Identification of Residues in the C-terminal Domain of HIV-1 Integrase That Mediate Binding to the Transportin-SR2 Protein*

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Background: The molecular details of the TRN-SR2/HIV-1 IN interaction are not known.

Results: Crucial amino acids in IN for the interaction with TRN-SR2 are located in the CTD, Arg-262/Arg-263/Lys-264, and Lys-266/Arg-269.

Conclusion: TRN-SR2 primarily interacts with the CTD domain of IN.

Significance: Understanding of the IN/TRN-SR2 interaction is necessary to guide drug discovery efforts targeting the nuclear entry step of replication.

Transportin-SR2 (TRN-SR2 and TNPO3) is a cellular cofactor of HIV replication that has been implicated in the nuclear import of HIV. TRN-SR2 was originally identified in a yeast two-hybrid screen as an interaction partner of HIV integrase (IN) and in two independent siRNA screens as a cofactor of viral replication. We have now studied the interaction of TRN-SR2 and HIV IN in molecular detail and identified the TRN-SR2 interacting regions of IN. A weak interaction with the catalytic core domain (CCD) and a strong interaction with the C-terminal domain (CTD) of IN were detected. By dissecting the catalytic core domain (CCD) of IN into short structural fragments, we identified a peptide (INIP1, amino acids 170EHLKTAVQMA-228FRVYYR) that comes together in the CTD tertiary structure to form an exposed antiparallel β-sheet. Through site-specific mutagenesis, we defined the following sets of amino acids in IN as important for the interaction with TRN-SR2: Phe-185/Lys-186/Arg-187/Lys-188 in the CCD and Arg-262/Arg-263/Lys-264 and Lys-266/Arg-269 in the CTD. An HIV-1 strain carrying K266A/R269A in IN was replication-defective due to a block in reverse transcription, confirming the study of nuclear import. Insight into the IN/TRN-SR2 interaction interface is necessary to guide drug discovery efforts targeting the nuclear entry step of replication.

The human immunodeficiency virus type 1 (HIV-1) belongs to the family of Lentivirinae, characterized by the ability to infect both dividing and nondividing cells (1, 2). After virus entry and reverse transcription, the preintegration complex (PIC) translocates to the nucleus, and the viral DNA is integrated into the host chromatin. HIV crosses the nuclear membrane through active nuclear import of the PIC (3). As efficient integration into human chromatin depends on nuclear import, and only few PICs seem to enter the nucleus, this process constitutes a true bottleneck for viral replication (4). Until recently, the mechanism of HIV nuclear import, albeit intensely studied, remained obscure (for recent reviews see Refs. 5, 6). Several viral elements, including matrix, viral protein R, capsid, IN, and the central polypurine tract have been suggested to play a role in the nuclear import of HIV (5, 7–10). Next to different viral components, multiple nuclear import factors have been implicated in the nuclear translocation of the PIC, such as importin-α/β (11–13), importin-7 (14–16), and importin-α3 (17). Recently, transportin-SR2 (TRN-SR2, TNPO3) has been proposed as a cellular cofactor of HIV-1 replication (18–20) mediating nuclear import of the PIC (19, 20). Independent of two genome-wide siRNA screens (18, 20), TRN-SR2 was identified as an interaction partner of HIV-1 integrase in a yeast two-hybrid screen (19). A reverse screen confirmed the interaction and demonstrated that none of the other HIV proteins directly interact with TRN-SR2. Using RNAi technology, the crucial role of TRN-SR2 in HIV replication was demonstrated (18–20). A specific reduction in two long terminal repeat (LTR) circles and integration was measured by quantitative PCR (Q-PCR) (19, 21, 22). Direct evidence for TRN-SR2-mediated nuclear import of HIV was obtained by using fluorescently labeled PICs (19). The importance of TRN-SR2 for HIV replication has been confirmed by multiple reports, but its exact mechanism of action is still under debate (7, 18–28). HIV-1 integrase (IN) mediates the integration of the viral DNA into the host chromatin (29). It consists of three domains connected by flexible

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The abbreviations used are: PIC, preintegration complex; CCD, catalytic core domain; CTD, C-terminal domain; NTD, N-terminal domain; IN, integrase; PDB, Protein Data Bank; co-IP, co-immunoprecipitation; NLS, nuclear localization sequence; Q-PCR, quantitative PCR; AZT, azidothymidine.
linkers as follows: 1) the N-terminal HH-CC zinc-binding domain (NTD); 2) the catalytic core domain (CCD); and 3) the C-terminal domain (CTD) (30). Integrase has been implied in nuclear import of HIV in multiple studies and various nuclear localization sequences have been reported. First, a bipartite nuclear localization signal in the CTD was described that consisted of amino acids 211KELQKQTIK219 and 261PRRKAQ266 (11). Later, a nonclassical NLS 161IIQVQDRQAEHLK173 was described in the CTD (31). Although TRN-SR2 was identified in a yeast two-hybrid screen as an interaction partner of HIV-1 IN (19), the question still remains whether the interaction between IN and TRN-SR2 mediates HIV nuclear import. As a first step in answering this question, we analyzed the interaction of TRN-SR2 and HIV-IN in molecular detail to identify the minimal interacting regions and the protein–protein interaction hot spots in HIV IN.

EXPERIMENTAL PROCEDURES

Recombinant Protein Purification—Recombinant proteins were expressed in Escherichia coli BL21-CodonPlus (DE3). Recombinant His$_6$-tagged HIV-1 integrase was purified as described previously (32). We thank Dr. Woan-Yuh Tarn (Institute of Biomedical Sciences, Taiwan) for the pGEX-TRN-SR2 expression plasmid. Recombinant GST-tagged and His$_6$-tagged TRN-SR2 were purified as described previously (19). For the expression of the GST peptides, bacteria were grown to an OD of 0.6, and protein expression was induced with 0.5 mM isopropyl-$\beta$-D-thiogalactopyranoside. After incubation at 37°C for 2 h, the bacteria were harvested, washed, and stored at −20°C. For purification of the GST peptides, the cells were resuspended in lysis buffer (PBS (pH 7.4), 0.5 mM NaCl, 1 mM DTT, 1 mg/ml lysozyme, 0.1 mM PMSF, 1 µl of DNase/10 ml). After complete lysis by sonication, the supernatant was cleared by centrifugation, and recombinant proteins were bound to glutathione-Sepharose resin (GE Healthcare). After washing of the resin with 20 volumes of washing buffer (PBS (pH 7.4), 0.5 mM NaCl, 1 mM DTT), the GST-tagged protein was eluted with 10 mM of elution buffer (PBS (pH 7.4), 0.5 µM NaCl, 1 mM DTT, 20 mM reduced glutathione). The fractions were analyzed by SDS-PAGE for protein content, pooled, and dialyzed (overnight, 4°C) against storage buffer (PBS (pH 7.4), 1 mM DTT, 10% (v/v) glycerol).

