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Citation
Engelman, Alan, and Peter Cherepanov. 2008. The lentiviral integrase binding protein LEDGF/p75 and HIV-1 replication. PLoS Pathogens 4(3): e1000046.

Published Version
doi:10.1371/journal.ppat.1000046

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Accessibility
The Lentiviral Integrase Binding Protein LEDGF/p75 and HIV-1 Replication

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Abstract: Retroviral replication proceeds through a stable proviral DNA intermediate, and numerous host cell factors have been implicated in its formation. In particular, recent results have highlighted an important role for the integrase-interactor lens epithelium-derived growth factor (LEDGF)/p75 in lentiviral integration. Cells engineered to over-express fragments of LEDGF/p75 containing its integrase-binding domain but lacking determinants essential for chromatin association are refractory to HIV-1 infection. Furthermore, both the levels of HIV-1 integration and the genomic distribution of the resultant proviruses are significantly perturbed in cells devoid of endogenous LEDGF/p75 protein. A strong bias towards integration along transcription units is a characteristic feature of lentiviruses. In the absence of LEDGF/p75, HIV-1 in large part loses that preference, displaying concomitant integration surges in the vicinities of CpG islands and gene promoter regions, elements naturally targeted by other types of retroviruses. Together, these findings highlight that LEDGF/p75 is an important albeit not strictly essential cofactor of lentiviral DNA integration, and solidify a role for chromatin-associated LEDGF/p75 as a receptor for lentiviral preintegration complexes. By now, one of the best characterized viral–host interactions, the integrase-LEDGF/p75 interface opens a range of opportunities for lentiviral vector targeting for gene therapy applications as well as for the development of novel classes of antiretroviral drugs.

Introduction

A key step in the retroviral lifecycle is the formation of the provirus, the integrated form of the viral cDNA that is produced during reverse transcription. Retroviral integration is promoted by the viral integrase (IN) enzyme, which enters the cell as a component of the virion particle. IN catalyzes two spatially and temporally distinct reactions within the context of the preintegration complex (PIC), a large structure derived from the virus core [1,2]. During the initial reaction, which is called 3’ processing and happens soon after the cDNA is made, IN hydrolyzes a dinucleotide from each end of HIV-1 DNA [2,3] (Figure 1). The second reaction, DNA strand transfer, takes place at the site of integration in the cell nucleus. IN uses the recessed 3’-OH groups created during 3’ processing to cut opposing strands of chromosomal DNA in a staggered fashion, concomitantly connecting the viral DNA 3’ ends to the generated 5’ overhangs [4]. The resultant DNA recombination intermediate harbors single-strand discontinuities that must be repaired to complete provirus formation [Figure 1]. See [5] for a thorough overview of the mechanism of HIV-1 integration as well as the host cell factors that are implicated in the final DNA repair step.

Retroviral IN enzymes purified from a variety of sources display 3’ processing and DNA strand transfer activities in vitro [6–10]. These seminal results highlighted that IN alone sufficed to form DNA strand transfer reaction products; however, numerous subsequent studies indicated that other proteins play important auxiliary roles in the context of virus infection (see [5,11–13] for recent reviews). This review focuses on the IN interactor lens epithelium-derived growth factor (LEDGF)/transcriptional co-activator p75, whose critical role in lentiviral DNA integration has been highlighted by a number of recent studies [14–18].

LEDGF/p75: A Cellular Interactor of Lentiviral INs

LEDGF/p75, a member of the hepatoma-derived growth factor (HDGF) related protein (HRP) family, was initially implicated in lentiviral biology through its association with ectopically expressed HIV-1 IN in 293T cells [19]. Significantly, purified recombinant LEDGF/p75 protein stimulated HIV-1 IN catalytic function in vitro [19]. The interaction was independently discovered by analyzing proteins associated with HIV-1 IN in HeLa cells [5] and in a yeast two-hybrid screen for HIV-1 IN interactors [20].

