The Interaction of LEDGF/p75 with Integrase Is Lentivirus-specific and Promotes DNA Binding*

Katrien Busschots,%% Joel Vercammen§, Stéphane Emiliani**, Richard Benarous**, Yves Engelborghs, Frauke Christ, and Zeger Debyser‡‡

From the ‡Laboratory for Molecular Virology and Gene Therapy, Katholieke Universiteit Leuven and Interdisciplinary Research Center Katholieke Universiteit Leuven Campus Kortrijk, Kapucijnenvoer 33, B-3000 Leuven, Flanders, Belgium, and Laboratory of Biomolecular Dynamics, Katholieke Universiteit Leuven, Celestijnenlaan 200D, B-3001 Leuven, Flanders, Belgium, and **Institut Cochin, U567 INSERM, UMB8104 CNRS, Department of Infectious Diseases, 27 Rue du Faubourg St. Jacques, 75104 Paris, France

We have previously shown that the p75 isoform of the transcriptional co-activator lens epithelium-derived growth factor (LEDGF) interacts tightly with human immunodeficiency virus (HIV)-1 integrase (IN) and is essential for nuclear targeting of this protein in human cells (Cherepanov, P., Maertens, G., Proost, P., Devreese, R., Van Beuemen, J., Engelborghs, Y., De Clercq, E., and Debyser, Z. (2003) J. Biol. Chem. 278, 372–381; Maertens, G., Cherepanov, P., Pluymers, W., Busschots, K., De Clercq, E., Debyser, Z., and Engelborghs, Y. (2003) J. Biol. Chem. 278, 33528–33539). Here the interaction between recombinant LEDGF/p75 and HIV-1 IN was examined in a pull-down binding test. LEDGF/p75 was shown to increase the solubility of HIV-1 IN. Next, fluorescence correlation spectroscopy was used to measure the interaction of LEDGF/p75 or the complex of HIV-1 IN and LEDGF/p75 with a specific double-stranded DNA oligonucleotide. Whereas LEDGF/p75 displayed only a moderate affinity for DNA, it strongly promoted the binding of HIV-1 IN to DNA. This effect was specific for the p75 isoform of LEDGF and was not seen with p52. In the pull-down assay LEDGF/p75 interacted with HIV-1, HIV-2, and feline immunodeficiency virus IN, but not with the IN of human T-cell lymphotropic virus type 2, Moloney murine leukemia virus, or Rous sarcoma virus. These results strongly suggest that the interaction of LEDGF/p75 with IN is specific to lentiviridae. LEDGF/p75 stimulated the binding of HIV-1 and HIV-2 IN, but not Moloney murine leukemia virus or Rous sarcoma virus IN, to an aspecific DNA. These results provide supporting evidence for our hypothesis that LEDGF/p75 plays a role in the tethering of lentiviral IN to the chromosomal DNA.

Human immunodeficiency virus (HIV)-1 belongs to the retroviridae that are characterized by reverse transcription of the diploid viral RNA genome into a double-stranded linear DNA molecule that is subsequently inserted into a host cell chromosome. Reverse transcription takes place in the cytoplasm of the infected cell and results in the formation of a compact and stable pre-integration complex (PIC) containing the viral reverse-transcribed genome and a number of virion-derived and cellular proteins. The family of the retroviridae can be broadly divided into simple and complex retroviridae, depending on their genomic composition and replication cycle. The retroviridae are then further subdivided into seven groups defined by evolutionary relatedness. Five of these groups represent retroviruses with oncogenic potential, and the other two groups are the lentiviruses and the spumaviruses (for a review, see Ref. 1). Lentiviridae (e.g. HIV-1, HIV-2, feline immunodeficiency virus (FIV), or simian immunodeficiency virus) are able to productively infect non-dividing cells, a feature that distinguishes them from oncoretroviridae (e.g. Moloney murine leukemia virus (Mo-MuLV), Rous sarcoma virus (RSV), and human T-cell lymphotropic virus (HTLV)-2), which require cell division for productive infection (2, 3). The viral integrase (IN) catalyzes the integration of the viral cDNA into the host genomic DNA, a process that is essential for replication and results in a provirus that will remain present as long as the cell survives (for reviews, see Refs. 4–6).