AlphaScreen Binding Assay—The AlphaScreen binding assay was optimized for use in 384-well OptiPlate microplates (PerkinElmer Life Sciences) with a final volume of 25 µl. Proteins were all diluted to 5× working solutions in the assay buffer (25 mM Tris (pH 7.4), 150 mM NaCl, 1 mM MgCl$_2$, 2 mM DTT, 0.1% (v/v) Tween 20, and 0.1% (w/v) bovine serum albumin (BSA)). First, 10 µl of the TRN-SR2 was pipetted into the wells, followed by 5 µl of His$_6$-IN or a GST–peptide dilution series. The plate was sealed and left to incubate for 1 h at 4°C. Next, 10 µl of a mixture of Ni$^{2+}$ chelate acceptor and glutathione donor AlphaScreen beads (PerkinElmer Life Sciences) was added. This establishes final concentrations of 20 µg/ml for each of the beads. Plates were then incubated for 1 h at 30°C and analyzed using an EnVision Multilabel Reader (PerkinElmer Life Sciences) according to the manufacturer’s instructions. Each titration was performed in duplicate, and assays were repeated at least twice in independent experiments. The equilibrium dissociation constants (apparent $K_d$) were calculated with Prism 5 (GraphPad), by plotting the titration curves using a one-site-specific binding with Hill slope fit as shown in Equation 1.

$$Y = (B_{max}) \times (X_0 + Z_0 + K_d) - \sqrt{(X_0 + Z_0 + K_d)^2 - (4X_0Z_0)} / 2$$

(Eq. 1)

The background signal was subtracted from each value. Points for which quenching of the signal occurs through excess of either binding partner (“hooking” as referred to by the supplier), were excluded from the final plot.

Co-immunoprecipitation—Proteins were expressed by double polyethyleneimine-mediated transfection of 293T cells with 10 µg of each plasmid. 24 h after transfection, cells were harvested and lysed in co-IP buffer (50 mM Tris (pH 7.3), 300 mM NaCl, 1 mM MgCl$_2$, 10% glycerol (v/v), 0.5% Triton X-100 (v/v) and protease inhibitor mixture (EDTA-free)) after which lysates were incubated with anti-FLAG-agarose beads (Sigma). After 3 h of incubation, the agarose beads were recovered by centrifugation for 2 min at 4000 rpm at 4°C and washed three times with 500 µl of co-IP buffer. Bound proteins were analyzed by SDS-PAGE followed by Western blotting. Membranes were probed with monoclonal antibodies against 3×FLAG (1:10,000 dilution) (Sigma) and GFP (1:5000) (Abcam). Detection was performed using horseradish peroxidase-conjugated goat anti-mouse and rabbit anti-goat antibodies, respectively (Dako), and chemiluminescence (ECL+, Amersham Biosciences).

Integrase Activity Assays—The 3′-processing activity was measured in a standard assay using radioactively labeled oligodeoxynucleotide substrates resembling the viral LTR end, although a quantitative ELISA was used to measure the overall enzymatic activity (33).

Viruses and Viral Vectors—The viral molecular clones pNL4-3 and pNL4-3.Luc.R.E− were obtained through the AIDS Research and Reference Reagent Program. The pMD.G plasmid encodes the vesicular stomatitis virus glycoprotein (34, 35). Viruses were produced by single polyethyleneimine-mediated transfection of 293T cells using 20 µg of the viral molecular clone pNL4-3 per 10-cm dish. Vesicular stomatitis virus glycoprotein-pseudotyped viral vectors were produced by double polyethyleneimine-mediated transfection of 293T cells using 5 µg of the envelope expression plasmid pMD.G and 20 µg of the molecular clone pNL4-3.Luc.R.E− per 10-cm dish. Two and 3 days post-transfection, the supernatant was harvested, filtered with 0.22-µm pore-size syringe filters (Sartorius), concentrated by centrifugal filtration using VivaSpin concentrators (Millipore), and treated with DNase I (Roche Applied Science).

RESULTS

Determination of the Interaction Domain between HIV-1 Integrase and TRN-SR2—By yeast two-hybrid, we previously identified TRN-SR2 as a bona fide binding partner of HIV-1 IN (19). A reverse screen confirmed the interaction between TRN-SR2 and IN and demonstrated that no other viral protein interacts with TRN-SR2 under these conditions. By now, the inter-
action has independently been confirmed by co-IP, pulldown (7, 19), AlphaScreen (26), and surface plasmon resonance (7).

To define the minimal TRN-SR2 interaction domain in HIV-1 integrase, we now investigated its interaction with the NTD, the CCD, and the CTD. The different IN domains fused to GFP were expressed in 293T cells, and TRN-SR2 was expressed with a 3×FLAG tag (Fig. 1A). By co-IP, TRN-SR2 was shown to interact with both the catalytic core and the C-terminal domain of IN but not with the NTD (Fig. 1A). Next, deletion mutants of either the CCD or the CTD were generated and tested for interaction with TRN-SR2 in co-IP (data not shown). This approach failed to generate further insights because mutants bound nonspecifically to the agarose beads, most likely due to their reduced structural stability. We therefore opted for an in vitro approach.

We purified recombinant full-length IN and its domains carrying an N-terminal His6 tag as well as recombinant TRN-SR2 with an N-terminal GST tag to determine the interaction by AlphaScreen (Fig. 1B). The apparent $K_d$ value as well as the AlphaScreen counts are important to compare the affinity of two proteins tested in AlphaScreen. Both the CCD and the CTD of HIV-1 IN displayed affinity for TRN-SR2. The CCD of IN interacts with TRN-SR2 with a 2-fold lower affinity ($K_d = 40.2 \pm 15.1$ nM) and 10-fold lower counts than full-length IN ($K_d = 16.8 \pm 3.8$ nM), whereas the CTD of IN interacts with TRN-SR2 in a cooperative manner with a 6-fold lower affinity ($K_d = 94.2 \pm 2.6$ nM) but 4-fold higher counts than full-length IN (Fig. 1B). The data confirm direct protein-protein interactions between TRN-SR2 and both CCD and CTD of HIV-1 IN as shown by co-IP. Hence, we focused our subsequent analysis of the interaction interface on these two domains.

Defining the Minimal Interaction Domain in the CCD of HIV-1 IN—Nuclear localization signals are usually composed of short amino acid stretches and are commonly bipartite (36). Classical NLSs for instance are known to be composed of one or two basic-rich stretches (37). Therefore, single point mutations usually do not abolish the interaction of a cargo with its specific nuclear import factor (38, and multiple mutations are required.