HRPs are characterized by a conserved N-terminal PWPP domain, an ~90– to 135–amino acid module found in a variety of nuclear proteins [21,22]. Six human HRP family members have been described: HDGF, HRP1, HRP2, HRP3, LEDGF/p75, and LEDGF/p52 [23–25], of which two, LEDGF/p75 and HRP2, possess affinity for HIV-1 IN [25]. Significantly larger than the rest of the HRP family, LEDGF/p75 and HRP2 contain a second evolutionarily conserved domain within their extended C-termini. It is this domain that mediates the interaction with HIV-1 IN, hence the term “IN-binding domain (IBD)” [23,26] (Figure 2A). LEDGF/p75 and LEDGF/p52 are expressed from the same gene (human PSRP) [27]. The smaller p52 isoform, produced by alternative RNA splicing [27], lacks the IBD and fails to engage HIV-1 IN in vitro or in live cells [28] (Figure 2A).

LEDGF/p75 is a ubiquitous nuclear protein, tightly associated with chromatin throughout the cell cycle [19,26,28–31]. Chro-
matin association is primarily mediated by three conserved sequence elements within the N-terminal half of the protein: the PWWP domain, nuclear localization signal (NLS), and a dual copy of the AT-hook DNA binding motif [31,32] (Figure 2A). LEDGF/p75 binds a variety of DNA substrates in vitro, an activity that appears relevant to its association with chromatin [31]. We note that the sequence-specific DNA binding of LEDGF/p75 to stress response and heat shock elements [33] could not be independently verified [31]. Recent results have revealed that the association with chromatin is essential for LEDGF/p75 function during HIV-1 infection ([16,18]; see below), highlighting the importance of clarifying the mechanism of LEDGF/p75 chromatin binding.

The cellular functions of LEDGF/p75 and closely related HRP2 remain largely uncharacterized, although initial reports have indicated a role for LEDGF/p75 in transcriptional regulation [27,33,34]. The protein is not essential for cell survival [18], though the majority of LEDGF-null mice died soon after birth or showed a range of developmental abnormalities in adulthood [35]. Of note, the eye lens developed normally in LEDGF knockout mice [35], highlighting that its most often used name, which was coined during the second isolation of the gene [36], reflects the use of lens epithelial cells for cDNA isolation more so than biological function. The finding that LEDGF/p75 associates with JPO2, a known interactor of c-MYC, will hopefully help efforts to link its function to an established cellular mechanism [37,38].

HIV-1 and feline immunodeficiency virus (FIV) INs predominantly localize to nuclei upon ectopic expression in a variety of cell types [30,39–42]. In mitotic cells, and likely throughout the cell cycle, the lentiviral INs stably associate with chromatin [30,40,41]. Three important consequences on IN expression occurred when levels of endogenous LEDGF/p75 protein were reduced using RNA interference (RNAi). First, in interphase cells,
the vast majority of HIV-1 and FIV IN re-localized to the cell cytoplasm [28,30]. Second, and perhaps more significant, IN proteins lost their chromosomal association, as was most clearly observed in mitotic cells [28,30]. Finally, at least in case of HIV-1 IN, the redistribution was accompanied by significant reductions in steady-state levels of the viral protein [43]. These observations implicated LEDGF/p75 as the dominant cellular interactor of lentiviral INs and indicated that the cellular protein was essential for IN-chromatin association, likely acting as a receptor or molecular tether. LEDGF/p75 thus contributes to the karyophilic properties of lentiviral INs. Indeed, the cell factor contains a classical NLS (residues 148–156; Figure 2A) [26,44,45], and overexpression of a NLS defective form of LEDGF/p75 trapped HIV-1 IN in the cytoplasm [26,44]. However, the viral protein has not been formally proven to piggyback into the nucleus through its interaction with LEDGF/p75. In one report, a primarily nuclear IN population was observed when knockdown cells were treated with proteasome inhibitors [20]. Although the association of ectopically expressed HIV-1 IN with chromatin is attributable to the LEDGF/p75 interaction, these data suggest the viral protein might access nuclei in a LEDGF/p75-independent manner. The mechanisms of lentiviral IN/PIC nuclear import remain ongoing areas of investigation (see [46,47] for recent reviews).

