Integrase Mutants Defective for Interaction with LEDGF/p75 Are Impaired in Chromosome Tethering and HIV-1 Replication

Stéphane Emiliani, a,b Aurélie Mousnier, c,d,e Katrien Busschots, f,d Marlène Maroun, a,g Bénédicte Van Maele, f,d Denis Tempé, a Linos Vandekerckhove, f,h Fanny Moisant, i Lilia Ben-Slama, j Myriam Witvrouw, j Frauke Christ, i Jean-Christophe Rain, i Catherine Dargemont, i Zeger Debyser, f,h and Richard Benarous, a,d

From the aInstitut Cochin, U567 INSERM, UPR8104 CNRS, Université Paris VI et VII, Tour 43, 2e étage, couloir 43–44, 2 place Jussieu, F-75251 Paris cedex 05, France, Department of Infectious Diseases, 27 rue du Faubourg Saint Jacques, Batiment Gustave Roussy, 75014 Paris, France, the bInstitut Jacques Monod, UMR7592 CNRS, Paris, France, the cLaboratory for Molecular Virology and Gene Therapy, KULAK and KULEuven, Flanders, Kapucijnenvoer 33 B-3000, Belgium, and the dHybrigenics S.A. 3–5 impasse Reille, 75014 Paris, France

The insertion of a DNA copy of its RNA genome into a chromosome of the host cell is mediated by the viral integrase with the help of mostly uncharacterized cellular cofactors. We have recently described that the transcriptional co-activator LEDGF/p75 strongly interacts with HIV-1 integrase. Here we show that interaction of HIV-1 integrase with LEDGF/p75 is important for viral replication. Using multiple approaches including two-hybrid interaction studies, random and directed mutagenesis, we could demonstrate that HIV-1 virus harboring a single mutation that disrupts integrase-LEDGF/p75 interaction, resulted in defective HIV-1 replication. Furthermore, we found that LEDGF/p75 tethers HIV-1 integrase to chromosomes and that this interaction may be important for the integration process and the replication of HIV-1.

Integration is an essential step in HIV-1 replication catalyzed by the virus-encoded integrase (IN) protein. The choice of integration sites in cellular chromosomes is affected by the heterogeneous structure of the chromatin. While in vivo HIV-1 integration is not sequence-specific, transcriptionally inactive regions of the genome, such as centromeres and telomeres, are disfavored targets (1–3). Integration of proviral HIV-1 DNA occurs preferentially into transcriptional units of active genes while the oncoretrovirus murine leukemia virus prefers to integrate near the transcription start site of actively transcribed genes (4, 5). The differences observed between the integration profiles of these two viruses strongly suggest that cellular cofactors actively tether proviral DNA to specific regions of the genome (6). In vivo, integration is mediated by a large nucleoprotein complex called preintegration complex (PIC) containing the viral cDNA, together with viral proteins: matrix (MA), nucleocapsid (NC), reverse transcriptase (RT), and integrase (IN). The PIC carries out DNA cutting and joining reactions (7, 8). In addition, several cellular proteins join the PIC along its journey from the cytoplasm to the chromosomes such as the high mobility group protein HMGa1, which seems required for integration in vitro (9–12) by a still unknown mechanism, barrier to autointegration factor and Ku (13–15). In addition, INI-1/SNF5, a component of the chromatin remodeling complex SWI/SNF, is a binding partner for IN (16), and HIV-1 infection may induce the cytoplasmic relocation of INI-1/SNF5, leading to its association with the incoming PIC (17). We recently identified LEDGF/p75 as a new cellular binding partner for HIV-1 IN (18, 19). LEDGF/p75, a member of the hepatoma-derived growth factor family is a transcriptional co-activator which plays a protective role during stress-induced apoptosis (20). LEDGF/p75 has been reported as a component of the PIC, and when its expression is silenced IN is found mostly in the cytoplasm (21, 22).