FIGURE 1. TRN-SR2 interacts with the catalytic core domain and with the C-terminal domain of IN. A, GFP-IN and FLAG-TRN-SR2 were detected with anti-GFP and anti-3×FLAG antibodies, respectively, after Western blotting. GFP-tagged full-length IN or IN domains were co-expressed with 3×FLAG-TRN-SR2 or 3×FLAG in 293T cells (left panel). 3×FLAG-TRN-SR2 and 3×FLAG were immunoprecipitated (IP) with anti-FLAG antibody, in the presence of full-length IN, ΔNTD (IN deleted for NTD), ΔCTD (IN deleted for CTD), NTD, CCD, or CTD of IN (right panel). 3×FLAG serves as a control for aspecific co-immunoprecipitation. B, analysis of the interaction of GST-tagged TRN-SR2 with His6-tagged full-length integrase (upper left panel), the NTD (upper right panel), the CCD (lower left panel), and the CTD (lower right panel) of HIV-IN. Complexes of GST-TRN-SR2 and His6-IN were bound to glutathione donor beads and nickel-chelate acceptor beads. Light emission was measured using an EnVision Multilabel Reader. Each experiment was repeated three times. Representative graphs of single experiments are shown. The apparent equilibrium dissociation constants ($K_d$) were calculated with GraphPad Prism 5 and are indicated.
TABLE 1
Amino acid sequences of IN derived fragments

| Fragment name | Fragment sequence |
|---------------|-------------------|
| CCD(91–108)  | AETGQETAYFLKLAKGR |
| CCD(118–138) | GSNFTSTKVAAACWYAGIKQ |
| CCD(145–166) | SQGFVSMKELKKIIQVDR |
| CCD(170–191) | EHLKTVAVMQAFHNFKRGGL |
| CTD(214–228) | QKQTQIKFRYYVR |
| CTD(229–243) | DSRPDLWKGAKLLW |
| CTD(244–258) | KGEAVIQQDNSDIK |
| CTD(259–273) | VPVRKVKKIDRYGK |
| CTD(274–288) | QMAGDCCVASQED |

*a The fragment numbers refer to the positions of the amino acids in integrase and define the first and the last amino acid of each CTD fragment. The peptides were designed to match protein stretches (15–22 amino acids) of IN with at least some surface-exposed amino acids.

Likewise, abolishing the IN/TRN-SR2 interaction by single point mutations proved to be difficult in our hands, and deletion mutants of IN domains displayed poor solubility. Therefore, we opted for a different strategy, selecting short oligopeptide stretches that are lining the surface of the CCD of HIV-1 IN and screened those for interaction with TRN-SR2. For ease of expression and purification, we produced the small CCD fragments with a GST tag and analyzed their binding to His6-TRN-SR2. Reversal of the affinity tags did not affect the interaction of IN with TRN-SR2 (data not shown; GST-IN/His6-TRN-SR2, $K_d = 18.6 \pm 1.9$ nm; His6-IN/GST-TRN-SR2, $K_d = 16.8 \pm 3.8$ nm). We purified four distinct GST-tagged oligopeptide fragments representing the four α-helices lining the CCD surface (Table 1) and analyzed them for their affinity to His6-TRN-SR2 (Fig. 2A).

One of the four peptides (CCD(170–191)) displayed a moderate affinity for His6-TRN-SR2 (Fig. 2A). The AlphaScreen signal for CCD(170–191) allowed us to determine a $K_d$ of 401.4 ± 7.7 nm. We will refer to the CCD(170–191) peptide as integrase-derived interaction peptide 1 (INIP1). To map the amino acids involved in the interaction, we performed mutagenesis of selected residues in INIP1. The selection of mutants was guided by the general knowledge of NLS sequences and the surface exposure of amino acid residues in integrase (Fig. 2B). Therefore, we generated two mutants of INIP1 (INIP1R170Q/H171A/K173A and INIP1F185A/K186A/R187A/K188A) and assayed their affinity for TRN-SR2 (Fig. 2C). Both mutants of INIP1 displayed a reduced affinity for TRN-SR2. To confirm that these amino acids were the main contributors for the IN-TRN-SR2 interaction, we generated the corresponding mutants in the CCD domain (CCD170Q/H171A/K173A and CCDF185A/K186A/R187A/K188A). In agreement with the results obtained with the peptides, CCD170Q/H171A/K173A showed a reduced affinity for TRN-SR2, although CCDF185A/K186A/R187A/K188A did not interact any longer with TRN-SR2 in AlphaScreen (Fig. 2D).

Defining the Minimal Interaction Domain in the C-terminal Domain of HIV-1 IN—Following the same approach, we divided the C-terminal domain of HIV-1 IN into short oligopeptide stretches of 15 amino acids each (Table 1) and screened those for interaction with TRN-SR2. The small CTD fragments were purified with a GST tag. The five distinct GST-tagged oligopeptide fragments together covered amino acids 214–288 of the CTD and were analyzed for their affinity to His6-TRN-SR2 in the AlphaScreen assay (Fig. 3A).

Two out of five peptides (CTD(214–229) and CTD(259–274)) displayed affinity for His6-TRN-SR2. CTD(214–229) interacts with TRN-SR2 with an apparent $K_d$ of 288.3 ± 22.03 nm, whereas the interaction with CTD(259–274) was too weak to allow accurate $K_d$ determination (Fig. 3A). Mapping the sequences of both peptides onto the crystal structure of CCD-CTD integrase demonstrates a striking structural relationship between both peptides; CTD(214–229) and CTD(259–274) together form an antiparallel β-sheet in the CTD of IN (Fig. 3B).

We subsequently coupled fragments CTD(214–229) and CTD(259–274) through a flexible three-amino acid linker (DSG). Based on structural information (PDB code 1EX4 (39)) amino acids 259–261 were left out, resulting in the 30-mer CTD(214–228) (link-262–273) (Fig. 3B). The affinities of CTD(214–228), CTD(259–273), and the combined peptide were subsequently determined (Fig. 3C). The combined peptide CTD(214–228)-link-262–273 interacts with TRN-SR2 with a similar affinity ($K_d = 87.9 \pm 4.8$ nm) as the affinity of the entire CTD ($K_d = 108.5 \pm 3.6$ nm) (Fig. 3C). The combined peptide will be referred to as integrase-derived interaction peptide 2 (INIP2).