Additional studies revealed that LEDGF/p75 binds to a variety of lentiviral IN proteins, but, significantly, fails to interact with IN proteins derived from five (α, β, γ, and Spuma) of the six other tested retroviral genera [30,48,49]. These observations implied that the IN-LEDGF/p75 interaction underlies a unique aspect of lentiviral biology. Of note, lentiviral PICs access cell nuclei and target chromatin (Figure 1A, steps 6 and 7) using mechanisms that distinguish them from other retroviruses [46,47,50].

**Structural Aspects of the IN–LEDGF/p75 Interaction**

HIV-1 IN is composed of three functional domains: the N-terminal domain (NTD), the catalytic core domain (CCD), and the C-terminal domain (CTD) (Figure 2B). Initial mapping experi-
ments using fluorescent fusions expressed in live cells revealed that the CCD is minimally required for the interaction with LEDGF/p75, and highlighted a role for the NTD as an affinity enhancer [28]. A number of single amino acid substitutions within the CCD, including V165A [5], R166A [51], and Q168A [20], were soon thereafter shown to impair the IN–LEDGF/p75 interaction. Each of these changes rendered HIV-1 replication defective [20,52–54], suggesting that the IN–LEDGF/p75 interaction might be essential for HIV-1 replication [20,51,55]. However, many mutations in IN exert so-called “class II” pleiotropic effects, whereby poorly understood aspects of IN biology extending beyond its innate catalytic function contribute to the overall replication defect [55–57]. Recent results indicate that PICs formed in the absence of LEDGF/p75 protein in vivo are fully competent to integrate the endogenous cDNA made during reverse transcription into exogenous target DNA in vitro [18]. Based on this, one would predict that LEDGF/p75 mutant viruses defective for growth solely due to the inability to interact with LEDGF/p75 would yield PICs fully competent for integration in vitro. The replication defect caused by the Q168A mutation was suggested to result from the lack of cofactor binding [20], though a follow-up study indicated HIV-1Q168A behaved as a class II IN mutant virus [55]. PICs derived from class II mutants fail to support in vitro integration activity [58], indicating that PIC analyses would help to shed light on the specificity of the HIV-1Q168A replication defect.

The 3-D structure of the LEDGF/p75 IN interaction. (A) Cartoon representation of the CCD-IBD complex (the complete asymmetric unit) [60]. IN CCD molecules are colored green and blue, whereas the LEDGF/p75 IBDs are magenta and yellow. The side chains of IN active site residues Asp-64, Asp-116, and Glu-152 (Figure 2B) are shown as red sticks. The region within the dashed box is expanded in (B). (B) Details of the CCD-IBD interface. LEDGF/p75 hotspot residues Ile-365 and Asp-366, situated at the base of the loop between IBD helices 1 and 2, project into a pocket at the CCD dimer interface. The bidentate hydrogen bond contact between Asp-366 and the backbone amides of IN residues Glu-170 and His-171 is critical for the protein–protein interaction in vitro [51], in yeast cells [55], and during HIV-1 infection [18]. Ile-365 is buried into a hydrophobic pocket predominantly formed by IN residues Ala-128, Trp-132, Leu-102, and Met-178. Hydrogen bonds and salt bridges are shown as dotted lines. The figure was drawn using PyMOL [99].

