Multiple Importins Function as Nuclear Transport Receptors for the Rev Protein of Human Immunodeficiency Virus Type 1

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The Rev protein of human immunodeficiency virus type 1 is an RNA-binding protein that is required for nuclear export of unspliced and partially spliced viral mRNAs. Nuclear import of human immunodeficiency virus type 1 Rev has been suggested to depend on the classic nuclear transport receptor importin β, but not on the adapter protein importin α. We now show that, similar to importin α, Rev is able to dissociate RanGTP from recycling importin β, a reaction that leads to the formation of a novel import complex. Besides importin β, the transport receptors transportin, importin 5, and importin 7 specifically interact with Rev and promote its nuclear import in digitonin-permeabilized cells. A single arginine-rich nuclear localization sequence of Rev is required for interaction with all importins tested so far. In contrast to the importin β-binding domain of importin α, Rev interacts with an N-terminal fragment of importin β. Transportin contains two independent binding sites for Rev. Hence, the mode of interaction of importin β and transportin with Rev is clearly distinct from that with their classic import cargoes. Taken together, the viral protein takes advantage of multiple cellular transport pathways for its nuclear accumulation.

The machinery for transport of macromolecules across the nuclear envelope consists of the nuclear pore complex, which is embedded between the inner and outer nuclear membrane (1), and a large variety of soluble transport factors (for reviews see Refs. 2–4). The transport cargoes are characterized by recognition sequences for soluble transport receptors: nuclear export signals (NLSs) bind to importins, promoting transport of the heterogeneously nuclear ribonucleoprotein (hnRNP) A1 protein (6). Transportin is also involved in nuclear import of a number of basic proteins like histones (7) and ribosomal proteins (8) and the transcription factor c-Fos (9). Another importin that may function without an adapter is importin β itself. The T-cell protein tyrosine phosphatase (10), the parathyroid hormone-related protein (11), cyclin B1 (12), the sterol regulatory element-binding protein 2 (13), the transcription factors c-Jun, c-Fos, and cAMP-response element-binding protein (14), and the zinc finger protein Snail (15) directly interact with importin β and are thus imported into the nucleus in an importin β-dependent, yet importin α-independent, manner.

Another prominent example of a direct importin β cargo is the Rev protein of the human immunodeficiency virus type 1 (HIV-1). HIV Rev (for review see Ref. 16) is a shuttling protein that is required for nuclear export of unspliced and partially spliced viral mRNAs (17). It was one of the first proteins identified to contain a so-called leucine-rich nuclear export sequence (18) that links the protein to its exportin, CRM1 (19). A stretch of basic residues in the RNA-binding domain of Rev is required for nuclear import (20, 21). In contrast to the “classic” NLS, where lysine is the predominant amino acid, the Rev-NLS...
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is characterized by arginines as the basic component. Consequently, Rev does not bind to importin α and its nuclear import has been suggested to occur directly via importin β, independent of the adapter protein (22, 23).

We used in vitro nuclear import assays and biochemical binding studies to characterize nuclear import of Rev in detail. Our results clearly demonstrate that Rev binds specifically to the nuclear transport receptors importin β, transportin, importin 5, and importin 7 and, hence, can be imported into the nucleus via multiple transport pathways. Interaction of Rev with importin β and transportin is clearly distinct from that with the classic interaction partners of these transport receptors, importin α and the M9 sequence of the hnRNP A1 protein, respectively.

MATERIALS AND METHODS

Molecular Clones—HIV-1 Rev and HIV-1 RevM5 (24) were PCR amplified from pGEX-Rev and pGEX-RevM5, respectively, and cloned into the NdeI and EcoRI sites of a pGEX-2T vector. Constructs were verified by DNA sequencing.