Mapping of the Hot Spots of Interaction in INIP2—To pinpoint the actual protein-protein contacts, we performed mutagenesis of selected residues in INIP2. Selection of mutants was again based on the general knowledge of NLS sequences and amino acid surface exposure in IN (Fig. 4A). Because a typical NLS contains a set of basic residues, we first generated mutant INIP2 versions in which two or three basic residues were substituted with alanine. Some NLSs, like the M9 NLS (37), are rich in glycine and aromatic residues. Hence, we also mutated several aromatic residues into alanine. We then tested these mutant peptides for interaction with TRN-SR2 in the AlphaScreen assay (Fig. 4B). Substitutions of either positively charged or aromatic amino acids, affected the binding of INIP2 to TRN-SR2. INIP2R224A/Y226A ($K_d = 173.8 \pm 10.9$ nm) displayed a slightly lower affinity for TRN-SR2 than wild type INIP2 ($K_d = 113.0 \pm 0.6$ nm), INIP2K266A/R269A ($K_d = 298.5 \pm 28.8$ nm) and INIP2R262A/R263A/K264A ($K_d = 384.1 \pm 23.7$ nm) displayed a 3–4-fold lower affinity than wild type INIP2, respectively (Fig. 4B). The binding of INIP2F223A/Y226A was too weak to allow confident $K_d$ determination.

As none of the initial substitutions led to a complete loss of binding, we generated two CTD mutants each containing two sets of substitutions generated in INIP2 (CTDF223A/R224A/Y226A/R228A and CTDK266A/R269A). CTDF223A/R224A/Y226A/R228A showed a reduced affinity for TRN-SR2, although CTDK266A/R269A completely lost binding to TRN-SR2 in AlphaScreen (Fig. 4C). To narrow down the number of substitutions needed to abrogate the binding of the CTD to TRN-SR2, we generated two more CTD mutants (CTD262A/R263A/K264A and CTDK266A/R269A). CTDK266A/R269A showed a reduced affinity for TRN-SR2 although CTD262A/R263A/K264A completely lost binding to TRN-SR2 (Fig. 4C).

IN Mutants Defective for Interaction with TRN-SR2—To prove the importance of the selected residues in the CCD and CTD, we finally inserted these sets of substitutions into full-length IN. Interaction of both INF185A/K186A/R187A/K188A and INK262A/R263A/K264A with TRN-SR2 was reduced in comparison.

S. De Houwer and W. Thys, unpublished data.
with WT IN (Fig. 5A). The F185A/K186A/R187A/K188A substitutions only moderately affected TRN binding (50% reduction of AlphaScreen signal), whereas the R262A/R263A/K264A substitutions clearly mediated the largest effect (95% reduction in signal), although residual binding subsists (Fig. 5A). To control for correct folding of the interaction-defective mutant integrases (IN(F185A/K186A/R187A/K188A) and IN(R262A/R263A/K264A)), we analyzed their interaction with LEDGF/p75 (32) and IN (Fig. 5B). LEDGF/p75 is a validated cofactor of integrase that interacts with integrase through its CCD domain (40–42). The development of a robust AlphaScreen to measure interaction between LEDGF/p75 and IN has been described before (33). IN(R262A/R263A/K264A) bound LEDGF/p75 with a similar affinity as wild type IN (Fig. 5B) demonstrating that the overall structure of IN is not altered by these substitutions. Not surprisingly, IN(F185A/K186A/R187A/K188A) did not bind to LEDGF/p75 (Fig. 5B) because the substitutions K186A/R187A are known to inhibit tetramerization and LEDGF/p75 binding (43). To additionally control for the tertiary and quaternary structure, we tested the mutant integrases by size exclusion chromatography (Fig. 5C). The F185A/K186A/R187A/K188A substitutions clearly affected the IN oligomerization as no tetramers could be measured, only dimers of IN were present. The R262A/R263A/K264A substitutions, however, did not affect IN oligomerization (Fig. 5C). Because the F185A/K186A/R187A/K188A substitutions clearly affect IN multimerization (Fig. 5C), we cannot exclude that the impact on TRN-SR2 binding is due to the altered multimerization equilibrium. Therefore and because the F185A/K186A/R187A/K188A substitutions (located in the CCD) only modestly affected TRN-SR2 binding, we focused on the substitutions in C-terminal IN for the remainder of our study.

Analysis of the Replication of HIV-1 Defective for Interaction with TRN-SR2—Because IN substitutions are known to have pleiotropic effects on HIV replication, we tried to find a set of mutations that only affects interaction with TRN-SR2. We screened different sets of double point mutants for their effect on binding to TRN-SR2 (Fig. 6A). Ultimately, we selected two double mutant INs (IN(R263A/K264A) and IN(K266A/R269A)) with a 10-fold reduced affinity for TRN-SR2. We next assayed these two mutants for 3′-processing and overall integrase activity. IN(R263A/K264A) was defective (<25% of WT activity) for 3′-processing and strand transfer, whereas IN(K266A/R269A) could catalyze 3′-processing (>75% of WT activity) but not strand transfer (data not shown). As productive DNA binding is a prerequisite of catalytic activity, this assay indirectly reflects on the DNA binding capacity of IN(K266A/R269A). Because IN(K266A/R269A) showed a strongly reduced affinity for TRN-SR2 peptide; ○, CCD(91–108); ■, CCD(118–138); ▲, CCD(145–167); ▼, CCD(170–191). B, monomer of the catalytic core of IN is shown (PDB code 1E4X (36)). The aromatic amino acid Phe-185 is colored green, the acidic amino acid Glu-170 is red, and basic amino acids His-171/Lys-173/Lys-186/Arg-187/Lys-188 are blue. AlphaScreen analysis of the affinity of the different mutant INIP1 (C) and mutant CCDs (D) for TRN-SR2 is given. ○, wild type; ■, Glu-170/His-171/Lys-173; ▲, Phe-185/Lys-186/Arg-187/Lys-188. Complexes were bound to glutathione donor beads and nickel-chelate acceptor beads. The signal, light emission, was measured using an EnVision Multilabel Reader (in counts/s). Each experiment was repeated three times. Representative graphs of single experiments are shown.

FIGURE 2. Critical amino acids at the surface of the INIP1 in the CCD of HIV-1 IN. A, analysis of the interaction between TRN-SR2 and the different CCD peptides. The fragment numbers refer to the positions of the amino acids in integrase and define the first and the last amino acid of each CCD peptide.
and retained 3’-processing activity, we selected this mutant for further virological studies. To study the impact of the loss of interaction between INK266A/R269A and TRN-SR2 on the nuclear import of HIV, the INK266A/R269A mutations were inserted into a molecular clone of HIV (pNL4-3). Mutant and wild type viruses were harvested after transient transfection of 293T cells and normalized both on p24 levels and RT activity, which showed comparable ratios. HeLaP4 cells were infected in parallel with WT or INK266A/R269A virus, and supernatants were collected at days 3–7 post-infection. The INK266A/R269A virus proved to be replication-deficient (Fig. 6B). To pinpoint at which step the viral replication was inhibited, we analyzed a single round HIV-1 infection using Q-PCR. Late reverse transcripts were measured by Q-PCR at different time points after infection (44, 45). Reduced levels of late reverse transcripts were evidenced for NL4-3-INK266A/R269A virus compared with
We also produced a vesicular stomatitis virus glycoprotein-pseudotyped INK266A/R269A NL4-3.Luc.R-E/H11002 vector and compared it with a WT pseudotyped NL4-3.Luc.R-E/H11002 vector using Q-PCR (Fig. 6D). Again only background levels of reverse transcription were evidenced confounding the study of nuclear import.