doi:10.1371/journal.ppat.1000046.g003
LEDGF/p75 and HIV-1 Replication

Initial RNAi-based studies were central to establish important links between endogenous LEDGF/p75 protein and lentiviral IN expression levels and subcellular localization, yet they failed to reveal an important role for the cell factor in HIV-1 replication. In some RNAi-based studies, despite achieving what appeared to be very efficient reductions in cellular protein [30,61], specific HIV-1 replication defects were not observed despite rigorous effort to identify them. At the time it was suggested that intracellular LEDGF/p75 levels might significantly exceed those required to effect normal lentiviral DNA integration [61], a hypothesis supported by subsequent RNAi-based work [16,62,63] and a gene knockout study [18]. Llano and colleagues [16] performed an elegant study whereby the expression of short-hairpin RNA (shRNA) was linked to that of green fluorescence protein (GFP) within the same lentiviral-based vector. Sorting the brightest green cells therefore ensured for selection of potent LEDGF/p75 knockdowns. Selected cells were moreover fractionated to analyze levels of chromatin-bound protein. In this way, HIV-1 infectivity levels were correlated to residual levels of chromatin-associated LEDGF/p75. In the absence of detectable protein, infection was reduced to 3.5% of that observed in the presence of normal LEDGF/p75 levels. Similarly significantly reduced levels of HIV-1 infection were observed in mouse embryo fibroblasts (MEFs) derived from LEDGF knockout as compared to littermate control animals [18]. The block in both cases was at integration: reverse transcription and the formation of two long terminal repeat (LTR)-containing DNA circles, a surrogate marker for PIC nuclear import, were normal, whereas integration was severely reduced [16,18]. Although these results would seem to exclude a role for LEDGF/p75 in the nuclear import of the PIC, the baffling ability of lentiviruses to infect non-dividing cell types with high efficiencies calls for further scrutiny of lentiviral PIC nuclear import, were normal, whereas integration was severely reduced [16,18].

The isolated IBD competitively inhibited LEDGF/p75-dependent HIV-1 infection [16,18], although important for lentiviral integration, LEDGF/p75 is clearly not strictly essential. The infectivity of Moloney murine leukemia virus (Mo-MLV), a γ-retrovirus, importantly did not depend on the presence of LEDGF/p75 in target cells [16,18], providing biological relevance to the observations of lentiviral specificity in the IN–LEDGF/p75 interaction.

HIV-1 infection was fully restored to LEDGF/p75-depleted cells by ectopic expression of the cell factor [16,18,62], allowing detailed mutational analyses of cofactor function. Mutagenesis studies highlighted two regions within LEDGF/p75, the IBD and N-terminal PWWP domain/AT-hook DNA binding motifs that mediate chromatin binding (Figure 2A), as crucial for HIV-1 infection [16,18]. The requirement for the N-terminal sequence elements lent credence to early conjectures that LEDGF/p75 might primarily function to tether HIV-1 PICs to chromatin for integration [19,28,30].

The isolated IBD competitively inhibited LEDGF/p75-dependent stimulation of IN activity in vitro [25], and GFP-IBD fusion proteins over-expressed in target cells rather potently restricted HIV-1 infection [13,16]. The inhibitory effect was specific, since Mo-MLV was not affected, and altering IBD hotspot residue Asp-366 negated the block to HIV-1 infection. Importantly, reverse transcription and nuclear localization proceeded normally, with a significant reduction in total HIV-1 DNA levels observed after approximately six cell divisions. These data were fully consistent with an integration block, though the lentiviral vectors used to create cell lines precluded direct measurements of HIV-1 integration [15]. These observations indicate that IBD binding in large part precluded IN from engaging chromatin-associated LEDGF/p75. It is noteworthy that IBD over-expression, when combined with shRNA-mediated depletion, yielded a multiplicative antiviral effect [16] that would appear to exceed that observed in cells completely devoid of LEDGF/p75 protein [18]. It seems plausible that IBD-bound IN is crippled in its capacity to effectively engage target DNA in the absence of effective levels of competing chromosomal LEDGF/p75.

