclear migration of MA but not that of Vpr and prevents the infection of terminally differentiated macrophages by *vpr*-defective virus but not wild-type virus. These results are consistent with a model in which Rch1 or another member of the karyopherin-α family, through the recognition of the MA NLS, participates in docking the HIV-1 nucleoprotein complex at the nuclear pore. In addition, our data suggest that Vpr governs HIV-1 nuclear import through a distinct pathway.

The matrix (MA) and Vpr proteins contribute to the karyophilic properties of the human immunodeficiency virus type 1 (HIV-1) nucleoprotein complex, thereby allowing replication in nonproliferating targets, such as terminally differentiated macrophages (4, 5, 10, 18, 43), as well as the establishment of a stable infection intermediate in quiescent T lymphocytes (43). Mutant viruses lacking only one of these two functions conserve the ability to infect macrophages (18), indicating that the two proteins act in a partly redundant manner.

MA is the N-terminally myristoylated cleavage product of the viral Gag precursor, whose membrane association is crucial for particle assembly and release (42). MA has intrinsic karyophilic properties conferred by a highly conserved stretch of basic residues which functions as a nuclear localization signal (NLS) (4, 10). During virion maturation, a subset of MA is phosphorylated on C-terminal tyrosine by a membrane-associated cellular tyrosine kinase (10). Tyrosine-phosphorylated MA molecules then bind to integrase (IN), which triggers their incorporation into the viral core (11). After entry, this subset of MA, still bound to IN, becomes part of the uncoated viral nucleoprotein complex, in which its karyophilic potential is manifested (11).

Vpr, recruited in virions by the p6 C-terminal product of Gag (22–25, 38), is another component of the viral nucleoprotein complex that exhibits karyophilic properties. However, Vpr does not contain a canonical NLS; attempts to map a domain critical for its nuclear localization have yielded conflicting results. One report suggested that the arginine-rich C-terminal portion of the protein was both necessary and sufficient for Vpr nuclear migration (25). In contrast, three independent studies have failed to detect a role for this sequence in Vpr nuclear import, finding instead that residues in one or both of two putative alpha-helices situated more proximally played a major role in this process (9, 26, 46).

By using digitonin-permeabilized mammalian cells and NLS-containing proteins, a number of cellular factors necessary for nuclear transport have been isolated from the cytosol (reviewed in reference 28). A heterodimeric complex, termed karyopherin (39), is necessary to target NLS-bearing substrates to the nuclear pore, where two additional proteins, the GTPase Ran/TC4 (29, 31) and p10/NTF2 (32, 37), are required for transport into the nucleus. The karyopherin complex consists of two subunits, α and β (1, 14, 16, 33, 34). Either karyopherin-α1 (also known as hSRP1, NPI-1, and importin 60) (7, 15, 20, 33, 36) or karyopherin-α2 (Rch1, hSRP1α, or NPI-3) (8, 35, 45) serves as the NLS receptor, whereas karyopherin-β (p97 or importin 90) (6, 14) functions as an adapter that mediates binding to nucleoporins.

The present study was designed to analyze the cellular factors involved in transporting the HIV-1 nucleoprotein complex to the nucleus of a nondividing cell. Our results reveal that an early step of this process is the recognition of the MA NLS by a member of the karyopherin-α family. Furthermore, they suggest that MA and Vpr govern HIV-1 nuclear transport through distinguishable pathways.

MATERIALS AND METHODS

DNA constructions. Plasmid R7 contains HIV-1_{HXB2} proviral DNA with a full-length *nef* reading frame (21). R8 was constructed by replacing a *Bss*HII-to-*Bam*HI fragment in R7, extending from U5 to the 3′ portion of *env*, with a corresponding fragment from HIV-1_{NL4-3} proviral DNA. In contrast with R7, R8 thus contains functional *vpr* and *vpu* reading frames. R8BaL was generated by replacing a *Sal*I-*Bam*HI piece in R8, extending from the distal portion of *vpr* to the middle of the gp41-coding sequence, with the equivalent fragment from the macrophagetropic HIV-1 clone BaL (19). The MA NLS mutation was previously described (43) and consists of two lysine-to-alanine changes at positions 26 and 27. To mutate *vpr*, R8 and R8BaL were digested with *Eco*RI and the DNA free ends were filled in with the Klenow fragment of DNA polymerase I. The resulting translational frameshift results in a Vpr protein of only 68 amino acids, instead of 96 amino acids for the wild type. Nonmyristoylated MA was expressed from a modified R7 proviral DNA construct ($MA_{G^2A/STOP}$) in which the glycine codon at the 5′ end of *gag* was changed to one for alanine and in which a stop