Here, we report that the interaction of IN with LEDGF/p75 is involved in integration and replication of HIV-1. A single mutation in IN, Gln168 to Ala, disrupted the interaction with LEDGF/p75 without affecting its catalytic activity and abolished the chromosomal targeting of IN resulting in integration and replication-deficient viruses. Furthermore, the mutation did not affect the nuclear import of HIV-1 integrase. Taken together, our data indicate that integration of HIV-1 is under the control of the cellular cofactor LEDGF/p75.

MATERIALS AND METHODS

Integrase Mutant Library—Integrase bait plasmid was amplified in mutagenic conditions (0.2 mM dGTP, 0.2 mM dATP, 1 mM dCTP, 1 mM dTTP, 3 mM MgCl2) according to Cadwell and Joyce (23) using the

See supplemental material for additional methods.

Received for publication, February 7, 2005, and in revised form, April 22, 2005
Published, JBC Papers in Press, April 25, 2005, DOI 10.1074/jbc.M501378200
following oligonucleotides: GGCGCTGGGTTGGTTATCGCCAC-GGCGACTGCTGATTGC and ATCTAAGAATTTAGCGGGAAT- TAGCTTGTCGTCAGTTC. PCR product was digested by DpnI and transfected in yeast with an open empty plasmid. The resulting plasmid was used for a cell-to-cell mating protocol (24). A test screen was performed for each bait to adapt the screening conditions. The activity of the HIS3 reporter gene was eventually modulated with 3-aminotriazole (Sigma) to obtain a maximum of 285 histidine-positive clones for 50 million diploids screened. For all the selected clones, LacZ activity was evaluated by overlay assay. (ii) 192 white and light blue colonies were selected from 1,100 diploid yeast transformants containing as bait and LEDGF/p75 as prey (amino acids 168–473). (iii) Inserts were amplified by PCR, sequenced, and analyzed. (iii) Plasmids of interesting mutants were extracted and phenotypes confirmed by retransformation.

**Plasmid Construction and Mutagenesis**—All mutations were generated by using the QuikChange mutagenesis kit (Stratagene). Mutations of the pBru molecular clone were performed as described previously (25). The IN synthetic gene (In+) (26) was PCR-amplified using the following primers: 5'-GGAGAGATCTGGTCGAATTC and 5'-CGCGACTGGCTGGAATTC. The resulting PCR product was subcloned between the BglIII and EcoRI sites of pEGFP-C1 vector (Clontech) thus generating the GFP-IN expression vector. The recombinant INQ616L and INQ68A mutants were generated by mutagenesis of the plasmid used for retransformation into yeast. The IN+ gene was pRP1012 coding for integrase with a N-terminal His-tag (R. Plasterk, Dutch Cancer Institute, Amsterdam, The Netherlands). The following primers were used: INTQ168L, 5'-GTAAGAGATCTGGCTGAATTC; and INTrev2, 5'-GTAAGAGATCTGGCTGAATTC. The resulting PCR fragments were then digested with DpnI, generating the pKBINQ168L and pKBINQ68L plasmids.

**In Vitro Integration Assay**—The DNA substrate used in the enzymatic assay corresponds to the U5 LTR end of the HIV-1 genome. The INT1 (5'-GGTCGAGACTGGCTGGAATTC) and INT2 (5'-AAGGACTGGCTGGAATTC) oligonucleotides were purified by gel electrophoresis on a denaturing urea gel. The resulting PCR products were separated in a 15% denaturing polyacrylamide gel and visualized with a PhosphorImager.

**Cell Transfection and Immunoprecipitation Experiments**—293 cells were transiently transfected by electroporation with 10 μg of IN-FLAG expression vectors alone or co-transfected with 10 μg of a HA-SN5 expression vector (a kind gift from C. Muchardt, Pasteur Institute, Paris, France). The vectors were co-transfected twice in phosphate-buffered saline and lysed in 400 mM NaCl, 50 mM Tris-HCl (pH 7.6), 5 mM EDTA, 1% Triton X-100, containing 1 mM DTT and standard protease inhibitors (Sigma) for 30 min on ice. Cell lysates were sonicated twice for 20 s, then debris was pelleted by centrifugation at 4°C. Precleared supernatants were incubated with protein G-Sepharose loaded with anti-Flag M2 antibody (Sigma) at 4°C overnight. The beads were washed four times in lysis buffer and analyzed by Western blotting.