An HIV-1 mutant selected for its ability to replicate in MT-4 T cells engineered to express the C-terminal portion of the p75 isoform (residues 326–530; Figure 2A) acquired two mutations in IN, A128T and E170G [17]. Predictably, the mutations affected residues whose side chains directly participate in the LEDGF/p75-binding interface. As illustrated by the IBD-CCD crystal structure, Glu-170 is involved in a salt bridge with Lys-364 of LEDGF/p75, whereas Ala-128 contributes to the hydrophobic pocket that buries LEDGF/p75 hotspot residue Ile-365 [60] (Figure 3B). As expected, these mutations reduced the apparent affinity of the interaction with LEDGF/p75 [17]. Altering IN residue Ala-128 to the bulkier Gln had been previously shown to reduce the affinity of the protein–protein interaction [55]. Intriguingly, HIV-1ΔA128T/E170G was partially defective, and its replication capacity was reduced further upon LEDGF/p75 knockdown, suggesting that the mutant IN still depended on LEDGF/p75 for integration [17]. The estimated KD of the interaction between wild type HIV-1 IN and LEDGF/p75 is notably in the low nM range (PC and AE, unpublished data). Conceivably, by slightly detuning the LEDGF/p75-binding interface, the escape mutations afforded the dissociation of PIC-born IN from non-productive complex formation with the LEDGF/p75 fragment. Following multiple cycles of association/dissociation, the PIC would eventually engage a functional cofactor molecule.

These results provided physiological evidence for previous contentions that LEDGF/p75 is the dominant cellular binding partner of HIV-1 IN. Indeed, cell factors that potentially bind to other regions of IN were clearly unable to compensate for the loss in LEDGF/p75 binding caused by the A128T/E170G mutations. One interpretation is that LEDGF/p75 is the only cell protein involved in tethering HIV-1 to chromosomes for integration [17], though it remains plausible that other factors that bind IN with lower affinity could play roles in this process. As the ability of HRP2 to stimulate HIV-1 IN activity in vitro was inhibited by an excess of the LEDGF/p75 IBD fragment, both HRP protein family members are predicted to bind IN in similar manners [25].

Over-expression of human HRP2 in LEDGF-null cells rescued HIV-1 infection [18], although the analogous experiment failed to reveal a significant infectivity boost in severely knocked-down human SupT1 cells [16]. The generation of mouse HRP2 knockout cells, currently under way in the Engelman laboratory, should help to clarify whether this IBD-containing protein plays a significant role in HIV-1 integration.

LEDGF/p75 and PIC Targeting during Lentiviral Integration

Establishment of the stably integrated provirus is a hallmark of retroviral replication, fundamental to the persistence of infection.
However, mammalian genomic DNA is a vast target, a significant proportion of which is not transcriptionally active. What’s more, when integrated, the transcriptional activity of viral cDNA becomes sensitive to the local chromosomal environment [64]. Hence, it is not surprising that retroviruses do not leave integration entirely to chance, having evolved mechanisms for selecting suitable target loci. Indeed, the observed distributions of integrated proviruses along host chromosomal DNA are not random, and biases at the level of local DNA sequences as well as on the genomic scale have been described (reviewed in [13] and [30]). These biases appear to be genus-specific, and although the differences are sometimes subtle, three retroviral genera appear to stand out most distinctly. Lentiviruses, including HIV (both type 1 and type 2) [65,66], simian immunodeficiency virus (SIV) [67,68], FIV [69], and equine infectious anemia virus (EIAV) [70], are strongly biased towards integration into transcription units (TUs), with a preference for highly expressed genes. The γ-retrovirus Mo-MuLV, in contrast to lentiviruses, prefers to integrate in the vicinity of transcription start sites and CpG islands [67,71], while a spumaretrovirus, simian foamy virus (SFV), is biased against integrating into genes, yet nonetheless displays significant preferences for gene start sites and CpG islands [72,73].