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codon was placed immediately after the MA sequence (10). For the production of recombinant molecules in *Escherichia coli*, wild-type or NLS-mutated HIV-1 MA sequences were cloned into the pGEX-2T vector (Pharmacia) to produce glutathione *S*-transferase (GST) fusion proteins. Cytomegalovirus (CMV)-based plasmids expressing two N-terminally truncated forms of Rch1 were previously described (8). A CMV-based vector expressing the Vpr protein was created by cloning the PCR-amplified Vpr-coding sequence of R8 into the previously described CMX plasmid (2).

Cells. Human kidney fibroblastic 293 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Monocyte-derived macrophages were obtained from blood samples of seronegative donors by centrifugation over Ficoll-Hypaque. Macrophages were further purified by adherence to plastic and cultivated in RPMI medium supplemented with 10% fetal calf serum for 2 weeks before infection.

Transfections and infections. Transfections were performed by the calcium phosphate method, as previously described (2). Viruses were isolated from the filtered supernatant of transfected cells by ultracentrifugation through a 20% sucrose cushion as previously described (44). Virus concentrations were determined by p24 antigen capture enzyme-linked immunosorbent assay (ELISA; DuPont). To test the effects of Rch1 derivatives on HIV-1 nuclear transport, 293 cells were transfected with a CMV-based vector expressing CD4 and with plasmids encoding $Rch1_{33/529}$, $Rch1_{244/529}$, or control DNA. At 48 h posttransfection, cells were infected with 100 ng of p24 of wild-type (R8) or *vpr*-defective (ΔVpr.R8) HIV-1 or with a variant mutated in both *vpr* and the MA NLS ($MA_{KK^{27}TT}$.ΔVpr.R8). At 2, 8, and 24 h postinfection, the generation of linear and circular HIV-1 reverse transcripts was monitored by PCR analysis as described below. Infections of macrophages (0.5×10^6 cells) were performed with 20 and 100 ng (for growth curves and PCR analyses, respectively) of p24 from 293 cell-produced R8BaL-derived viruses. To measure viral replication, cells were washed extensively after 6 h of adsorption and p24 antigen production was monitored by ELISA. Experiments were repeated several times with cells isolated from different donors with similar results. To block nuclear import in macrophages, the high-pressure liquid chromatography (HPLC)-purified peptide corresponding to the prototypic simian virus 40 T-antigen NLS motif (PKK KRKVEDPYC) was used at a concentration of 500 μM, as previously described (17). The reverse peptide (PDEVKRKKKPYC) corresponding to the inverted T-antigen NLS sequence was used as a negative control.

In vitro binding experiments. Cytoplasmic extracts of 293 cells (30×10^6) transfected by the calcium phosphate method with 120 μg of CMV-based vectors encoding $Rch1_{33/529}$ or $Rch1_{244/529}$ were loaded on GST-MA agarose beads for 30 min at room temperature in phosphate-buffered saline containing 0.05% Nonidet P-40, as previously described (36). After five washes with the same buffer, bound material was eluted with 50 μl of sodium dodecyl sulfate (SDS) sample buffer and loaded on an SDS–15% polyacrylamide gel. The presence of Rch1 was examined by Western blot (immunoblot) analysis with an anti-myc monoclonal antibody (Oncogene Science). For inhibition experiments, cytoplasmic extracts were incubated for 1 h at room temperature with 500 μM HPLC-purified NLS or reverse peptide prior to incubation with GST-MA beads.