**DISCUSSION**

Recently, multiple research groups have reported on the importance of TRN-SR2 as a cellular cofactor of HIV replication (7, 18–28). Although the exact role of TRN-SR2 in HIV replication is still under debate, there is a consensus that even moderate knockdown of this cofactor results in a strong inhibition of HIV replication at an early replication step prior to integration. Because TRN-SR2 belongs to the importin-β family of nuclear import factors (46–49), it is not far-fetched to imagine a direct or indirect role in the nuclear import of the viral PIC (7, 19, 21). Recently, the hypothesis was formulated that TRN-SR2 also acts as a nuclear export factor for tRNA and HIV capsid (27), but this model awaits independent confirmation.

We identified TRN-SR2 as an interaction partner of HIV-1 IN in a yeast two-hybrid screen (19). Reverse yeast two-hybrid screening with TRN-SR2 only revealed IN as a viral binding partner. By employing Q-PCR and imaging of fluorescent viral particles, we could demonstrate that knockdown of TRN-SR2 blocks HIV replication at the step of nuclear import, a bottleneck for the infection of the host cell (19). Whether the physical interaction of TRN-SR2 with HIV-1 IN is the sole determinant for the nuclear import of the PIC and whether TRN-SR2 exerts other roles prior to and/or after nuclear import of the PIC still await further investigation (7, 18–28). Here, we studied the molecular interaction between TRN-SR2 and HIV-1 IN to define the NLS in IN. Because IN has been proposed before to play a role in HIV nuclear import (5, 6, 11, 12, 50), several attempts have been made to define potential NLSs in IN. A bipartite NLS composed of two stretches ranging from amino acids 211KELQKQITK219 and 261PRRKAK266 (11) and a nonclassical NLS composed of amino acids 161IIGQVRDQAEHLK173 were described (31). Multiple IN mutations were inserted into viruses and tested for their impact on viral replication. IN mutants affecting HIV nuclear import were assayed for reduced two-LTR circle formation (51, 52). Despite these extensive studies, so far no single NLS was unambiguously identified as the determinant of HIV nuclear import. These older studies however were hampered by the lack of knowledge on the identity of the nuclear import factor.

Extended NLS sequences that result in the recognition of structural motifs in import factors are commonly found in

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**FIGURE 5.** Analysis of INF185A/K186A/R187A/K188A and INR262A/R263A/K264A with reduced affinity for TRN-SR2. A, analysis of the interaction between INF185A/K186A/R187A/K188A and wild type LEDGF/p75. B, analysis of the interaction between INR262A/R263A/K264A and wild type LEDGF/p75. C, size exclusion chromatography elution profiles of WT IN, INF185A/K186A/R187A/K188A, INR262A/R263A/K264A, and INF185A/K186A/R187A/K188A and INR262A/R263A/K264A. Complexes were bound to glutathione or anti-FLAG donor beads (for GST-TRN-SR2 and LEDGF/p75, respectively) and nickel-chelate acceptor beads. The signal, light emission, was measured using an EnVision Multilabel Reader (in counts/s). The experiment was repeated three times. Representative graphs of single experiments are shown. C, size exclusion chromatography elution profiles of WT IN, INF185A/K186A/R187A/K188A, and INR262A/R263A/K264A. 15 µg of each IN was loaded onto a Superose 6 10/300 GL column connected to an Akta FPLC. Peaks corresponding to tetrameric (Tet) IN or dimeric (Dim) IN are indicated.
cargo. To ensure a certain redundancy in cargo binding, it is unlikely that a single point mutation in the cargo will abrogate the interaction. With this in mind, we searched for the interaction domain in IN by assaying IN peptide stretches rather than using scanning or deletion mutagenesis. Mapping of the IN/TRN-SR2 interface was based on dissecting a large interaction domain into several small peptides and assaying those for their affinity for TRN-SR2. This method solved problems encountered with protein stability and solubility and allowed us to define INIP₁ and INIP₂. Of note, the GST-peptide approach can be recommended for other protein-protein interaction studies when protein deletions are unstable or multipartite interaction regions occur.

Co-immunoprecipitation experiments demonstrated that TRN-SR2 interacts with both the catalytic core and the C-terminal domain of IN. Interaction studies with recombinant proteins confirmed the lack of firm contact with the NTD but revealed strong binding between TRN-SR2 and the CTD and weaker binding with the CCD prompting us to focus on these domains for further analysis.

In the original TRN-SR2 yeast two-hybrid paper, we described a virtual selected interaction domain based on the overlap between multiple clones that were picked up (19). The identified selected interaction domain covered the CCD, but a lot of the clones that were picked up also contained the CTD (19). As such, the identified clones further corroborate the present identification of a major interaction spot in the CTD and a weak second interaction spot in the CCD.

Here, we identified one stretch of amino acids in the CCD (170EHLKTAVMAVFHNFKRG) defined as the first integrase-derived interaction peptide (INIP₁) and two stretches of amino acids (214QKQITKIQNVRVFYR and 259VVPRVKIRIDYKG) in the CTD. Of note, the peptides identified contain the potential NLS sequences that were previously identified (11, 31) but not confirmed due to a lack of an established interaction partner. By combining the two stretches of the CTD, we defined the second integrase-derived interaction peptide (INIP₂). By mutagenesis of INIP₁ and INIP₂, we identified two stretches of amino acids that are crucial for the interaction. Phe-185/Lys-186/Arg-187/Lys-188 determine interaction in the CCD although Arg-262/Arg-263/Lys-264 and Lys-266/Arg-269 are hot spots in the CTD. In the accompanying paper by Larue et al. (53), the role of the CTD residues Arg-262, Arg-263, and Lys-264 in binding to TRN-SR2 was independently confirmed.