**Western Blotting**—Proteins were separated by SDS-PAGE and revealed by Western blotting using anti-FLAG M2 peroxidase-coupled antibody (Sigma), anti-LEDGF (BD Transduction Laboratories), or anti-HA (Roche Applied Science).

**Quantification of Three Different Viral DNA Forms during HIV-1 Replication**—CEM-A301 cells were infected with HIV-1 Bru WT or mutants viruses in presence of 1 μM Saquinavir, to limit viral replication to a single round, and harvested at 3, 9, 24, and 48 h post-infection. Samples were washed in phosphate-buffered saline and treated with 500 units of DNase I (Roche Diagnostics) for 1 h at 37°C, prior to DNA extraction using a Qiagen blood DNA minikit (Qiagen). The amounts of total HIV-1 DNA, two-LTR circles, and integrated HIV-1 DNA were quantified using real-time PCR with the Light Cycler instrument (Roche Applied Science) as described previously (28). Each sample was analyzed in duplicate. Briefly, the total HIV-1 DNA copy number was determined using primer that annealed in the U5 region of the LTR (MH531) and in the 5' region of the gag gene (MH532) (29). Two-LTR circles were amplified using primers spanning the LTR-LTR junctions (HIV-1 and HIV R1) (28). Integrated DNA was quantified using an Alu-LTR-based nested PCR procedure. In a first round of PCR, integrated HIV-1 sequences were amplified with two outward facing Alu primers and a HIV-1 LTR-specific primer (L-M667) containing a λ phage-specific sequence at the 5'-end of the oligonucleotide. In a second round of PCR, we used specific primers for the λ sequence (A T) and the LTR region (AAS5) (28). To eliminate the signal due to primer extension carried out by the L-M667 primer during the first round PCR, a nested PCR assay was performed without Alu primers. The signal of the nested PCR obtained in the absence of Alu primers was subtracted from the integrated HIV-1 DNA signal. Copy numbers of total viral DNA two-LTR circles and integrated DNA were determined in reference to standard curves prepared by amplification of cloned DNA with matching sequences (28). Results were normalized by the number of cells and the amount of cellular DNA quantified by PCR of the β-globin gene according manufacturer’s instructions (Roche Applied Science).

**RESULTS**

**Glutamine 168 of IN Is Involved in Interaction with LEDGF/p75**—To demonstrate the role of the interaction of HIV-1 IN with LEDGF/p75 in the viral replication cycle, we first mapped by yeast two-hybrid screening the IN interacting domain on LEDGF/p75 to a discrete region of 102 amino acids in the C-terminal domain of the p75 isoform of LEDGF, located between amino acids 340 and 442 (data not shown). These data
are in agreement with the results from Cherepanov et al. (30) showing that the interacting binding domain of LEDGF/p75 with IN is comprised between residues 347 and 429. Then, we characterized the interacting domain for LEDGF/p75 on HIV-1 IN using yeast two-hybrid screening of a highly complex library of HIV-1 random fragments obtained after nebulization of the HIV-1 DNA and inserted in the prey plasmid (data not shown and see additional methods given in the supplemental material). By this technique, we could map the LEDGF/p75 interacting domain in the catalytic core of IN between amino acids 56 and 182. Then, to identify the amino acids of IN required for interaction with LEDGF/p75, we screened by two-hybrid a library of HIV-1 IN random mutants obtained by PCR random mutagenesis using LEDGF/p75 as bait. Several mutations impairing LEDGF/p75 interaction were characterized in the core region of IN, in particular two different mutants at position Gln168. These mutations were introduced in molecular clones of HIV-1 Bru, and viral stocks were tested for replication in A301 cells. With the notable exception of the Gln168 mutants, all the other mutations affected the synthesis of viral cDNA (data not shown). So we choose to focus our studies on the mutants Q168P and Q168L, since the isolation of two independent mutants at this position is a strong indication about the importance of this residue for interaction with LEDGF/p75. Effectively, both of these mutants were impaired for LEDGF/p75 interaction, as estimated by a quantitative β-galactosidase assay (Fig. 1A) and by the lack of co-immunoprecipitation of LEDGF/p75 with IN-Flag168L (Fig. 1B and data not shown). In addition, we generated a more conservative mutation Q168A to confirm that the lack of interaction with LEDGF/p75 is linked to the absence of the WT residue Gln168 (Fig. 1, A and B). We verified that these mutants were defective for interaction with LEDGF/p75 but were still able to interact with SNF5/Ini1, another partner of IN (Fig. 1C). We next tested whether these mutants remained enzymatically active in vitro. Recombinant WT and mutant integrases were purified from Escherichia coli, and both 3′ processing and strand transfer activities were assayed in the presence of Mg2+. Reaction products were separated in a denaturing urea gel and visualized with a PhosphorImager.