Subcellular fractionation and protein analysis. To determine the subcellular localization of MA and Rch1, 293 transfected cells were lysed in cold hypotonic buffer (20 mM potassium HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid] [pH 7.8], 5 mM potassium acetate, 0.5 mM $MgCl_2$, 0.5 mM dithiothreitol, 1 μg of leupeptin per ml, 1 μg of aprotinin per ml, 1 μg of pepstatin A per ml, 1 mM phenylmethylsulfonyl fluoride) by using several strokes of a Dounce homogenizer as previously described (10). Nuclear preservation during cell lysis was monitored by microscopy. Cytoplasmic and nuclear extracts were separated by centrifugation at $750 \times g$ for 5 min. Nuclei were then extracted with a hypertonic buffer (20 mM potassium HEPES [pH 7.8], 0.5 mM $MgCl_2$, 500 mM potassium acetate, 0.5 mM dithiothreitol, 1 μg of leupeptin per ml, 1 μg of aprotinin per ml, 1 μg of pepstatin A per ml, 1 mM phenylmethylsulfonyl fluoride). HIV-1 viral nucleoprotein complexes were purified from SupT1 cells acutely infected with strain IIIB viruses at 5 h postinfection as previously described, with 0.025% Brij 96 as the detergent (30). Western blot analyses were performed as previously reported (2) with an enhanced chemiluminescence detector kit (DuPont). Rabbit anti-HIV-1 MA serum was obtained by immunization with a previously described recombinant histidine-tagged HIV-1 MA protein (10). Rch1 was detected with a rabbit polyclonal antiserum or a myc-specific monoclonal antiserum in the case of myc-tagged proteins. Reverse transcriptase (RT) activity was monitored by a so-called exogenous RT assay (13), with minor modifications (3). Vpr was detected with an antiserum provided by the National Institutes of Health AIDS Research and Reference Program, the kind gift of L. Ratner.

PCR analysis. PCR analysis was performed essentially as previously described (44). The sequences of the HIV-specific primers are as follows (the positions of nucleotides in the $HIV\text{-}1_{HXB2D}$ sequence according to Ratner et al. [40] are indicated in parentheses): Vif 6, GGGAAAGCTAGGGGATGGTTTTAT (5136 to 5159); Vif 7, CAGGGTCTACTTGTGTGCTATTC (5340 to 5317); LTR5, GGCTAACTAGGGAACCCACTGCTT (496 to 516); 5NC2, CCGAG TCCTGCGTCGAGAGAGC (698 to 677); LTR8, TCCCAGGCTCAGATCT GGTCTAAC (488 to 465 and 9572 to 9549); LTR9, GCCTCAATAAAGCTT GCCTTG (522 to 542 and 9606 to 9626). Vif 6 and Vif 7 amplify elongated minus-strand DNA (early linear product), LTR5 and 5NC2 amplify double-stranded molecules generated after the second template switch (late linear product), and LTR8 and LTR9 amplify 2-LTR circles. PCR amplification products were visualized after Southern blot transfer and hybridization with a ^{32}P-labeled DNA probe, as previously described (41).

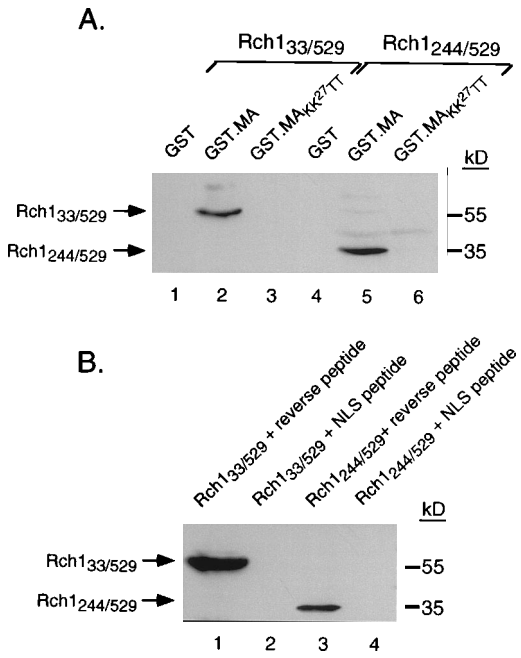

FIG. 1. NLS-dependent binding of MA to Rch1 in vitro. (A) Cytoplasmic extracts from 293 cells expressing myc-tagged forms of $Rch1_{33/529}$ or $Rch1_{244/529}$ were incubated with wild-type or NLS-mutated GST-MA fusion proteins coupled to agarose beads. Bound material was analyzed by Western blot with anti-myc antibody. (B) Same experiment, except for the preincubation of cytoplasmic extracts with peptide corresponding to the large-T-antigen NLS in the sense (PKKKRKVEDPYC) or reverse (PDEVKRKKKPYC) orientation. kD, kilodaltons.