Disengagement of HIV-1 IN from chromatin upon knockdown of endogenous cellular LEDGF/p75 strongly suggested that the cofactor acts as a chromosomal receptor or molecular tether for IN [28,30]. In addition, the interaction with LEDGF/p75 is conserved among and unique to Lentivirus [30,48,49], essentially paralleling the genus’ notable bias towards integration into TUs. The anticipation that LEDGF/p75 is the lentiviral targeting factor was initially confirmed by Ciuffi et al., who reported statistically significant albeit modest reductions of HIV-1 integration into TUs in two of three human cell lines following LEDGF/p75 knockdown [14]. More recently, a novel genetic knockout model afforded the study of HIV-1 integration under LEDGF-null conditions [18]. The observed frequency of HIV-1 integration into TUs in LEDGF knockout cells was significantly lower than in the littermate control condition. Importantly, the selectivity of HIV-1 for TUs in LEDGF-null cells was marginally less than the levels observed for γ- and α-retroviruses, as well as the adeno-associated parvovirus, in normal human cells [18]. Integration distribution and frequency positively correlated with local transcription activity in the absence of LEDGF/p75, though this correlation was significantly reduced compared with cells expressing the host factor [18]. The frequency of HIV-1 integration in the vicinity of CpG islands and transcription start sites was augmented in the absence of LEDGF/p75 [18], yielding profiles more similar to α-, γ-, δ-, and spuma-retroviruses, which naturally display affinity for these genomic features [71–76]. These data suggest that Retroviridae might exploit a common mechanism for intranuclear trafficking and integration site selection, which lentiviruses have evolved to override by the virtue of their interaction with LEDGF/p75. These findings solidified that LEDGF/p75 is the principal Lentivirus-specific integration targeting factor [18]. Nevertheless, in our minds, it is impossible to rule out that other cellular factors would contribute to the observed integration site distribution.

The Model for LEDGF/p75 Function in HIV-1 Integration

The question of how important LEDGF/p75 is to HIV-1 replication has caused fierce debates and remained controversial until recently. On one hand, viral replication defects caused by mutations in and around the LEDGF/p75-binding interface of HIV-1 IN indicated that the cofactor might play an essential role [20,51,55]. On the other hand, LEDGF/p75 depletion via RNAi significantly reduced but fell short of abrogating HIV-1 integration [16,62,63]. Since LEDGF knockout cells supported residual levels of HIV-1 integration, we must conclude that the cofactor is not essential for integration [18]. We concede that it is possible that some additional mechanisms rescue HIV-1 integration in the mouse knockout system, or that murine HR2 takes over the role of LEDGF/p75. Nevertheless, these results are in agreement with a large body of experimental evidence demonstrating that the isolated HIV-1 IN protein can perform its catalytic functions [7,10] (reviewed in [5] and [13]). Furthermore, PICs assembled in LEDGF-null cells were fully functional in vitro, indicating that the cofactor is not essential for the assembly or intrinsic activity of the HIV-1 complex. Though the endogenous cellular protein readily co-immunoprecipitated lentiviral PICs [30], the equivalent activities of PICs isolated from normal and LEDGF-null cells strongly suggest that it is the chromatin-bound pool of the protein that is functionally relevant (Figure 4).

In our model, chromatin-associated LEDGF/p75 acts as a receptor for incoming PIC particles, and although the PICs possess the capacity to engage target DNA, the interaction with LEDGF/
p75 encourages IN’s DNA strand transfer activity, thereby directing integration to a nearby genomic locus (Figure 4A). Concordantly, the cofactor robustly stimulates the enzymatic activities of lentiviral INs in vitro [19,25,31,49,77,78]. The model suggests that when the pool of chromatin-associated LEDGF/p75 is reduced or ablated, a larger fraction of PICs will rely on a slower, cofactor-independent pathway (Figure 4B). Conceivably, this will extend the time a PIC lingers in the non-integrated state, widening the window of opportunity for the cell to destroy it, and hence affecting overall integration levels. Indeed, the nucleoprotein complexes generated during reverse transcription [79,80] as well as IN itself [41,43,81] are subject to degradation by the cellular ubiquitin-proteasome system. The model accounts for the retention of the weakly favored target DNA consensus sequence at integration and replication [62,63], it is not essential, since stringently knocked-down human SupT cells and knockout MEFs supported residual provirus formation (approximately 10% of that seen in the presence of endogenous LEDGF/p75 levels) [16,18].