**Fig. 1. Gln^{168} of IN is involved in LEDGF/p75-interaction.** A, interaction of wild type, mutated Q168L, Q168P, or Q168A IN with LEDGF/p75 was tested using a two-hybrid quantitative β-galactosidase assay. B, 293 cells were transiently transfected with IN'-FLAG expression vectors coding for the wild type, mutated Q168A, or Q168L IN, as indicated. Cells extracts were immunoprecipitated using anti-FLAG M2 antibody followed by immunoblotting using anti-FLAG-horseradish peroxidase or anti-LEDGF antibodies as indicated. C, IN Q168L and Q168A interact with SNF5/Ini1. 293 cells were transiently co-transfected with IN'-FLAG and HA-SNF5 expression vectors as indicated. Cell extracts were immunoprecipitated using anti-FLAG M2 antibody followed by immunoblotting using anti-FLAG-horseradish peroxidase or anti-HA-horseradish peroxidase antibodies as indicated. D, activities of recombinant integrases were tested in an oligonucleotide based assay. Both 3′ processing and strand transfer activities were evaluated for Q168A and Q168L IN in comparison with WT IN. Asterisks indicate E. coli exonuclease activity. Reaction products were separated in a denaturing urea gel and visualized with a PhosphorImager.
clones of HIV-1 Bru, and viral stocks were produced. No difference in virus release was observed between these mutants and the WT virus, suggesting that none of these mutations impair virus assembly or release (data not shown). In contrast, both of these viruses were found completely defective for replication over 2 weeks in A301 cells and other T cell lines including Jurkat (Fig. 2A and data not shown). Taken together, these results demonstrate that disruption of the IN-LEDGF/p75 interaction by a single mutation in integrase completely inhibits the replication of HIV-1.