RESULTS

NLS-dependent binding of MA to Rch1 (karyopherin-α2). Originally identified through its interaction with the RAG-1 recombination-activating protein (8), Rch1 was recently recognized as a member of the karyopherin-α family and shown to function as an NLS receptor (45). The possibility that Rch1 binds HIV-1 MA was thus investigated. Recombinant GST-MA fusion protein could capture two forms of Rch1 overexpressed in transfected 293 human fibroblastic cells, a fragment encompassing amino acids 33 to 529 of Rch1 ($Rch1_{33/529}$) and a derivative lacking 243 N-terminal residues ($Rch1_{244/529}$) (Fig. 1A). In contrast, neither version of Rch1 associated with a GST-MA variant whose NLS sequence carried a mutation previously shown to abrogate MA nuclear import (Fig. 1A). Furthermore, the peptide corresponding to the prototypic simian virus 40 large-T-antigen NLS blocked MA-Rch1 complex formation, whereas the reverse peptide had no effect (Fig. 1B). This confirms that Rch1 interacts with MA in an NLS-dependent manner.

A truncated form of Rch1 inhibits MA nuclear import. Nonmyristoylated HIV-1 MA and $Rch1_{33/529}$ localized to both the cytoplasm and nuclei of transfected 293 cells, whether expressed separately or together (Fig. 2A). In contrast, $Rch1_{244/529}$ was found exclusively in the cytoplasm. Furthermore, this protein prevented MA nuclear translocation in cotransfected cells (Fig. 2A). MA-specific antibodies precipitated $Rch1_{33/529}$ from both the cytoplasm and nuclei of cotransfected cells, whereas

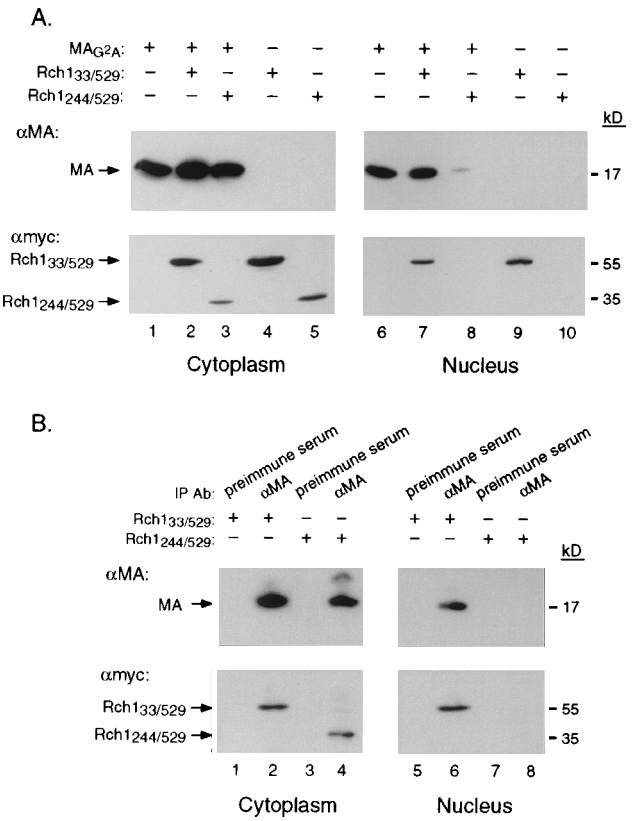

FIG. 2. A truncated form of Rch1 blocks MA nuclear import. (A) Subcellular localization of nonmyristoylated MA, Rch1$_{33/529}$, and Rch1$_{244/529}$ in singly and dually transfected 293 cells; (B) detection of MA-Rch1 complexes in cotransfected cells. 293 cells were transfected with 20 μg of a vector encoding a nonmyristoylated form of MA (MA$_{G^2A}$) or a control DNA, with or without constructs producing myc-tagged Rch1$_{33/529}$ or Rch1$_{244/529}$. MA and Rch1 were detected in cytoplasmic and nuclear fractions by Western blot with a mixture of MA- and myc-specific antibodies (αMA and αmyc, respectively) either directly (A) or after immunoprecipitation (IP) with MA-specific antiserum (B). kD, kilodaltons; Ab, antibody.

MA-Rch1$_{244/529}$ complexes were restricted to the cytoplasm (Fig. 2B). Rch1$_{244/529}$ can thus interfere with MA nuclear import by sequestering this protein in the cytoplasm. The previously reported inhibitory effect of Rch1$_{244/529}$ on RAG-1-mediated recombination (8) probably stems from a similar mechanism.