Purification and Labeling of Recombinant Proteins—GST-Rev was expressed in BL21(-DE)-RIL (Stratagene). Bacterial pellets were resuspended in Rev buffer (50 mM sodium phosphate, pH 6.5, 150 mM NaCl, 10 mM K$_3$SO$_4$) containing 0.4 mg/ml of lysozyme, 2 mM dithiothreitol, and 1 μg/ml each of leupeptin, pepstatin, and aprotinin. After sonification, the lysates were cleared by centrifugation at 100,000 × g at 4 °C for 30 min and incubated with glutathione-Sepharose beads (Amersham Biosciences) for 12–16 h at 4 °C. The beads were washed three times with Rev buffer, and the bound protein was eluted with Rev buffer containing 15 mM reduced glutathione. Rev was bound to heparin-Sepharose (Amersham Biosciences), the beads were washed with Rev buffer containing 450 mM NaCl, and the protein was eluted with Rev buffer containing 1.5 M NaCl. Rev was desalted using PD10-columns (Amersham Biosciences). For RanGAP assays and for in vitro nuclear import using Rev as a competitor, Rev was cleaved from the GST tag with Tev protease. RanBP1 was expressed in BL21(DE3). Bacteria were pelleted and resuspended in buffer A (50 mM Tris, pH 8.0, 75 mM NaCl, 1 mM MgCl$_2$, 1 mM dithiothreitol, 1 mg/ml of lysozyme, 0.1 mM phenylmethylsulfonyl fluoride, and 1 μg/ml each of leupeptin, pepstatin, and aprotinin), subjected to one freeze-thaw cycle, and centrifuged at 100,000 × g for 30 min. A precipitate resulting from a 50–75% saturation ammonium sulfate cut of the supernatant was resuspended in buffer B (50 mM Tris, pH 7.4, 100 mM NaCl, 1 mM dithiothreitol, and protease inhibitors as above) and subjected to anion exchange chromatography on a POROS HQ column (PerSeptive Biosystems). RanBP1 (95% pure) eluted at ~250 mM NaCl and was dialyzed against transport buffer (TP buffer; 20 mM potassium phosphate, pH 7.5, 200 mM KOAc, 1 mM MgCl$_2$, 250 mM sucrose, and 1 mM EGTA) for Rev or TP buffer for other substrates. A standard 20-μl reaction contained 250 nM import substrate, 4 μM Ran or RanQ69L, 2 mM GTP, an ATP regenerating system (28), and either 2 mg/ml of HeLa cytosol and 1.5 M import receptors. In some experiments, HeLa cytosol was preincubated with 3 μl of a monoclonal α-importin β antibody (clone 3E9; Affinity BioReagents; see Ref. 28), 20 μM MBP-M9, 4.5 μM zz-L23, 12.5 μM histone H2B (Roche Applied Science), or 40 μM cleaved Rev. Import reactions were performed for 40 min at 18 °C for Rev or for 30 min at 30 °C for other substrates. For detection of GST-M9 and GST-NLS, cells were subjected to indirect immunostaining using an anti-GST-antibody as described (9). Alternatively, Cy3-labeled GST-M9 was used. The import substrates HIV-Rev, BSA-NLS, and histone H1.2 were fluorescently labeled. Cells were analyzed by fluorescence microscopy using a Zeiss Axioskop2 microscope. Pictures were processed using Adobe PhotoShop.

RanGAP Assays—Labeling of Ran with [$\gamma$-32P]GTP and RanGAP assays were essentially performed as described (9, 30). A standard reaction in 50 μl of KP buffer contained 40–80 nm [$\gamma$-32P]RanGTP, preincubated with 50–80 nm importin β (titrated to obtain maximal inhibition of GTP hydrolysis in the absence of importin β-binding proteins), 10 nm RanBP1, 4–8 nm RanGAP, unless otherwise indicated, and 200–400 μM GTP. Reactions in the presence of untagged Rev contained 0.5% Nonidet P-40. After 10 min at 20 °C, reactions were stopped by adding 1 ml of stop solution (7% charcoal, 10% ethanol, 0.1 M

![Image](313x627 to 565x734)

FIGURE 1. Nuclear import of Rev in vitro. Digitonin-permeabilized cells were incubated with Cy3-labeled GST-Rev in the presence of cytosol, wild-type Ran, or RanQ69L at 4 or 18 °C as indicated.
HCl, 10 mM NaH$_2$PO$_4$). After centrifugation, the released $^{32}$P-phosphate in the supernatant was measured by scintillation counting. GTP hydrolysis was expressed as the percentage of the maximal value of recovered radioactivity as obtained in a reference reaction containing a high concentration of importin α. Values obtained in the absence of importin β-binding proteins were subtracted as background.

**SDS-PAGE and Western Blotting**—Proteins were separated by SDS-PAGE and subjected to Western blotting using standard methods. Rabbit anti-His antibodies (Santa Cruz) or rabbit anti-MBP antibodies (E8030S; New England Biolabs) were used for the detection of His-tagged importin β or MBP-tagged transportin, respectively. Horseradish peroxidase-coupled goat anti-rabbit IgG (Dianova) was used as secondary antibody. The ECL system (Pierce) was used for visualization of proteins.