**Viruses Defective for the LEDGF/p75 Interaction Are Predominantly Blocked at Integration**—To determine whether in the absence of interaction of IN with LEDGF/p75 the integration step is specifically blocked during the replication cycle of HIV-1, total HIV-1 DNA, two-LTR circles, and integrated forms of proviral DNA were measured by quantitative PCR on cell extracts from A301 cells infected, respectively, with two LEDGF/p75 interaction deficient mutant viruses Bru IN Q168A (HIVQ168A) and Bru IN Q168L (HIVQ168L), while HIVWT Bru and the catalytically IN inactive D116A mutant (Bru HIVD116A) were used as controls (Fig. 2B). In this experiment, HIV-1 replication was restricted to a single round infection by addition of a protease inhibitor. At 3-h post-infection, reverse transcription products peaked, and levels of early reverse transcriptase (strong stop cDNA) (data not shown) or late reverse transcriptase (total HIV cDNA) were similar (HIVD116A and HIVQ168L) or even higher (HIVQ168A) than that of the WT virus (Fig. 2B). These results show that none of these mutations impaired reverse transcription. At 9 h post-infection, the amount of total HIV cDNA dropped for the mutant HIVQ168L, while the amount of cDNA remained high for Q168A until 24 h post-infection. We next monitored the formation of two-LTR circles that are generally accepted to reflect PIC nuclear import. All viruses were able to form two-LTR circles at about the same level at 9 h post-infection (Fig. 2C), indicating that all PICs were imported into the nucleus. Compared with the WT virus, a 2–3-fold reduction in two-LTR circles formation was observed for the HIVQ168L virus after 24 h, while the HIVQ168A virus displayed normal levels. Because HIVQ168A made twice as much total HIV cDNA as the WT virus, it was also 2-fold defective for two-LTR circles. The catalytic mutant D116A accumulated about 5 times more two-LTR circles than the WT virus, as described previously (31). Finally, by quantifying integrated proviruses at 24 and 48 h post-infection, both HIVQ168L and HIVQ168A mutants were deficient for integration much like the D116A mutant (Fig. 2D). These results show that the mutant virus HIVQ168A encoding a fully active integrase that does not interact with LEDGF/p75 is specifically blocked at the integration step.

These results suggest that the IN-LEDGF/p75 interaction is involved in the mechanism controlling integration of the proviral DNA. In addition they suggest that this interaction does not participate per se to the nuclear translocation of the PIC.

**LEDGF/p75 Tethers IN to Chromatin**—We previously reported that in the absence of endogenous LEDGF/p75, nuclear localization of IN was aborted (19), suggesting a potential role of LEDGF/p75 in IN nuclear import. However, we also noticed that silencing of endogenous LEDGF/p75 greatly decreased GFP-IN expression level in the nucleus (19). Here we show that treatment by the proteasome inhibitor MG132 restored a normal level of nuclear GFP-IN WT in cells that were silenced for LEDGF/p75 expression (Fig. 3). This suggests that the defect in nuclear accumulation of GFP-IN observed after transient silencing of LEDGF/p75 expression could be an indirect consequence of proteasome-dependent degradation of IN within the nucleus in the absence of LEDGF/p75 expression. Using an in vitro nuclear import assay based on digitonin-permeabilized HeLa cells (27), we found that recombinant IN was imported with the same efficiency in cells treated with LEDGF/p75 siRNA or control siRNAs (Fig. 4A, compare upper and lower
right panels). In addition, both Q168L and Q168A IN mutants fused to GFP accumulated also in the nucleus even if some diffuse pattern is visible in the cytoplasm (Fig. 4B). This con-
firms that interaction with LEDGF/p75 does not seem to be required for nuclear import of IN. Alternatively, LEDGF/p75, which is known to be strongly associated with chromosomes in mitotic cells (19, 32), could act at the level of retention of IN within the nucleus rather than at the level of nuclear import per se. Of note, the retention phenomenon seems particularly crucial when IN is not fused to GFP, since the Q168L IN-Flag mutant protein is more homogeneously distributed between nuclear and cytoplasmic compartments compared with the WT IN-Flag or to the GFP-IN Q168L mutant (Fig. 4B).

To further analyze the role of LEDGF/p75 in the nuclear localization of IN, we studied the binding of the Q168L IN mutant fused to GFP to chromosomes in mitotic HeLa cells. While GFP-IN WT fully co-localized with chromosomal DNA in mitotic cells (Fig. 5), strikingly mutants GFP-IN Q168L and Q168A displayed a diffuse staining and were no longer able to bind with condensed chromosomes (Fig. 5). In sharp contrast, the enzymatically inactive form of integrase, GFP-IN D116A, is still able to bind to mitotic chromosomes. From these experiments, we conclude that LEDGF/p75 is not required for nuclear localization of IN. A, knock-down of p75 by specific siRNA does not inhibit IN nuclear import in vitro. HeLa cells transfected with p75-
specific or control (p75inv) siRNA, as indicated, were digitonin-perme-
abilized and incubated with Cy3-labeled IN and an energy-regenerating
system (ATP, GTP, creatine phosphate, and creatine phosphokinase).