MA is bound to Rch1 in the uncoated viral nucleoprotein complex. To ask whether MA interacts with Rch1 during the early stages of the HIV-1 life cycle, cytoplasmic extracts of freshly infected T-lymphoid cells were fractionated on a sucrose gradient. Fractions were then analyzed for their contents of viral and cellular proteins as well as of viral DNA (Fig. 3). The viral envelope and the bulk of MA were found in the region of the gradient with the lowest density, consistent with the membrane association of these two proteins. A peak of RT activity, together with the viral genome, was detected at a density of 1.20 to 1.26 g/cm^3. These fractions, likely corresponding to partially purified uncoated viral nucleoprotein complexes, also contained a subset of MA, as previously described (11). By using an MA-specific antiserum, these MA molecules could be coimmunoprecipitated with a cellular protein whose molecular weight and immune reactivity were those of Rch1. In contrast, no association between Rch1 and plasma membrane-associated MA was detected. Rch1 was not found in virions (data not shown), indicating that MA interacts with its cytoplasmic receptor only after HIV-1 enters target cells.

A dominant negative form of Rch1 blocks MA- but not Vpr-mediated HIV-1 nuclear import. To assess the functional significance of Rch1-MA interaction, 293 cells were transfected with a CD4 expression vector and plasmids encoding either Rch1$_{33/529}$, Rch1$_{244/529}$, or a control DNA. Two days later, cells were infected with either wild-type HIV-1 (R8), a *vpr*-defective variant (ΔVpr.R8), or one mutated in both *vpr* and the MA NLS (MA$_{KK^{27}TT}$.ΔVpr.R8). The synthesis and processing of viral DNA was monitored by PCR. Linear forms of viral DNA were efficiently generated in all cases, indicating that the Rch1 derivatives did not interfere with the early steps of viral replication, including entry and reverse transcription (Fig. 4A). Nucleus-specific (42) 2-LTR viral DNA circles were equally abundant in control and Rch1$_{33/529}$-expressing cells, regardless of the virus used as the inoculum, reflecting normal rates of viral nuclear import in these cells. In contrast, in Rch1$_{244/529}$-producing cells, the generation of DNA circles by *vpr*-defective HIV-1 was inhibited, whereas wild-type and MA NLS-mutated viruses normally induced these forms of the viral genome (Fig. 4). Rch1$_{244/529}$ thus exerts a dominant negative effect on HIV-1 nuclear import in a manner which is strictly dependent on the presence of the MA NLS and can be overcome by a functional *vpr* gene product. Interestingly, this effect appears to be manifested even in the context of proliferating cells, since mitosis was not prevented in these experiments.

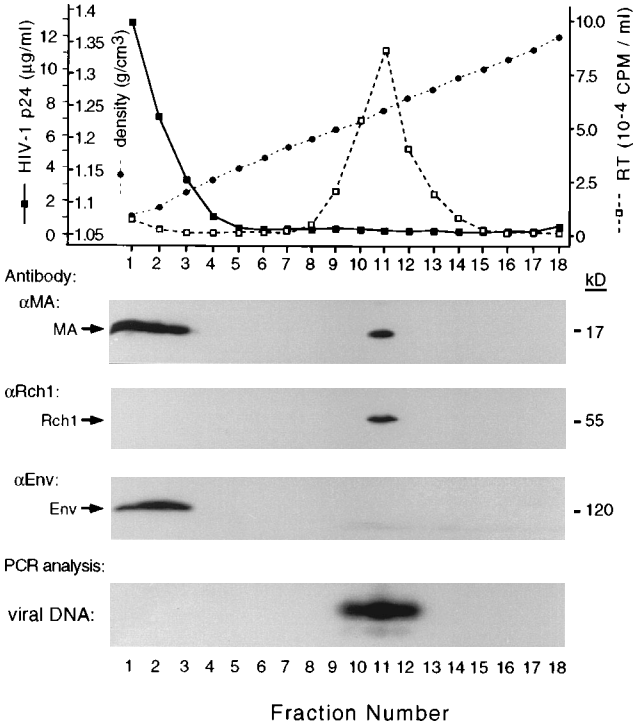

FIG. 3. Rch1 is bound to MA within the HIV-1 nucleoprotein complex. HIV-1 viral nucleoprotein complexes were purified from the cytoplasm of acutely infected SupT1 cells by partial lysis and sucrose gradient fractionation. Fractions were analyzed by a combination of p24 ELISA, RT assay, Western blot, and PCR with virus-specific primers. To test the possibility of an interaction between MA and Rch1, each fraction was immunoprecipitated with anti-MA immunoglobulin G covalently cross-linked to CNBr-activated agarose beads and the bound material was submitted to Western blot analysis with MA and Rch1-specific antibodies (αMA and αRch1, respectively). αEnv, env-specific antibody; kD, kilodaltons.