HIV-1 IN is a 32-kDa protein that consists of three distinct structural domains (7): the N-terminal zinc-binding domain required for oligomerization (8–10), the central catalytic core, and the less highly conserved C-terminal domain thought to be involved in DNA binding (11) and oligomerization of IN in vitro (12). The functional holoprotein required for concerted integration of two long terminal repeat ends is believed to exist as a homodimer of two tetramers (13, 14). In cells stably overexpressing IN, the enzyme remains stably associated with condensed chromosomes during mitosis (15, 16).

The insight has grown that HIV relies on cellular proteins for completion of its replication cycle. Identification and characterization of these cellular cofactors will increase our understanding of the viral replication cycle and aid in the development of new antiviral drugs. Various cofactors of the lentiviral integration process have been proposed. Integrase interactor 1, a component of the SWI/SNF chromatin remodeling complex (17–19), interacts directly with HIV-1 IN and stimulates the integration

* This work was supported in part by European Commission Targeting Replication and Integration of HIV (TRIOH) Project LSHB-CT-2003-503480 and Grant 530-030239 from the Strategic Basic Research Program of the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Both authors contributed equally to this work.

‡ Supported by a grant from the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT).

§ To whom correspondence should be addressed. Tel.: 32-16-332183; Fax: 32-16-332121; E-mail: zeger.debyser@med.kuleuven.ac.be.

The abbreviations used are: HIV, human immunodeficiency virus; FCS, fluorescence correlation spectroscopy; FIV, feline immunodeficiency virus; HTLV, human T-cell lymphotropic virus; IN, integrase; LEDGF, lens epithelium-derived growth factor; Mo-MuLV, Moloney murine leukemia virus; PIC, pre-integration complex; RSV, Rous sarcoma virus; CHAPS, 3-(3-cholamidopropyl)dimethylammonio-1-propanesulfonic acid; Ni-NTA, nickel-nitrilotriacetic acid.

Received for publication, October 14, 2004, and in revised form, February 17, 2005
Published, JBC Papers in Press, March 4, 2005, DOI 10.1074/jbc.M411681200

This paper is available online at http://www.jbc.org

17841
Lentivirus-specific Tethering of IN to DNA by LEDGF/p75

reaction in vitro (20, 21). Other cellular host factors might participate in HIV integration. The high mobility group protein HMG-1a, a non-histone chromosomal protein involved in transcriptional control and nuclear architecture, was identified as a cellular host factor essential for PIC activity in vitro (22). Human EED, a cellular protein interacting with HIV-1 matrix protein and acting as a transcriptional repressor and gene silencer, was found to interact with HIV-1 IN and to stimulate the integration reaction in vitro (23). Other cellular factors, such as the barrier to autointegration factor and Ku, have been shown to co-purify with PICs. Barrier to autointegration factor is thought to prevent intramolecular interaction (24). The exact function of all these factors during in vivo integration remains to be determined.

Recently, we have identified the p75 isoform of lens epithelium-derived growth factor (LEDGF/p75) as a tight binding partner of HIV-1 IN in human cells (13). LEDGF/p75 was shown to enhance the enzymatic activity of recombinant HIV-1 IN. Expression of LEDGF/p75 is required for the association of IN with mitotic chromosomes, suggesting a role in tethering IN to DNA (16). Although the exact cellular function of LEDGF/p75 is still unknown, it has been suggested to play a role in transcriptional regulation because it is isolated from HeLa cell extracts as an interactor of the transcriptional co-activator PC4 (25). LEDGF/p75 is a member of the hepatoma-derived growth factor family and has been proposed to play a protective role in the cytoplasm during stress-induced apoptosis (26–28). DNA binding of LEDGF/p75, with specificity for stress response DNA elements, has been reported (29). A second protein product, p52, is generated from the LEDGF/p75 gene as a result of alternative splicing of the pre-mRNA (25, 30). Although p52 was found to be a more general and stronger transcriptional co-activator in vitro than LEDGF/p75 (31), it does not interact with HIV-1 IN in vivo or in living cells (16).