DISCUSSION

By a combination of multiple complementary approaches based on interaction studies using random and directed mu-
tageneis, we could demonstrate that interaction of IN with LEDGF/p75 is important for integration and replication of HIV-1. Our results show that LEDGF/p75 plays the role of a chromosomal ligand allowing the binding of IN to the chromo-
somes and could be involved in targeting the HIV-1 PIC to favorable chromatin regions (6).

The LEDGF/p75 interacting domain maps within the core region of IN where the catalytic site is also located. Using two-hybrid screening, we found that mutations of Glu$^{168}$ of IN impaired LEDGF/p75 interaction. Although the Q168A mutation that disrupts the IN-LEDGF/p75 interaction has no effect on the catalytic activity of IN in vitro, the proximity of these two domains could explain why, at least in vitro, LEDGF/p75 is able to modulate and enhance the catalytic activity of IN (18, 30). Importantly, we found that the mutant Q168A was still catalytically active, although it was defective for tethering IN to cellular chromosomes. On the other hand, the mutant D116A was still able to be targeted to chromosomes, although it was catalytically dead. These results demonstrate that the tether-
ing of IN to the chromosomes and the catalytic activity of integration are governed by two independent determinants in the protein. These results also underline the importance of LEDGF/p75 function in targeting IN to cellular chromosomes, as an independent step prior to the enzymatic reaction that integrates the proviral cDNA into the host genome. However, one cannot rule out that, once the HIV-1 preintegration complex is targeted via LEDGF/p75 at the site of integration, LEDGF/p75 could also act as a cofactor for the enhancement of the enzymatic activity of HIV-1 integrase.

Our results on the nuclear import of IN using either in vitro nuclear import or GFP-IN fusion proteins indicate that LEDGF/p75 is not required for active nuclear import of IN. In fact, we observed a 2-fold reduction of two-LTR circle formation (relative to the amount of total HIV-1 cDNA), as well as an inhibition of integrated copies of HIV-1 Q168A and HIV-1 Q168L. This phenotype is different from that observed with the catalytic mutant of IN, which displayed a 5-fold increase of two-LTR circles formation as well as inhibition of integrated forms (Fig. 2, B and C). LEDGF/p75 silencing led to proteasome-dependent degradation of IN (Fig. 3). Furthermore, we observed a decrease in the half-life of IN Q168L and IN Q168A compared with the WT protein, while IN D116A was stable.3 These data suggest that by inhibiting its interaction with LEDGF/p75, IN becomes more prone to degradation in the nucleus, leading to a decrease of the two-LTR circles forms associated with preintegration complexes. Although we cannot rule out that the isolated IN and the PIC could display distinct karyophilic behavior, our results show that HIV-1 Q168L and HIV-1 Q168A are predominantly blocked at the integration step, suggesting that IN-LEDGF/p75 interaction is not directly required for importing the HIV-1 PIC in the nucleus. Maertens et al. (22) found that overexpression of LEDGF/p75 mutated in the nuclear localization signal resulted in the aggregation of IN-LEDGF/p75 complexes in the cytoplasm. This is fully consistent with our own conclusions in favor of a role of LEDGF/p75 in the targeting of IN to chromatin rather than in nuclear import. In fact, overexpression of a nuclear localization signal mutant of LEDGF/p75 excluded from the nucleus but still able to bind to IN will act as a transdominant, trapping IN in the cytoplasm, even if the nuclear import of IN is not controlled by interaction with LEDGF/p75.