After confirming their role in the CCD and the CTD, we combined the mutations in full-length IN and generated an IN mutant INF₁₈₅ₐ/K₁₈₆ₐ/R₁₈₇ₐ/K₁₈₈ₐ-R₂₆₂ₐ/R₂₆₃ₐ/K₂₆₄ₐ that does not bind TRN-SR₂. Because the F₁₈₅ₐ/K₁₈₆ₐ/R₁₈₇ₐ/K₁₈₈ₐ mutant virus is replication-deficient due to a reverse transcription block. A, comparison of the interaction between mutant integrases and TRN-SR₂. ○, wild type IN; ▲, IN₁₈₅ₐ/R₂₆₂ₐ; ■, IN₁₈₅ₐ/K₂₆₄ₐ; ○, IN₂₆₆ₐ/R₂₆₉ₐ; ▲, IN₂₆₆ₐ/R₂₆₉ₐ. Complexes were bound to glutathione donor beads and nickel-chelate acceptor beads. The signal, light emission, was measured using an EnVision Multilabel Reader (in counts/s). The experiment was repeated three times. Representative graphs of single experiments are shown. B, HeLaP₄ cells were infected with 5 × 10⁶ pg of p24 of HIV NL4-3 WT or IN₂₆₆ₐ/R₂₆₉ₐ mutant virus. From 2 days postinfection, supernatants were sampled daily for p24 measurements. One of two independent experiments each performed in duplicate is shown. ○, HIV NL4-3 WT; ■, HIV NL4-3 WT or IN₂₆₆ₐ/R₂₆₉ₐ mutant virus.

D, HeLaP₄ cells were infected with 10 × 10⁶ pg of p24 of HIV NL4-3 WT or IN₂₆₆ₐ/R₂₆₉ₐ mutant vector. At distinct time points after infection, DNA was extracted from cells, and viral DNA species were identified by Q-PCR.

C, HeLaP₄ cells were infected with 10 × 10⁶ pg of p24 of HIV NL4-3 WT or IN₂₆₆ₐ/R₂₆₉ₐ mutant vector. At distinct time points after infection, DNA was extracted from cells, and viral DNA species were identified by Q-PCR. ○, HIV NL4-3 WT with AZT; ■, HIV NL4-3 WT with DMSO; ▲, HIV NL4-3 IN₂₆₆ₐ/R₂₆₉ₐ with AZT; ▼, HIV NL4-3 IN₂₆₆ₐ/R₂₆₉ₐ with DMSO. D, HeLaP₄ cells were infected with 10 × 10⁶ pg of p24 of HIV NL4-3.Luc.R-E WT or IN₂₆₆ₐ/R₂₆₉ₐ mutant vector. At distinct time points after infection, DNA was extracted from cells, and viral DNA species were identified by Q-PCR. ○, HIV NL4-3.Luc.R-E WT with AZT; ■, HIV NL4-3.Luc.R-E WT with DMSO; ▲, HIV NL4-3.Luc.R-E IN₂₆₆ₐ/R₂₆₉ₐ with AZT; ▼, HIV NL4-3.Luc.R-E IN₂₆₆ₐ/R₂₆₉ₐ with DMSO. AZT, azidothymidine.
substitutions affect IN multimerization (Fig. 5C), we cannot exclude that the impact on TRN-SR2 binding is due to a modified multimerization equilibrium. Therefore and because the CCD substitutions only modestly affected TRN-SR2 binding, we focused on substitutions in the CTD for the remainder of our study. Because IN substitutions are known to have pleiotropic effects on HIV replication, we tried to find a set of mutations affecting only the interaction with TRN-SR2. We screened different sets of double point mutations for their binding to TRN-SR2. We selected two mutants (IN[K263A/K264A] and IN[R269A/R269A]) with a strongly reduced affinity for TRN-SR2. Next, we assayed these two mutants for 3’-processing and overall integrase activity. Because IN[K263A/R269A] showed 10-fold reduced affinity for TRN-SR2 and retained 3’-processing activity, we selected this mutant for further virological studies. We introduced the mutations in a molecular clone and studied HIV replication. The K266A/R269A substitutions in IN rendered HIV replication-defective. However, due to a reverse transcription defect, we could not assess the impact of loss of IN/TRN-SR2 interaction on the nuclear import. The pleiotropic effects of IN mutations, especially in the CTD, are well known (51). First, the region between amino acids 220 and 270 of HIV IN plays a role in DNA binding (54). Furthermore, IN is a key protein within the viral particle that is not only responsible for integration catalysis but also interacts with other viral and cellular proteins to ensure integrity of the PIC. Within these nucleoprotein complexes, IN is known to interact with the reverse transcriptase. The interface of this interaction has been mapped to three crucial amino acids (K258A, W243E, and V250E) (55), but IN mutations outside of this interface (e.g., K186Q, R228A, R262A/R263A, R262A/K264A, K266A, and R269A) were shown to affect reverse transcription as well (51, 52, 56). Unfortunately, all mutants identified in this study are known to display reduced reverse transcription during replication (51, 52, 56), confounding the study of their role in the nuclear import of HIV in cellular replication assays. Still, insight into the hot spots that define the protein-protein interaction between IN and TRN-SR2 is necessary to guide future drug discovery efforts targeting the nuclear entry step of replication.

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REFERENCES

1. Lewis, P., Hensel, M., and Emerman, M. (1992) Human immunodeficiency virus infection of cells arrested in the cell cycle. EMBO J. 11, 3053–3058

2. Weinberg, J. B., Matthews, T. J., Cullen, B. R., and Malim, M. H. (1991) Productive human immunodeficiency virus type 1 (HIV-1) infection of nonproliferating human monocytes. J. Exp. Med. 174, 1477–1482

3. Bukrinsky, M. I., Sharova, N., Dempsey, M. P., Stanwick, T. L., Bukrinsky, A. G., Haggerty, S., and Stevenson, M. (1992) Active nuclear import of human immunodeficiency virus type 1 preintegration complexes. Proc. Natl. Acad. Sci. U.S.A. 89, 6580–6584

4. Albanese, A., Arosio, D., Terreni, M., and Cereseto, A. (2008) HIV-1 preintegration complexes selectively target decondensed chromatin in the nuclear periphery. PLoS One 3, e2413

5. De Rijck, J., Vandezande, L., Christ, F., and Debayer, Z. (2007) Lenti-viral nuclear import. A complex interplay between virus and host. BioEssays 29, 441–451

6. Suzuki, Y., and Craigie, R. (2007) The road to chromatin. Nuclear entry of retroviruses. Nat. Rev. Microbiol. 5, 187–196

7. Krishnan, L., Matreyek, K. A., Ozturk, I., Lee, K., Tipper, C. H., Li, X., Dar, M. J., KewalRamani, V. N., and Engelman, A. (2010) The requirement for cellular transportin 3 (TNP03 or TRN-SR2) during infection maps to human immunodeficiency virus type 1 capsid and not integrase. J. Virol. 84, 397–406

8. Lee, K., Ambrose, Z., Martin, T. D., Ozturk, I., Mulkey, A., Julias, J. G., Vanden boss, N., Baumann, J. G., Wang, R., Yuen, W., Takemura, T., Shelton, K., Taniuchi, I., Li, Y., Sodroski, J., Littman, D. R., Coffin, J. M., Hughes, S. H., Unutmaz, D., Engelman, A., and Kewal-Ramani, V. N. (2010) Flexible use of nuclear import pathways by HIV-1. Cell Host Microbe 7, 221–233