In this report, we used fluorescence correlation spectroscopy (FCS) to study the effect of LEDGF/p75 on the binding of IN to different DNA substrates. LEDGF/p75, but not p52, clearly stimulated the binding of HIV-1 IN to DNA. Moreover, in both pull-down and FCS assays, the interaction of LEDGF/p75 with IN proved to be specific for lentiviridae.

EXPERIMENTAL PROCEDURES

Plasmids for Bacterial Expression—The constructs pCPmH1 and pKBmIN6H were over-expressed in the bacteria (E. coli). The non-tagged LEDGF/p75 and p52 proteins as described previously (16). The plasmid pKBM-IN6H was used for the bacterial expression of the C-terminal His-tagged form of HIV-1 IN (16), and the N-terminal-tagged form of HIV-1 IN was expressed from pRP1012 (32). DNA fragments containing the IN open reading frame of HIV-2 (33), HIV-3 (34), and 35 were cloned into PET-20b (+) vector (Novagen, VWR, Leuven, Belgium) as fusions to a C-terminal His tag, using the Ndel and SalI sites. The fragments were PCR-amplified using the following primers and templates: 5′-CGCGTCGACTGCTACATTCCTGACCATCCTCATCCT, 5′-GGCCATATGTCCTCGGACCATATCCCTGCAGGAAGGACGGCAGTGGTTACGAAATGGAAGGACGGCAGTGGTTACGAAATGGAAGGCAGTGGTTACGAAATGGAAGGACGGCAGTGGTTACGAAATGGAAGGACGGCAGTGGTTACGAAATGGAAGGACGGCAGTGGTTACGAAATGGAAGGACGGCAGTGGTTACGAAATGGAAGGACGGCAGTGGTTACGAAATGGAAGGACGGCAGTGGTTACGAAATGGAAGGACGGCAGTGGTTACGAAATGGAAGGACGGCAGTGGTTACGAAATGGAAGGACGGCAGTGGTTACGAAATGGAAGGACGGCAGTGGTTACGAAATGGAAGGACGGCAGTGGTTACGAAATGGAAGGACGGCAGTGGTTACGAAATGGAAG
and BSA, LEDGF/p75, and His6-IN were loaded in the same amounts more pronounced in the presence of 150 mM NaCl (Fig. 2). This indicated. The complexes were bound on a Ni2

C terminus.

further experiments were carried out with IN tagged at the enhances the affinity of IN for LEDGF/p75 (16). Therefore, all our previous findings that the N-terminal domain of IN en-

tered from the supernatant, which was precipitated with

binding assay, based on the ability of Ni-NTA-agarose to bind IN. Finally, the proteins in the complex are identified in a

preparation was verified with SDS-PAGE, and the proteins

solubility (data not shown). The purity of the different IN preparations was identical for all INs, except for Mo-MuLV and HTLV-2 IN. Of Mo-MuLV c-terminal His-tagged IN was used. Addition of MgCl2 increased the binding of LEDGF/p75 to IN (compare lane 4 with lane 3).

measured 10 times. The data were subsequently analyzed as described previously, using the quantile plot analysis method (12).

RESULTS

Interaction of HIV-1 Integrase with LEDGF/p75 as Analyzed by a Pull-down Binding Assay—To study the interaction between IN and LEDGF/p75 in vitro we optimized a specific binding assay, based on the ability of Ni-NTA-agarose to bind His6-tagged proteins (16). We have previously demonstrated that LEDGF/p75 but not p52 specifically interacts with HIV-1 IN in this test (16). After pre-incubation of His6-tagged HIV-1 IN with recombinant LEDGF/p75, HIV-1 IN is bound to Ni-NTA-agarose, washed, and eluted with imidazole. Recombinant LEDGF/p75 is readily recovered in a stable complex with IN. Finally, the proteins in the complex are identified in a SDS-PAGE gel stained with Coomassie Blue R-250.