Surprisingly, it was recently reported that replication of HIV-1 in Jurkat cells silenced for LEDGF/p75 was not impaired, despite the fact that in these conditions, integrase was found delocalized in the cytoplasm (21). One cannot rule out that the functions of LEDGF/p75 in viral replication and chromosomal targeting of IN could be redundant and that another protein could substitute for LEDGF/p75 in certain cell types. Interestingly, it has been proposed that HRP2 (hepatoma-derived growth factor protein 2), another hepatoma-derived growth factor-related protein, is also able to interact with HIV-1 integrase and could be a substitute for some of LEDGF/p75 functions (30). However, we have generated several cell lines stably depleted for LEDGF/p75, and we constantly observed a reduction of HIV-1 replication in the absence of LEDGF/p75 that was restored when the protein was re-expressed.4 Furthermore, as indicated above, we found that HIV-1 Q168L and HIV-1 Q168A viruses were also replication-defective in Jurkat cells.

Two other mutations of IN impairing interaction with LEDGF/p75 were recently described. The point mutation H12N in the zinc binding domain of IN was shown to reduce its affinity for LEDGF/p75 in vitro (19). V165A, another IN mutant, was also shown to be defective for LEDGF/p75 interaction (9). A virus harboring the V165A mutation was replication-deficient (31). Analysis of the IN structure shows that residues Val165 and Gin168 are in close contact within the monomer. However, Val165 is partially buried within the protein, therefore less accessible for interaction with LEDGF/p75 than Gin168, which is exposed at the surface of the protein.

Altogether, these findings support the notion that LEDGF/ p75 is an important cofactor of HIV-1 integrase involved in its chromosomal targeting and required for integration and replication of HIV-1. Taking into account the full defect in viral replication resulting from lack of interaction of IN with LEDGF/p75, one can postulate that a compound capable of disrupting or preventing the interaction of IN with LEDGF/p75 would display a very potent anti-viral activity.

Acknowledgments—We thank Caroline Petit and Lang-Xia Liu for helpful discussions, Rik Gijbers for critical reading of the manuscript and Emmanuelle Ségalard, and Linda Desender and Nathalie Simoes for excellent technical assistance.

REFERENCES

1. Carette, S., Hoffmann, C., and Bushman, F. (1998) J. Virol. 72, 4005–4014
2. Jordan, A., Defechereux, P., and Verdin, E. (2001) EMBO J. 20, 1726–1738
3. Jordan, A., Biagrove, D., and Verdin, E. (2003) EMBO J. 22, 1686–1677
4. Schroder, A. R., Shinn, P., Chen, H., Berry, C., Ecker, J. R., and Bushman, F. (2002) Cell 110, 521–529
5. Wu, X., Li, Y., Crise, B., and Burgess, S. M. (2003) Science 300, 1749–1751
6. Bushman, F. D. (2003) Cell 115, 135–138
7. Bukrinsky, M. I., Sharova, N., McDonald, T. L., Pushkarskaya, T., Tarpley, W. G., and Stevenson, M. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 6125–6129
8. Miller, M. D., Farnet, C. M., and Bushman, F. D. (1997) J. Virol. 71, 5382–5390
9. Turlure, F., Devroe, E., Silver, P. A., and Engelmann, A. (2004) Front. Biosci. 9, 3187–3208
10. Farnet, C. M., and Bushman, F. D. (1997) Cell 88, 483–492
11. Hindmarsh, P., Ridsky, T., Reeves, R., Andrade, M., Skalka, A. M., and Leis, J. (1999) J. Virol. 73, 2994–3003
12. Li, L., Yoder, K., Hansen, M. S., Olivera, J., Miller, M. D., and Bushman, F. D. (2000) J. Virol. 74, 10985–10974
13. Chen, H., and Engelmann, A. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 15270–15274
14. Lin, C. W., and Engelmann, A. (2003) J. Virol. 77, 5030–5036
15. Li, L., Olivera, J. M., Yoder, K. E., Mitchell, R. S., Butler, S. L., Lieber, M., Martin, S. L., and Bushman, F. D. (2001) EMBO J. 20, 3272–3281
16. Kalpana, G. V., Marmon, S., Wang, W., Crabtree, G. R., and Goff, S. P. (1994) Science 266, 2092–2096
17. Turelli, P., Doucas, V., Craig, E., Mangeat, B., Klages, N., Evans, R., Kalpana, G., and Trono, D. (2001) Mol. Cell 7, 1245–1254
18. Cheerepanov, P., Maertens, G., Proost, P., Devreeze, B., Van Beeumen, J., Engelborghs, Y., De Clercq, E., and Debyser, Z. (2003) J. Biol. Chem. 278, 372–381
19. Maertens, G., Cheerepanov, P., Pluymers, W., Busschots, K., De Clercq, E., Debyser, Z., and Engelborghs, Y. (2003) J. Biol. Chem. 278, 33528–33539