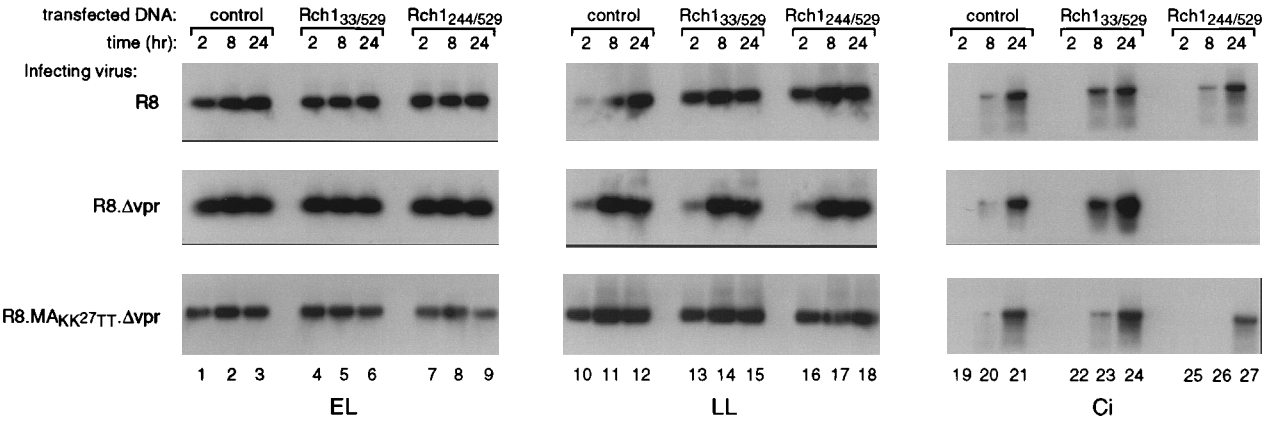

FIG. 4. A truncated form of Rch1 blocks MA-mediated HIV-1 nuclear import. Synthesis and circularization of viral DNA in cells expressing $Rch1_{33/529}$, $Rch1_{244/529}$, or a control vector, infected with wild-type (R8), *vpr*-defective (ΔVpr.R8), and MA NLS/*vpr* double mutant ($MA_{KK^{27}TT}$.ΔVpr.R8BaL) HIV-1. PCR analysis was carried out with primers specific for early linear (EL), late linear (LL), and nucleus-specific circular (Ci) reverse transcription products. $Rch1_{244/529}$ selectively blocks the nuclear import of *vpr*-defective, MA NLS-competent HIV-1.

NLS peptide inhibits MA- but not Vpr-mediated HIV-1 nuclear import. Vpr does not contain a prototypic NLS; therefore, it was not expected to interact directly with Rch1. The lack of inhibition of Vpr-mediated HIV-1 nuclear import by $Rch1_{244/529}$ further demonstrates that the karyophilic properties of Vpr are not derived from its binding to an NLS-containing cellular protein. In confirmation of this point, NLS peptide blocked MA- but not Vpr-mediated nuclear import in transiently transfected 293 cells (Fig. 5). Furthermore, NLS peptide inhibited the replication of *vpr*-defective HIV-1 (ΔVpr.R8BaL) but not wild-type HIV-1 (R8BaL) in terminally differentiated macrophages (Fig. 6A). This reflected a block in nuclear import of this virus. *vpr*-defective HIV-1 specifically failed to induce circular forms of viral DNA in NLS peptide-treated macrophages, as observed with a variant mutated in both the MA NLS and *vpr* ($MA_{KK^{27}TT}$.ΔVpr.R8BaL) in untreated macrophages (Fig. 6B).

DISCUSSION

This work reveals that the recognition of the MA NLS by Rch1, or probably another member of the karyopherin-α family in some cells, constitutes the initial step of a pathway leading to HIV-1 nuclear import. First, we find that Rch1 binds MA in vitro in an NLS-dependent manner. Second, we demonstrate that the two proteins form a complex in cotransfected cells, as well as in the context of the uncoated viral nucleoprotein complex. The functional significance of this interaction is supported by the results obtained with a dominant negative form of Rch1 lacking 243 N-terminal residues. Not only does this mutant sequester MA in the cytoplasm of cotransfected cells, but it also strongly interferes with the nuclear import of *vpr*-defective HIV-1 in a manner which requires the presence of the MA NLS.