**RESULTS**

**Nuclear Import of HIV Rev Does Not Depend on Importin β or Transportin**—The aim of this study was to investigate the requirements for nuclear import of HIV Rev with respect to soluble nuclear transport factors. Nuclear import of Rev has been suggested to depend on importin β as a nuclear import receptor (22, 23). To analyze the nuclear transport of Rev in detail, we established an import assay in digitonin-permeabilized cells. Import of Rev required somewhat different buffer conditions and lower temperatures compared with the classic transport system (31). Fig. 1 shows that GST-Rev was imported efficiently into nuclei of permeabilized cells at 18 °C in the presence of cytosol and wild-type Ran, yielding a characteristic nucleolar staining pattern that can also be observed in cells upon transfection (Ref. 32 and data not shown). Import was inhibited at 4 °C in the absence of cytosol or in the presence of RanQ69L, a Ran mutant that is predominantly in the GTP-bound form (33). RanQ69L promotes the disassembly of nuclear import complexes (34) and is often used to demonstrate the specificity of nuclear import reactions in vitro. Thus, our results show that Rev can be specifically imported into the nucleus in vitro in a reaction that appears to depend on Ran-binding proteins of the importin β superfamily.

Rev has been shown to directly interact with the name-giving member of this family, importin β (22, 23). We therefore tested whether inhibition of the importin β-dependent import pathway would affect nuclear import of Rev. In permeabilized cells, importin α/β-dependent transport can be specifically inhibited by a monoclonal antibody against importin β (35). As shown in Fig. 2A, preincubation of cytosol with this antibody strongly inhibited nuclear import of GST-NLS, a substrate for the classic importin α/β-dependent pathway. Inhibition was specific, as transport of GST-M9, a substrate for the import receptor transportin, was not affected. Strikingly, import of GST-Rev was almost as efficient in the presence of the antibody as in the control reaction, suggesting that other transport receptors besides importin β are present in the cytosolic extract and mediate nuclear import of Rev. Of course we cannot completely exclude the possibility that the monoclonal anti-importin β antibody inhibits importin α-dependent, but not importin α-independent, import.

Another import receptor known to be involved in transport of basic proteins is the importin β family member transportin. We therefore tested whether inhibition of transportin would affect nuclear import of the basic Rev protein. For inhibition we now used an excess of a transportin-dependent import substrate, the M9 sequence fused to the maltose-binding protein (MBP-M9). Nuclear import of GST-M9 was strongly inhibited in the presence of an excess of MBP-M9 (Fig. 2B), demonstrating the saturation of the pathway. We also showed that under similar conditions the transportin-dependent import of c-Fos is inhibited (9). Inhibition was specific for the transportin pathway, as import of BSA-NLS (a substrate for importin α/β) was not affected by

![FIGURE 2. Individual importins are not required for nuclear import of Rev. A, nuclear import reactions in permeabilized cells were performed with GST-NLS, GST-M9, or GST-Rev as import substrate with cytosol that had been preincubated with (+ α-imp β) or without (− α-imp β) a monoclonal antibody against importin β. Import reactions were performed in the presence of cytosol with GST-M9, fluorescein isothiocyanate-BSA-NLS, or GST-Rev as import substrate, with (+) or without (−) MBP-M9 as a competitor of transportin-dependent import. C, import reactions were performed in the presence of cytosol with GST-Rev as import substrate with or without ribosomal protein L23 (left panel) or histone H2B (right panel) as broad range transport competitors. D, import reactions were performed in the presence of cytosol with GST-M9 or histone H1.2 as import substrate and cleaved Rev as competitor as indicated.](https://doi.org/10.1074/jbc.M605623200)
MBP-M9 (Fig. 2B). Likewise, GST-Rev was imported into the nuclei whether or not MBP-M9 was present in the reaction. We conclude from these results that neither importin β nor transportin is absolutely required for nuclear transport of Rev.