9. Piller, S. C., Caly, L., and Jans, D. A. (2003) Nuclear import of the preintegration complex (PIC). The Achilles heel of HIV? Curr. Drug Targets 4, 409–429

10. Yama shita, M., and Emerman, M. (2004) Capsid is a dominant determinant of retrovirus infectivity in nondividing cells. J. Virol. 78, 5670–5678

11. Gallay, P., Hope, T., Chin, D., and Trono, D. (1997) HIV-1 infection of nondividing cells through the recognition of integrase by the importin/ karyopherin pathway. Proc. Natl. Acad. Sci. U.S.A. 94, 9825–9830

12. Heaps, A. C., and Jans, D. A. (2006) HIV-1 integrase is capable of targeting DNA to the nucleus via an importin α/β-dependent mechanism. Biochem. J. 398, 475–484

13. Nitahara-Kashara, Y., Kamata, M., Yamamoto, T., Zhang, X., Miyamoto, Y., Muneta, K., Iijima, S., Yoned, Y., Tsunetsugu-Yokota, Y., and Aida, Y. (2007) Novel nuclear import of Vpr promoted by importin-α is crucial for human immunodeficiency virus type 1 replication in macrophages. J. Virol. 81, 5284–5293

14. Ao, Z., Huang, G., Yao, H., Xu, Z., Lahine, M., Cochrane, A. W., and Yao, X. (2007) Interaction of human immunodeficiency virus type 1 integrase with cellular nuclear import receptor importin 7 and its impact on viral replication. J. Biol. Chem. 282, 13456–13467

15. Fassati, A., Görlch, D., Harrison, L., Zaytseva, L., and Mingot, J. M. (2003) Nuclear import of HIV-1 intracellular reverse transcription complexes is mediated by importin 7. EMBO J. 22, 3675–3685

16. Zaitseva, L., Cerepovan, P., Leyens, L., Wilson, S. J., Rasaiyaah, J., and Fassati, A. (2009) HIV-1 exploits importin 7 to maximize nuclear import of its DNA genome. Retrovirology 6, 11

17. Ao, Z., Danappa Jayappa, K., Wang, B., Zheng, Y., Kung, S., Rassart, E., Depping, R., Kohler, M., Cohen, E. A., and Yao, X. (2010) Importin α3 interacts with HIV-1 integrase and contributes to HIV-1 nuclear import and replication. J. Virol. 84, 8650–8663

18. Brass, A. L., Dykhoorn, D. M., Benita, Y., Yan, N., Engelman, A., Xavier, R. J., Lieberman, J., and Elledge, S. J. (2008) Identification of host proteins required for HIV infection through a functional genomics screen. Science 319, 921–926

19. Christ, F., Thys, W., De Rijck, J., Gijssbers, R., Albanese, A., Arosio, D., Emiliani, S., Rain, J. C., Benavides, R., Cereseto, A., and Debayer, Z. (2008) Transportin-3/SR2 imports HIV into the nucleus. Curr. Biol. 18, 1192–1202

20. König, R., Zhou, Y., Elleder, D., Diamond, T. L., Bonamy, G. M., Irelan, J. T., Chiang, C. Y., Tu, B. P., De Jesus, P. D., Lilley, C. E., Seidel, S., Opaluch, A. M., Caldwell, J. S., Weitzman, M. D., Kuhnen, K. L., Bandoyo- padhyay, S., Ideker, T., Orth, A. P., Miraglia, L. J., Bushman, F. D., Young, J. A., and Chanda, S. K. (2008) Global analysis of host-pathogen interactions that regulate early stage HIV-1 replication. Cell 135, 49–60

21. Logue, E. C., Taylor, K. T., Goff, P. H., and Landau, N. R. (2011) The cargo-binding domain of transportin 3 is required for lentivirus nuclear import. J. Virol. 85, 12950–12961

22. Schaller, T., Ociewiea, K. E., Rasaiyaah, J., Price, A. J., Brady, T. L., Roth, S. L., Hué, S., Fletcher, A. J., Lee, K., KewalRamani, V. N., Noursadeghi, M.,
HIV-1 IN C-terminal Residues Interacting with TRN-SR2

Jenner, R. G., James, L. C., Bushman, F. D., and Towers, G. J. (2011) HIV-1 capsid-cytoplasm interactions determine nuclear import pathway, intervention, and replication efficiency. PLoS Pathog 7, e1002439

23. Cribier, A., Ségéral, E., Delelis, O., Parisi, V., Simon, A., Ruff, S., Benarous, R., and Emiliani, S. (2011) Mutations affecting interaction of integrase with TNPO3 do not prevent HIV-1 cDNA nuclear import. Retrovirology 8, 104

24. De Iaco, A., and Luban, J. (2011) Inhibition of HIV-1 infection by TNPO3 depletion is determined by capsid and detectable after viral cDNA enters the nucleus. Retrovirology 8, 98

25. Ocwieja, K. E., Brady, T. L., Ronen, K., Huegel, A., Roth, S. L., Schaller, T., James, L. C., Towers, G. J., Young, J. A., Chanda, S. K., König, R., Malani, N., Berry, C. C., and Bushman, F. D. (2011) HIV-1 integration targeting: a pathway involving Transportin-3 and the nuclear pore protein RanBP2. PLoS Pathog 7, e1001313

26. Thys, W., De Houlwer, S., Demeulemeester, J., Taltynov, O., Vancraenenbroeck, R., Gérad, M., De Rijck, J., Gijsbers, R., Christ, F., and Debayer, Z. (2011) Interplay between HIV entry and transportin-SR2 dependency. Retrovirology 8, 7

27. Zhou, L., Sokolskaia, E., Jolly, C., James, W., Cowley, S. A., and Fassati, A. (2011) Transportin 3 promotes a nuclear maturation step required for efficient HIV-1 integration. PLoS Pathog 7, e1002194

28. Valle-Casuso, J. C., Di Nuncio, F., Yang, Y., Reszka, N., Lienraf, M., Arhel, N., Perez, P., Brass, A. L., and Díaz-Griffero, F. (2012) TNPO3 is required for HIV-1 replication after nuclear import but prior to integration and binds the HIV-1 core. J Virol 86, 5931–5936

29. Engelman, A., Mizzuuchi, K., and Craigie, R. (1991) HIV-1 DNA integration. Mechanism of viral DNA cleavage and DNA strand transfer. Cell 67, 1211–1221