First we examined the effect of a N- versus C-terminal His6

tag on the interaction between LEDGF/p75 and HIV-1 IN as well as the effect of the NaCl concentration in the buffer. Heretofore we cloned the His6 tag at the C terminus or the N terminus of HIV-1 IN. In comparison with the protein with the His6 tag at the C terminus, N-terminal-tagged HIV-1 IN pulled down less LEDGF/p75 (Fig. 1). This result is in agreement with our previous findings that the N-terminal domain of IN enhances the affinity of IN for LEDGF/p75 (16). Therefore, all further experiments were carried out with IN tagged at the C terminus.

The binding assays were performed in both 150 and 400 mM NaCl. Although the interaction of IN with LEDGF/p75 is strongest in 150 mM NaCl (Fig. 1, compare the band for LEDGF/p75 in lanes 3 and 4 with lanes 6 and 7 and in lanes 10 and 11 with lanes 13 and 14), the precipitation of IN is also more pronounced in the presence of 150 mM NaCl (Fig. 2). This effect was minimal in the experiment shown in Fig. 1, but it was more pronounced with the other retroviral INs used in the present study. Therefore, the following binding experiments were always performed in both 150 and 400 mM NaCl.

LEDGF/p75 Enhances the Solubility of HIV-1 IN—To study the effect of LEDGF/p75 on the solubility of HIV-1 IN, we performed a precipitation experiment to determine the NaCl concentration required to keep IN in solution. We used the buffer of the pull-down assay, with NaCl concentrations ranging between 100 and 500 mM (Fig. 2). After 30 min of incubation, the samples were centrifuged, and the pellets were separated from the supernatant, which was precipitated with trichloroacetic acid. All samples were redissolved and run in a SDS-PAGE gel. In the absence of LEDGF/p75, IN precipitated in salt conditions of ≤350 mM. In the presence of LEDGF/p75, however, IN remained soluble at a NaCl concentration above 200 mM. Addition of bovine serum albumin did not increase the solubility of IN, indicating that the increase in solubility of IN by LEDGF/p75 is a specific effect.

LEDGF/p75 Enhances the Binding of HIV-1 IN to DNA—We have previously established an FCS-based assay to analyze the binding of HIV-1 IN to a fluorescence-labeled 20-mer double-stranded DNA resembling the HIV-1 U5 long terminal repeat sequence (12). In this study at a final NaCl concentration of 50 mM, the dissociation constant (Kd) for the IN-DNA complex was 208 ± 26 nM. We then investigated the effect of the addition of recombinant LEDGF/p75 on the formation of the IN-DNA complex. The Kd for this LEDGF-DNA complex was estimated at 110 ± 86 nM. Next, increasing concentrations of LEDGF/p75 were added to the DNA at various concentrations of HIV-1 IN. By plotting the calculated amounts of free proteins and complexes bound to the DNA, we could demonstrate a clear stimulation by LEDGF/p75 of the binding of HIV-1 IN to DNA (Fig. 3). From the slope of the binding plot at low IN concentrations, the dissociation constant for the IN-LEDGF/p75-DNA complex was calculated at 6.17 ± 0.88 nM, corresponding to a >33-fold increase in binding. We also investigated the binding of the p52 isoform to DNA. This protein bound to DNA with a higher affinity than LEDGF/p75. The calculated apparent Kd was 763 ± 303 nM. Upon addition of p52, no stimulation of the binding of IN to DNA was seen (Fig. 3). Overall binding merely represented the additive effect of the binding of each protein to the DNA (data point at IN = 0 concentration).

The Interaction between LEDGF/p75 and IN Is Lentivirus-specific—The specificity of binding of various retroviral INs to human LEDGF/p75 was tested in the pull-down binding assay. The INs of the following lentiviridae were included in the experiment: HIV-1, HIV-2, and FIV, as well as the INs of the retrovirdiae HTLV-2, Mo-MuLV, and RSV. All C-terminal His6-tagged INs were expressed in bacteria and purified by Ni-NTA and Heparin chromatography. The purification scheme is described under “Experimental Procedures” and was identical for all INs, except for Mo-MuLV and HTLV-2 IN. Of note, HTLV-2 IN displayed a very low solubility and precipitated even in 1 x NaCl, but LEDGF/p75 did not enhance its solubility (data not shown). The purity of the different IN preparations was verified with SDS-PAGE, and the proteins were used at equimolar concentrations in the pull-down binding assay.