Some basic nuclear proteins have been shown to be imported into the nucleus by multiple transport receptors. We therefore used the ribosomal protein L23 and the histone H2B, which interact with importin β, transportin, importin 5, and importin 9 (8, 36), in combination with GST-Rev in nuclear import reactions. As shown in Fig. 2C, L23 and H2B efficiently inhibited nuclear accumulation of GST-Rev. In a reciprocal approach, we performed nuclear import reactions with various substrates to test whether their nuclear import can be inhibited by Rev. In the absence of competing Rev, GST-M9 was efficiently imported into the nucleus (Fig. 2D). When high concentrations of Rev were included in the reaction, nuclear import of GST-M9 was clearly reduced. Similarly, histone H1.2, which uses the importin β/importin 7 heterodimer as an import receptor (56), was imported into the nucleus in the absence, but not in the presence, of Rev (Fig. 2D). Nuclear import of BSA-NLS, in contrast, was hardly affected by Rev (data not shown).

Taken together, these results suggest that multiple transport receptors of the importin β superfamily serve as importins for the viral protein HIV-Rev.

**HIV-Rev Can Be Imported into the Nucleus by Various Transport Receptors**—We directly tested this hypothesis, using bacterially expressed transport receptors. Recombinant importin β, transportin, importin 5, and importin 7 all promoted nuclear import of GST-Rev (Fig. 3A). Transport was specific, as no nucleolar accumulation of Rev was observed when cells were incubated at 4 °C. Furthermore, RanQ69L inhibited import mediated by importin β, transportin, and importin 5 (Fig. 3B). Import of GST-Rev in the presence of importin 7 was not sensitive to RanQ69L, probably reflecting the low affinity of RanGTP for this specific transport receptor (37). For histone H1, individual transport receptors do not promote nuclear import and a heterodimer of importin β and importin 7 has been shown to function as the physiological import receptor (38). For Rev, no further stimulation of import was observed when these two importins were added together to the reaction (data not shown), suggesting that dimer formation is not required for nuclear import of Rev.

Rev has been shown to directly bind to importin β (22, 23). We now tested whether other import receptors would specifically interact with Rev as well. As shown in Fig. 4, not only importin β, but also transportin, importin 5, and importin 7, bound to immobilized GST-Rev, but not to immobilized GST. Furthermore, binding of Rev to importin β, transportin, and, to some extent, importin 5 was sensitive to RanQ69L, demonstrating the specificity of the interaction. Only a small reduction in binding of importin 7 to GST-Rev was observed when RanQ69L was included in the reaction, probably reflecting the low affinity of importin 7 for RanGTP (compare Fig. 3B).

The arginine-rich domain of Rev is required for its nuclear import (20, 21) as well as for the interaction with importin β (22, 23). We therefore tested whether the interaction of Rev with other import receptors would also depend on this basic region. In the Rev mutant M5, two arginines are exchanged for aspartic acid and leucine, respectively. This mutation was shown to inhibit nuclear import of Rev as well as Rev function in general (24). Importin β, transportin, importin 5, and importin 7 all interacted with GST-Rev (wild type), but not with the mutant GST-Rev-M5 (Fig. 5). This result
shows that the arginine-rich NLS of Rev serves as a recognition sequence for various transport receptors. Mutations in regions besides the arginine-rich NLS have been described as leading to reduced nuclear import of Rev. We tested the Rev mutants MA4 and MA5 (39) for their interaction with the transport receptors importin β, transportin, importin 5, and importin 7 but did not detect significant differences compared with the wild-type protein (data not shown). Possibly, these mutations affect the general conformation of the Rev protein without perturbing the receptor-substrate interaction. The reasons for reduced nuclear accumulation of these mutants remain to be investigated.

How does the interaction of importins with Rev compare to that with their established import cargoes? To answer this question for the two best described importins, we expressed N- and C-terminal fragments of importin β and transportin and analyzed their binding to immobilized GST-Rev. As control cargoes, we immobilized GST-IBB (the importin β-binding domain of importin α) for importin β and the M9 sequence of the hnRNP A1 protein for transportin. As expected, the IBB domain bound to full-length importin β and to the C-terminal, but not to the N-terminal, fragment (Fig. 6A). Rev, in contrast, interacted with full-length importin β and both its N- and C-terminal fragments. Binding of Rev to full-length importin β is inhibited in the presence of RanQ69L (compare Fig. 4), suggesting that its interaction with the fragments is specific, too. We could confirm this assumption for the N-terminal fragment of importin β, which contains the Ran-binding site. As shown in Fig. 6B, RanQ69L prevented the interaction of GST-Rev with this importin β fragment. As a control, RanQ69L did not affect binding of Rev to the C-terminal fragment of importin β, as this part does not contain a Ran-binding site. Taken together, binding of importin β to Rev is clearly distinct from that to the IBB domain of importin α. In similar experiments, the M9 sequence of hnRNP A1 bound exclusively to a C-terminal fragment of transportin, whereas GST-Rev interacted with both an N-terminal and a C-terminal fragment (Fig. 6C). Again, binding of full-length transportin to Rev was specific, as it could be blocked by RanQ69L (compare Fig. 4). We performed a specificity control for the N-terminal fragment of transportin, which contains the Ran-binding domain. As shown in Fig. 6D, binding...
of Rev to this N-terminal fragment was clearly specific, as it was strongly reduced when RanQ69L-GTP was included in the reaction. RanQ69L did not affect binding of Rev to the C-terminal transportin fragment (Fig. 6D). We conclude from these results that the interaction of transport receptors with Rev is fundamentally different from that with their standard import cargoes, as different regions in the importins participate in binding.