30. Esposito, D., and Craigie, R. (1999) HIV integration structure and function. Adv. Virus Res. 52, 319–333

31. Bouyac-Bertoia, M., Dvorin, J. D., Fouchier, R. A., Jenkins, Y., Meyer, B. E., Wu, L. I., Emerman, M., and Malim, M. H. (2003) Comparison of lentiviral vector titration methods. J Virol 77, 4654–4666

32. Maertens, G., Cherepanov, P., Pluymers, W., Busschots, K., De Clercq, E., Hofstra, R., Ge´rard, M., De Rijck, J., Gijsbers, R., Christ, F., and Debyser, Z. (2003) LEDGF/p75 is essential for nuclear import receptor for SR proteins. J Biol Chem 278, 33528–33539

33. Christ, F., Voet, A., Marchand, A., Nicolet, S., Desimme, B. A., Marchand, D., Bardiots, D., Van der Veken, N. J., Van Remoortel, B., Strelkov, S. V., De Maeyer, M., Chaltin, P., and Debayer, Z. (2010) Functional nuclear localization signal. Nat Chem Biol 6, 442–448

34. Naldini, L., Blomer, U., Gallay, P., Ory, D., Mulligan, R., Gage, F. H., Verma, I. M., and Trono D. (1996) In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. Science 272, 263–267

35. Geraerts, M., Willems, S., Baekelandt, V., Debayer, Z., and Gijsbers, R. (2006) Comparison of lentiviral vector titration methods. BMC Biotechnol 6, 34

36. Marfori, M., Mynott, A., Ellis, J. J., Mehdizadeh, A. M., Saunders, N. F., Curmi, P. M., Forwood, J. K., Bodén, M., and Kobe, B. (2011) Molecular basis for specificity of nuclear import and prediction of nuclear localization. Biochem. Biophys. Acta 1813, 1562–1577

37. NakaIwama, S., and Dreyfuss, G. (1999) Transport of proteins and RNAs in and out of the nucleus. Cell 99, 677–690

38. Robbins, I., Dilworth, S. M., Laskey, R. A., and Dingwall, C. (1991) Two interdependent basic domains in nucleoplasmin nuclear targeting sequence. Identification of a class of bipartite nuclear targeting sequence. Cell 64, 615–623

39. Chen, J. C., Krucinski, J., Miercke, L. J., Finer-Moore, J. S., Tang, A. H., Leavitt, A. D., and Stroud, R. M. (2000) Crystal structure of the HIV-1 integrase catalytic core and C-terminal domains. A model for viral DNA binding. Proc Natl Acad Sci U S A 97, 8233–8238

40. Cherepanov, P., Maertens, G., Proost, P., Devreeze, B., Van Beeumen, J., Engelborghs, Y., De Clercq, E., and Debayer, Z. (2003) HIV-1 integrase forms stable tetramers and associates with LEDGF/p75 protein in human cells. J Biol Chem 278, 372–381

41. Llanos, M., Delgado, S., Vanegas, M., and Poeschl, E. M. (2004) Lens epithelium-derived growth factor/p75 prevents proteasomal degradation of HIV-1 integrase. J Biol Chem 279, 55570–55577

42. Engelman, A., and Cherepanov, P. (2008) The lentiviral integrase-binding protein LEDGF/p75 and HIV-1 replication. PLoS Pathog 4, e1000046

43. Zheng, Y., Zhao, Z., Jayappa, K. D., and Yao, X. (2010) Characterization of the HIV-1 integrase chromatin- and LEDGF/p75-binding abilities by mutagenesis analysis within the catalytic core domain of integrase. Virol J 7, 68

44. Van Maele, B., De Rijck, J., De Clercq, E., and Debayer, Z. (2003) Impact of the central polyuracil tract on the kinetics of human immunodeficiency virus type 1 vector transduction. J Virol 77, 4685–4694

45. Butler, S. L., Hansen, M. S., and Bushman, F. D. (2001) A quantitative assay for HIV DNA integration in vivo. Nat Med 7, 631–634

46. Kataoka, N., Bachorik, J. L., and Dreyfuss, G. (1999) Transportin-SR, a nuclear import receptor for SR proteins. J Cell Biol 145, 1145–1152

47. Lai, M. C., Liu, R. L., Huang, S. Y., Tsai, C. W., and Tarn, W. Y. (2000) A human importin-β family protein, transportin-SR2, interacts with the phosphorylated RS domain of SR proteins. J Biol Chem 275, 7950–7957

48. Lai, M. C., Liu, R. L., and Tarn, W. Y. (2001) Transportin-SR2 mediates nuclear import of phosphorylated SR proteins. Proc Natl Acad Sci U S A 98, 10154–10159

49. Lai, M. C., Kuo, H. W., Chang, W. C., and Tarn, W. Y. (2003) A novel splicing regulator shares a nuclear import pathway with SR proteins. EMBO J 22, 1359–1369

50. Armon-Omer, A., Graessmann, A., and Loyer, A. (2004) A synthetic peptide bearing the HIV-1 integrase 161–173 amino acid residues mediates active nuclear import and binding to importin-α. Characterization of a functional nuclear localization signal. J Mol Biol 336, 1117–1128

51. Lu, R., Ghory, H. Z., and Engelman, A. (2005) Genetic analyses of conserved residues in the carboxyl-terminal domain of human immunodeficiency virus type 1 integrase. J Virol 79, 10356–10368

52. Mohammed, K. D., Topper, M. B., and Muesing, M. A. (2011) Sequential deletion of the integrase (Gag-Pol) carboxyl terminus reveals distinct phenotypic classes of defective HIV-1. J Virol 85, 4654–4666

53. Larue, R., Gupta, K., Wuenesch, C., Schriabai, N., Kessel, J. J., Danhart, E., Feng, L., Taltynov, O., Christ, F., Van Duyn, G. D., Debayer, Z., Foster, M. P., and Kvaratskhelia, M. (2012) Interaction of the HIV-1 intasome with transportin 3 (TNPO3 or TRN-SR2). J Biol Chem 287, 34044–34058

54. Lutzke, R. A., Vink, C., and Plasterk, R. H. (1994) Characterization of the minimal DNA-binding domain of the HIV integrase protein. Nucleic Acids Res 22, 4125–4131

55. Wilkinson, T. A., Januszyk, K., Phillips, M. L., Tekeste, S. S., Zhang, M., Miller, J. T., Le Grice, S. F., Chubb, R. T., and Chow, S. A. (2007) Identifying and characterizing a functional HIV-1 reverse transcriptase-binding site on integrase. J Biol Chem 284, 7931–7939

56. Lu, R., Limón, A., Devroe, E., Silver, P. A., Cherepanov, P., and Engelman, A. (2004) Class II integrase mutants with changes in putative nuclear localization signals are primarily blocked at a postnuclear entry step of human immunodeficiency virus type 1 replication. J Virol 78, 12735–12746