The results of these interaction studies are shown in Fig. 4. The IN of HIV-1, HIV-2, and FIV showed a strong and specific interaction with human LEDGF/p75 (Fig. 4A). No interaction was detected with the IN of human HTLV-2, murine Mo-MuLV, or avian RSV (lanes 4 and 6 on all gels in Fig. 4B) because no LEDGF/p75 was pulled-down for these INs. This lack of interaction with retroviral IN was confirmed by a more sensitive detection of LEDGF/p75 by Western blotting using a monoclonal anti-LEDGF p75/p52 antibody (data not shown). The results demonstrate that the binding of LEDGF/p75 to IN is lentivirus-specific. In a parallel yeast two-hybrid experiment, interaction was shown between LEDGF/p75 and the integrases of HIV-1 and simian immunodeficiency virus from rhesus macaques (SIVmac) (data not shown).

Stimulation of the Binding of IN to DNA by LEDGF/p75 Is Lentivirus-specific—Next, lentiviral specificity of the functional interaction between IN and LEDGF/p75 was examined in the FCS assay (Fig. 5). To model tethering of IN to chromosomal DNA, we first used a single aspecific double-stranded DNA construct of IN-HIV-1 IN containing a His6 tag at the N terminus of HIV-1 IN reduces inter-

action with LEDGF/p75. Recombinant LEDGF/p75 was incubated with HIV-1 IN containing a His6 tag at the N (lanes 10, 11, 13, and 14) or C terminus (lanes 3, 4, 6, and 7) in the salt and MgCl2 concentrations indicated. The complexes were bound on a Ni2-

agarose resin. Lanes 1 and 8 reflect the protein input in the reactions: bovine serum albumin (BSA), LEDGF/p75, and His6-IN were loaded in the same amounts present in the binding reactions. The gel was stained using Coomassie Blue R-250. There is less LEDGF/p75 detectable in lanes 10, 11, 13, and 14, where N-terminally His-tagged IN was used, compared with lanes 3, 4, 6, and 7, where C-terminally His-tagged IN was used. Addition of MgCl2 increased the binding of LEDGF/p75 to IN (compare lane 4 with lane 3).

FIG. 1. A His6 tag at the N terminus of HIV-1 IN reduces interaction with LEDGF/p75. Recombinant LEDGF/p75 was incubated with HIV-1 IN containing a His6 tag at the N (lanes 10, 11, 13, and 14) or C terminus (lanes 3, 4, 6, and 7) in the salt and MgCl2 concentrations indicated. The complexes were bound on a Ni2-

agarose resin. Lanes 1 and 8 reflect the protein input in the reactions: bovine serum albumin (BSA), LEDGF/p75, and His6-IN were loaded in the same amounts present in the binding reactions. The gel was stained using Coomassie Blue R-250. There is less LEDGF/p75 detectable in lanes 10, 11, 13, and 14, where N-terminally His-tagged IN was used, compared with lanes 3, 4, 6, and 7, where C-terminally His-tagged IN was used. Addition of MgCl2 increased the binding of LEDGF/p75 to IN (compare lane 4 with lane 3).
DNA for the different integrases tested. The final NaCl concentration in this assay was 130 mM. Whereas LEDGF/p75 stimulated the binding of both HIV-1 IN and HIV-2 IN to DNA (Fig. 5A), no stimulation of the binding of RSV IN or Mo-MuLV IN to the DNA was detected, even when LEDGF/p75 was added at a concentration of 166 nM (Fig. 5B). The respective dissociation constants are given in Table I. To compare stimulation of IN binding to specific DNA, each recombinant integrase was subsequently tested in the presence of its cognate oligonucleotide DNA mimicking the 5’/H11032 long terminal repeat end (Fig. 5, C and D). Again, DNA binding of lentiviral but not retroviral integrases was stimulated upon addition of LEDGF/p75.