Rch1 recognizes MA only after viral entry, not during virion formation. Furthermore, the karyopherin binds exclusively to MA molecules that are associated with the viral nucleoprotein complex, not to those remaining at the plasma membrane. The proposed tertiary structure of MA (27) suggests an explanation for this specificity. Indeed, the amino acids that make up the MA NLS are part of a platform localized on the face of MA, which juxtaposes the plasma membrane (27), and contribute to strengthening the association between MA and the phospholipid bilayer through electrostatic forces (47). Thus, as long as MA is associated with the membrane, the NLS is inaccessible to Rch1. In contrast, if MA is not myristoylated or when it is liberated from its membrane attachment through its interaction with IN, it can bind the karyopherin.

By analogy with the transport of other NLS-bearing karyophiles (reviewed in reference 28), one can propose that karyopherin-α, bound to MA molecules contained in the viral nucleoprotein complex, interacts with karyopherin-β and that the two proteins dock the complex at the nuclear pore. Subsequently, GTPase Ran and p10 must induce the translocation process per se (Fig. 7).

Both $Rch1_{33/529}$ and $Rch_{244/529}$ bind to MA. This implies that the NLS-recognizing domain of Rch1 resides in the C-terminal half of this protein. The dominant negative phenotype of $Rch_{244/529}$ had been suggested by previous experiments showing that it interfered with RAG-1-induced VDJ recombination in transiently transfected HeLa cells (8). The present work reveals the mechanism of this phenomenon by showing that $Rch1_{244/529}$ sequesters an NLS-bearing karyophile in the cytoplasm. This might reflect the failure of this variant to interact with karyopherin-β. Additionally, an active trapping phenomenon has to be invoked to explain how $Rch1_{244/529}$ manifests its inhibitory effect in dividing cells. To some degree, $Rch1_{244/529}$ must interfere with the nuclear import of all mol-

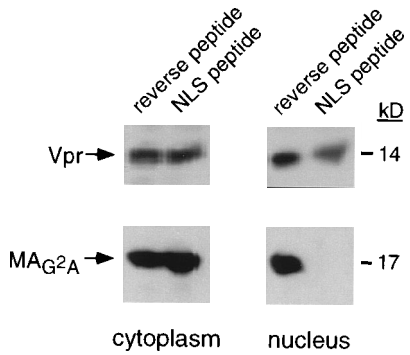

FIG. 5. NLS peptide blocks MA- but not Vpr-mediated nuclear import. Cytoplasmic and nuclear fractions from 293 cells transfected with plasmids encoding Vpr or nonmyristoylated MA (MA_{G^2A}) and exposed to NLS or reverse peptide at a concentration of 500 μM for a total of 16 h were analyzed by Western blot with MA- and Vpr-specific antibodies. kD, kilodaltons.