After one round of transport, import receptors are recycled back to the cytoplasm in a complex with RanGTP. Importantly, binding of import cargo and RanGTP to import receptors is mutually exclusive, so RanGTP has to dissociate from the importin prior to a second round of import. This dissociation is coupled to GTP hydrolysis on Ran, a reaction that is strongly stimulated by the GTPase activating protein RanGAP. Importin-RanGTP complexes, however, are resistant to RanGAP stimulated by the GTPase activating protein RanGAP. Importin prior to a second round of import. This dissociation is mutually exclusive, so RanGTP has to dissociate from the binding of import cargo and RanGTP to import receptors is back to the cytoplasm in a complex with RanGTP. Importantly, results that the interaction of transport receptors with Rev is more complicated by the fact that in addition to RanGAP and RanBP1, importin α is also needed for the dissociation of the importin-RanGTP complex (41, 42). As a consequence, the dissociation of the importin β-RanGTP complex appears to be coupled to the formation of a novel, import-competent importin α/β complex (30). However, Rev and importin α do not bind simultaneously to importin β (22). This raises the question whether import cargoes that bind directly to importin β are also able to promote the dissociation of importin β-RanGTP, like importin α. RanGAP assays where RanGAP-stimulated GTP hydrolysis on Ran is analyzed were performed with preassembled importin β-RanGTP complexes. In the presence of RanBP1, RanGAP alone was unable to promote efficient GTP hydrolysis on importin β-RanGTP. Upon addition of importin α to the reaction, however, strong GTP hydrolysis was observed (Fig. 7A), indicating an efficient dissociation of the importin β-RanGTP complex and, in all likelihood, a concomitant formation of a novel importin α/β complex. When we added Rev to the reaction, GTP hydrolysis was detected as well, albeit to a somewhat lower extent (Fig. 7A). In similar experiments, we added other importin β binding import cargoes like c-Fos (9, 14) or snurportin, an importin α-like adapter protein (43), to preassembled importin β-RanGTP complexes. Similar to importin α and Rev, both cargoes promoted GTP hydrolysis on Ran under our experimental conditions (Fig. 7, B and C). These results suggest that importin β binding import cargoes in general can disassemble importin β-RanGTP complexes, enabling them to compete with importin α for recycling importin β. These reactions are likely to lead to the formation of novel import-competent complexes containing importin β and either importin α, snurportin, or, for example, HIV Rev. In summary, we showed that multiple members of the importin β superfamily can function as import receptors for the Rev transactivator.

**DISCUSSION**

**Multiple Transport Receptors Function as Importins for Rev—**

The major nuclear import pathway involves recognition of a basic NLS by a complex of the adapter protein importin α and the import receptor importin β. In this study, we analyzed the importin α-independent nuclear import of the viral protein HIV Rev. Rev uses an arginine-rich NLS for nuclear import, i.e. a sequence that is clearly distinct from the “classic” lysine-rich NLS. We showed that Rev binds directly to importin β, independent of the adapter protein importin α, confirming previous results (22, 23). Importin β, however, is unlikely to be the only transport receptor for Rev, as inhibition of importin β with a monoclonal antibody did not prevent nuclear accumulation of the viral protein. Likewise, inhibition of the transportin-dependent import pathway with a competing substrate did not impede nuclear transport of Rev. To achieve import inhibition, the promiscuous substrates L23 or histone H2B, which are
known to bind to a variety of import receptors, were needed. In agreement with these results, the transport receptors importin β, importin 5, importin 7, and transportin were equally able to bind specifically to Rev and to promote its nuclear import in permeabilized cells. Rev can also inhibit nuclear import of some transport cargoes. As it potentially interacts with a number of different import receptors in a cytosolic extract, however, the degree of inhibition varies for individual substrates depending on the cytosolic concentrations of the receptor proteins and their relative affinities to Rev and their specific substrates.