DISCUSSION

The present data provide further evidence that LEDGF/p75 acts as a cellular cofactor for lentiviral integration. We proved the lentiviral specificity of the IN-LEDGF/p75 interaction by demonstrating that LEDGF/p75 interacts with the lentiviral INs of HIV-1, HIV-2, SIVmac, and FIV but not with the retroviral INs of HTLV-2, RSV, or Mo-MuLV. Furthermore, we shed light on the potential role of LEDGF/p75 during HIV replication by demonstrating a direct stimulation of the binding of lentiviral INs to DNA by LEDGF/p75, but not by p52. The molecular mechanism of this cofactor is apparently based on the stimulation of the binding of IN to the DNA. Our findings support our hypothesis that LEDGF/p75 plays a role in tethering IN to the chromosomal DNA (12, 15).

We have unambiguously demonstrated the specificity of interaction of LEDGF/p75 with lentiviral integrases both in a pull-down interaction assay and with FCS. While this article was in preparation, Llano et al. (43) reported as well on the role of LEDGF/p75 in the nuclear accumulation of HIV-1 and FIV but not Mo-MuLV IN.

In the FCS experiments, we first compared the DNA binding of the different INs by using the same nonspecific DNA substrate, thus modeling binding to the chromosomal DNA. Stimulation of binding to the DNA by LEDGF/p75 was observed for HIV-1 and HIV-2 IN but not for RSV or Mo-MuLV IN. When using specific DNA substrates, the same lentiviral specificity of LEDGF/p75 was observed.

Stimulation by LEDGF/p75 was more pronounced for HIV-1

**FIG. 2.** LEDGF/p75 enhances the solubility of HIV-1 IN. HIV-1 IN was added to the binding buffer of the pull-down assay in a variety of salt concentrations ranging from 100 to 500 mM NaCl. LEDGF/p75 was added in the experiment shown in the top panels. After 30 min of incubation, the samples were centrifuged, and the pellets were separated from the supernatant, which was precipitated with trichloroacetic acid. All samples were redissolved and separated by SDS-PAGE. Without LEDGF/p75, HIV-1 IN precipitated in buffers containing <350 mM salt, whereas the addition of LEDGF/p75 increased the solubility of IN.

**FIG. 3.** Stimulation of the DNA binding of IN by addition of LEDGF/p75. A fluorescence-labeled 20-mer specific double-stranded DNA was used as substrate for evaluating the binding of HIV-1 IN and the effect of the addition of LEDGF/p75. The amounts of free and bound DNA were calculated using FCS and spike analysis (12). This ratio was plotted against the initial IN concentration. Different concentrations of LEDGF/p75 were added, and the binding affinity of the IN-LEDGF/p75 complex formed was measured. An apparent binding constant was calculated from the slope of the curve at low IN concentrations ($K_d = 208 \pm 26$ nM for IN and $6.17 \pm 0.88$ nM for IN-LEDGF/p75-DNA). Each data point represents the average of 10 measurements. In the inset, the percentage of bound DNA is plotted against IN concentration.
than HIV-2 IN with both specific and aspecific DNA. The stimulation was also more pronounced with specific than aspecific DNA, although LEDGF/p75 by itself showed more affinity for the aspecific DNA substrate. Based on these data, we cannot rule out the possibility that LEDGF/p75 is also involved in tethering IN to the viral DNA ends. The evidence for the presence of LEDGF/p75 in the PIC (43) is in agreement with this hypothesis.

Interestingly, we observed a clear increase in the solubility of recombinant HIV-1 IN complexed with LEDGF/p75. This points to a potential strategy for crystallization of this holoprotein complex. The increased affinity of IN for DNA may facilitate co-crystallization of IN with a DNA substrate.