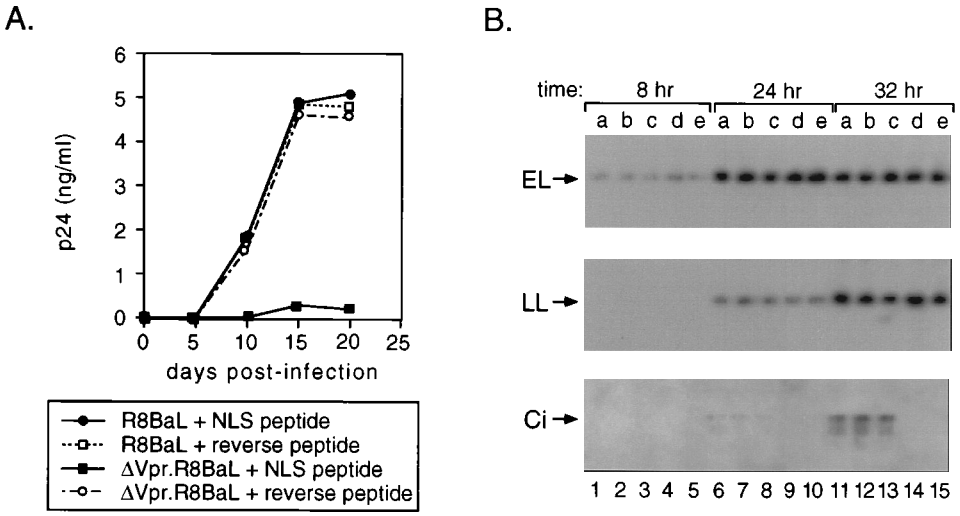

FIG. 6. Vpr bypasses NLS peptide-mediated blockade of HIV-1 nuclear import. (A) Replication of wild-type HIV-1 (R8BaL) and *vpr*-defective HIV-1 (ΔVpr.R8BaL) in terminally differentiated macrophages in the presence of NLS or reverse peptide. NLS peptide inhibits the growth of *vpr*-defective virus, not wild-type virus. (B) Synthesis and circularization of viral DNA in macrophages acutely infected with R8BaL or ΔVpr.R8BaL in the presence of reverse (lanes a and c, respectively) or NLS (lanes b and d, respectively) peptide, monitored as described in the legend to Fig. 4. Cells infected with the MA NLS/*vpr* double mutant $MA_{KK^{27}TT}$.ΔVpr.R8BaL, previously shown to be impaired for replication in macrophages (44), served as the control (lanes e). NLS peptide acts by blocking the nuclear import of *vpr*-defective HIV-1, not wild-type HIV-1.

ecules that utilize the karyopherin-specific pathway. It may thus appear surprising that the production of this protein did not kill cells. Most probably, this is due to the fact that it was expressed only transiently. Our data do not imply that $Rch1_{33/529}$, which lacks 32 N-terminal residues, is functionally equivalent to the wild-type full-length protein. However, it does not have a dominant negative phenotype in the type of assays utilized here.

The karyopherin complex was recently shown to be also involved in the nuclear import of influenza virus (35, 36). In that case, at least the viral nucleocapsid protein was recognized by karyopherin-α, thus allowing the nuclear translocation of viral RNA. Because three other NLS-containing proteins, the components of the heterotrimeric RNA polymerase complex, are associated with the influenza genome, it was hypothesized that they too participate in this process. In the case of HIV-1, either the MA NLS or Vpr is required for efficient import into the nuclei of terminally differentiated macrophages (4, 5, 10, 18, 43). However, we have obtained results suggesting that at least one additional NLS-bearing protein plays a role in other settings, such as γ-irradiated HeLa cells (12). The potential involvement of nucleocapsid protein and IN, both of which contain sequences reminiscent of a karyophilic motif, is currently being investigated.

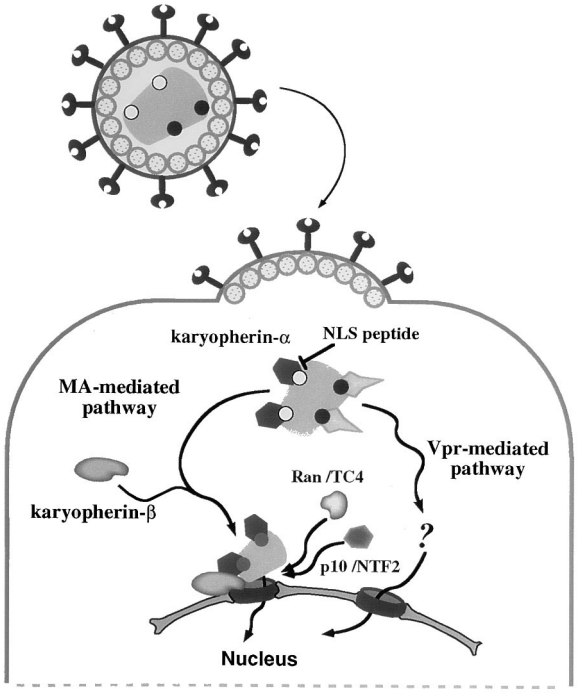

FIG. 7. Proposed model of HIV-1 nuclear import.

Two lines of evidence support a model in which MA and Vpr govern HIV-1 nuclear import through distinguishable pathways (Fig. 7). First, $Rch1_{244/529}$ fails to interfere with the nuclear import of *vpr*-competent virus. Second, NLS peptide affects MA but not Vpr subcellular localization and interferes with MA- but not Vpr-mediated HIV-1 nuclear import. Although it can be assumed that the Vpr-dependent pathway directs the viral nucleoprotein complex to the nuclear pore, its cellular components remain to be identified. In addition, cellular environments in which MA rather than Vpr, or vice versa, plays a dominant role have yet to be found. Nevertheless, it is likely that a dual mechanism for HIV-1 nuclear import was evolutionarily selected to ensure the completion of a process crucial for viral spread in vivo.

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