Our results are at variance with earlier observations (23) that suggested that transportin does not function as an importin for Rev. This discrepancy may result from differences in the way the nuclear transport assays were performed and/or the activity of the transport factors or import substrates.

The Interaction of Importins with Rev—Different importins appear to interact with Rev in a similar fashion, as point mutations in the arginine-rich NLS of the protein prevent all transport receptors from binding. Of course we cannot exclude the possibility that other regions in Rev that affect nuclear import are critical for the interaction with individual importins. We have recently described the nuclear import of the transcription factor c-Fos by more than one transport pathway (9). For this import substrate, however, different regions of the protein are used for the interaction with different importins. Furthermore, transportin appears to be a dominant transport receptor for c-Fos, as its inhibition leads to strongly reduced nuclear import of the transcription factor in permeabilized cells.

Which regions in import receptors are involved in binding to cargo molecules? For importin β, a number of receptor-cargo complexes have been crystallized (44–46). Importin α, the major cargo of importin β, interacts only with the C-terminal part of the import receptor (44). In contrast, Rev also binds to the N-terminal part of importin β. In this respect, it behaves similar to the parathyroid hormone-related protein (45). For transportin, no structures of receptor-cargo complexes have been reported so far. From binding studies it is known that the C-terminal part of transportin binds to its best characterized import cargo, the M9 sequence of the hnRNP A1 protein (47). c-Fos (9) and Rev, in contrast, specifically interact with the N-terminal part of transportin. The modes of interaction with this N-terminal fragment, however, appear to be different, as a basic stretch in Rev is involved in binding to transportin, which is not the case for c-Fos. We also detected substantial binding of Rev to the C-terminal fragment of transportin. Rev does not contain an obvious M9-like sequence, so the molecular basis for the interaction of this C-terminal part with Rev or the M9 sequence is probably very different. Rev has been shown to form homo-multimeric complexes on its Rev response element RNA target, and this oligomerization is required for Rev transactivation (48–50). Rev also forms homo-multimeric complexes in vivo in the absence of RNA binding (51, 52). It therefore appears conceivable that import receptors like transportin with more than one binding site for Rev serve as a scaffold for oligomerization of the viral protein, already in the cytoplasmic stage of nuclear import. Taken together, our results emphasize the striking capabilities of nuclear import receptors in recognizing different structural motives in a large variety of cargo molecules.

The retroviral Rev transactivator belongs to a group of basic proteins that can be imported into the nucleus via multiple transport pathways. Other members of this group are certain ribosomal proteins and the core histones. This raises the question of which transport receptor imports Rev into the nucleus in a living cell. Presumably, this depends on the concentration of the individual import receptors and of competing import cargoes. The cellular concentration of importin β is in the range of 1–2 μM, similar to that of its major import cargo, importin α (53). Rev can engage in the recycling of RanGTP-bound importin β that is coming out of the nucleus (Fig. 7), similar to importin α, snurportin, and c-Fos. In a reaction involving RanGAP and RanBP1, the dissociation of Ran from importin β is coupled to the formation of a novel import complex consisting of importin β and either importin α or Rev. For importin α, this reaction is further stimulated by NLS cargoes (54). Hence, the vast majority of importin β that is exported out of the nucleus may be rapidly incorporated into novel NLS-importin α complexes before ever encountering Rev, which is, as a regulatory protein, expressed to relatively low levels in a typical viral infection (55). Under conditions of limited availability of importin β (e.g., in certain cell types), Rev may therefore take advantage of its ability to interact with alternative transport receptors. It shares this property with abundant cellular proteins like ribosomal components or histones, which have to be imported into the nucleus very efficiently. In summary, the HIV-1 Rev protein uses all available cellular resources for its efficient nuclear import.

Acknowledgments—We thank Dr. Hans-Georg Kräusslich and Dr. Frauke Melchior for generous support of the project, Stephan Urban and Stefan Seitz for help with protein purifications, Dr. Jörg Kahle for the kind gift of importin 7, and Dr. Dirk Görlich, Dr. Tom Hope, and Dr. Larry Gerace for antibodies and plasmids. We acknowledge Dr. Eric Meulemeester for critical reading of the manuscript.

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