The specific interaction of LEDGF/p75 with lentiviral but not retroviral INs raises the question of whether retroviruses have a different mechanism of integration and/or interact with other host proteins. The human origin of the LEDGF/p75 used cannot be the culprit because interaction was found with FIV IN as well. Lentiviruses differ foremost from the other retroviruses in their ability to infect non-dividing cells (2, 3). This has resulted in a search for the possible import factors of the lentiviral pre-integration complex. Although the mechanism underlying the nuclear import of the lentiviral PIC has not been clarified, viral proteins such as Vpr, matrix protein, and IN as well as the central DNA flap have been implicated (44–49). Recent data by Maertens et al. (50) demonstrate that LEDGF/p75 contains a functional nuclear localization signal, but no role in the nuclear import of IN was detected. Devroe et al. (51) have previously shown that by fusing IN to a nuclear export signal, the nuclear localization of constitutively expressed HIV-1 IN was not abolished. They suggested that HIV-1 IN is trapped in the nucleus, possibly through interaction with chromatin or direct binding to DNA. We have previously reported that LEDGF/p75 is necessary for the nuclear localization of HIV-1 IN (16). By gene silencing of LEDGF/p75, the nuclear accumulation of IN was abolished. The recent data of Llano et al. (43) confirm our observations; a direct role of LEDGF/p75 in nuclear import was questioned, but a putative role in chromosomal targeting was put forward. Finally, our FCS analysis supports the view that LEDGF/p75 tethers IN to the chromosome by providing direct evidence for increased binding of HIV-1 IN to DNA in the presence of this cellular cofactor. Together, these findings point to LEDGF/p75 as the nuclear and chromosomal trap of IN rather than as a nuclear import factor for the PIC, although an additional role of LEDGF/p75 in nuclear import cannot be excluded at this moment.

Besides their different ability to infect cells, another important difference between lentiviral and retroviral integration is the selection of integration sites (for a recent review, see Ref. 52). Replication of retroviruses and retrotransposons depends on the selection of a favorable site for integration in the chromosome. Integration is known to occur in a non-sequence-specific manner, so many chromosomal sites can host integration. A recent study by Wu et al. (53) compared integration targeting in the human genome by HIV and Mo-MuLV vectors. For HIV, integration is favored in transcriptional units. Comparison with transcriptional profiling data supports the idea that active genes are preferred. Mo-MuLV integration preferentially occurs near the start of transcriptional units. This study has been confirmed and supplemented with data for avian sarcoma/leukosis virus by Mitchell et al. (54), showing that avian sarcoma/leukosis virus displays only weak preference for active genes and no preference for transcriptional start regions. Whereas the detailed mechanism of retroviral integration targeting is unknown, these studies can be easily accommodated in tethering models (for review, see Ref. 52). In one version of such a model, the binding of the PIC of Mo-MuLV to transcription factors or modified histones bound at or near the 5′ end of genes promotes local integration. HIV might similarly interact with the chromatin during the G2 phase of the cell cycle (55). We hypothesize that the temporal attachment of LEDGF/p75 to the chromatin targets the integration of HIV-1 proviral DNA to specific genomic sites of actively transcribed genes (56). Moreover, LEDGF/p75 was first identified as an interacting...
protein of the transcription co-activator PC4 (25). LEDGF/p75 has also been shown to interact with components of the general transcription machinery and with the transcription activation domain of VP16. By coupling the promotion of DNA binding of the HIV-1 PIC to the transcription machinery, LEDGF/p75 may provide the missing link between integration and transcription.

In conclusion, we have shed light on the potential role of LEDGF/p75 during HIV replication. We provide clear evidence for a lentivirus-specific mechanism of tethering IN to DNA.

**Acknowledgments**—We thank R. Plasterk for providing plasmids pRP1012, pRP1013, and pRP825; C. Jonsson for providing pHTLV2 and pETINH1 plasmids; and D. Grandgenett for providing the plasmid expressing RSV IN. Furthermore, we thank Martine Michiels for help with the IN activity assays, Sofie Janssen for the FCS measurements, and Bénédicte Van Maele for critical reading of the manuscript.

**REFERENCES**

1. Coffin, J. M., Hughes, S. H., and Varmus, H. E. (1997) in Retroviruses, pp. 1–25, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
2. Lewis, P. F., and Emerman, M. (1994) J. Virol. 68, 510–516
3. Roe, T., Reynolds, T. C., Yu, G., and Brown, P. O. (1993) EMBO J. 12,