<sup>3</sup> D. Tempe., S. Emiliiani, and R. Benarous, unpublished data.

<sup>4</sup> L. Vandekerckhove, F. Christ, M. Michiels, B. Van Maele, J. De Rijk, R. Gijbers, C. Vandenhoute, and Z. Debyser, submitted for publication.
HIV-1 Integrase Mutants Defective for LEDGF/p75 Interaction

20. Ganapathy, V., Daniels, T., and Casiano, C. A. (2003) *Autoimmun. Rev.* 2, 290–297
21. Llano, M., Vanegas, M., Fregoso, O., Saenz, D., Chung, S., Peretz, M., and Poeschla, E. M. (2004) *J. Virol.* 78, 9524–9537
22. Maertens, G., Cherepanov, P., Debyser, Z., Engelborghs, Y., and Engelman, A. (2004) *J. Biol. Chem.* 279, 33421–33429
23. Cadwell, R. C., and Joyce, G. F. (1999) *PCR Methods Appl.* 2, 28–33
24. Fromont-Racine, M., Rain, J. C., and Legrain, P. (2002) *Methods Enzymol.* 350, 513–524
25. Petit, C., Schwartz, O., and Mammano, F. (1999) *J. Virol.* 73, 5079–5088
26. Cherepanov, P., Pluymers, W., Claeyts, A., Proost, P., De Clercq, E., and Debyser, Z. (2000) *FASEB J.* 14, 1289–1299
27. Depienne, C., Mousnier, A., Leh, H., Le Rouzic, E., Dormont, D., Benichou, S., and Dargemont, C. (2001) *J. Biol. Chem.* 276, 18102–18107
28. Brussel, A., and Sonigo, P. (2003) *J. Virol.* 77, 10119–10124
29. Butler, S. L., Hansen, M. S., and Bushman, F. D. (2001) *Nat. Med.* 7, 631–634
30. Cherepanov, P., Devroe, E., Silver, P. A., and Engelman, A. (2004) *J. Biol. Chem.* 279, 48883–48892
31. Limon, A., Devroe, E., Lu, R., Ghory, H. Z., Silver, P. A., and Engelman, A. (2002) *J. Virol.* 76, 10598–10607
32. Nishizawa, Y., Usukura, J., Singh, D. P., Chylack, L. T., Jr., and Shinohara, T. (2001) *Cell Tissue Res.* 305, 107–114
33. Vojtek, A. B., and Hollenberg, S. M. (1995) *Methods Enzymol.* 255, 331–342
34. Fromont-Racine, M., Rain, J. C., and Legrain, P. (1997) *Nat. Genet.* 16, 277–282
Integrase Mutants Defective for Interaction with LEDGF/p75 Are Impaired in Chromosome Tethering and HIV-1 Replication

Stéphane Emiliani, Aurélie Mousnier, Katrien Busschots, Marlène Maroun, Bénédicte Van Maele, Denis Tempé, Linos Vandekerckhove, Fanny Moisant, Lilia Ben-Slama, Myriam Witvrouw, Frauke Christ, Jean-Christophe Rain, Catherine Dargemont, Zeger Debyser and Richard Benarous

J. Biol. Chem. 2005, 280:25517-25523.
doi: 10.1074/jbc.M501378200 originally published online April 25, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M501378200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2005/05/03/M501378200.DC1

This article cites 34 references, 21 of which can be accessed free at http://www.jbc.org/content/280/27/25517.full.html#ref-list-1