Therefore, it appears that HIV-1 does not entirely rely on the host factor, and furthermore, it can be assumed that there is a background of LEDGF/p75-independent integration under normal infection conditions. Conceivably, integration into transcriptionally repressed or gene-poor regions contributes to the establishment of latent viral reservoirs and hence to the persistence of clinical infection [86]. It remains to be determined if the residual levels of integration in LEDGF-null cells depend upon HRP2, a close kin of LEDGF/p75.

The potential for directed integration could alleviate concerns of insertional mutagenesis and in theory greatly improve the safety of retroviral vectors in gene therapy applications. Given the central role it plays in directing lentiviral integration [14,18] and its modular structural organization [25], LEDGF/p75 seems to be an ideal candidate for creating a designer targeting factor for lentiviral vectors. Indeed, a protein chimera containing the LEDGF/p75 IBD and the DNA-binding domain of bacteriophage λ repressor stimulated HIV-1 integration in the vicinity of λ operator sequences in vitro [87]. Undoubtedly, the future will see more work in this important direction. Also impending is focus on clarifying the cellular functions of LEDGF/p75 and the related HRP1s. Broad changes (both increases and decreases) in the transcriptional activity of ~2,000 genes were observed in silenced human 293T cells [14], with significantly fewer (<200) genes affected in LEDGF-null MEFs [18]. Although the chromosomal distribution of LEDGF/p75 has not been addressed directly, based on well-established HIV-1 integration site preferences [85], it is expected to be associated with a large body of active genes, distributed along the length of TUs [18,74]. A function in RNA polymerase II transcription elongation or splicing would agree with this distribution pattern.

The significant reductions in HIV-1 infectivity observed in cells extensively depleted for LEDGF/p75 protein [16,18] and in those engineered to over-express IBD-containing fragments [15–17], as well as the remarkable conservation of the interaction among divergent lentiviruses [49], highlight the protein–protein interaction as a novel target for the development of antiviral drugs. Though protein–protein interactions are traditionally more challenging than enzyme active sites for targeted drug development [88], LEDGF/p75 hotspot residues Ile-356 and Asp-366 notably extend down into a cleft formed at the IN dimer interface (Figure 3B). It is not difficult to envision a small molecule with sufficient binding affinity occupying the pocket and precluding LEDGF/p75 from binding. A major hurdle in identifying such a compound will be the strength of LEDGF/p75-IN interaction, although recent results seem to suggest this may not be insurmountable. A compound that ablated HIV-1 IN’s processing and DNA strand transfer activities in vitro at the relatively modest median inhibition concentration (EC50) of 150 μM bound the CCD at the IBD interaction site [60,89]. This region of IN is therefore amenable to small molecule binding. Peptides derived from the face of the IBD that interacts with the CCD were moreover reported to inhibit IN activity in a noncompetitive manner [90]. At present, development of clinically useful small molecules possessing sufficient affinity to preclude LEDGF/p75 binding and inhibit HIV-1 replication remains an inspiring challenge.

After the acceptance of this Review, Marshall et al. [91] reported that the frequency and distribution of HIV-1 as well as equine infectious anemia virus integration were significantly altered in human cells knocked down for LEDGF/p75 expression and in mouse cells carrying disrupted Pipp1 sequences.

Supporting Information

Accession Numbers

Detailed information on the following genes and proteins can be accessed at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) using the following accession numbers: HIV-1 IN (NP_705928), LEDGF/p75 (NP_150091), HDGF (NP_004485), HRP1 (NP_612641), HRP2 (NP_001001520), HRP3 (NP_057157), LEDGF/p52 (NP_066967), PSIP1 (GenecID 11168), JPO2 (Q96GN5), c-MYC (NP_002458), and FIV IN (NP_040973). The Protein Data Bank (http://www.rcsb.org/pdb) accession number for the CCD-IBD complex is 2B4J.

References

1. Bowerman B, Brown PO, Bishop JM, Varmus HE (1989) A nucleoprotein complex mediates the integration of retroviral DNA. Genes Dev 3: 469–478.
2. Miller M, Farnet C, Bushman F (1997) Human immunodeficiency virus type 1 preintegration complexes: Studies of organization and composition. J Virol 71: 5392–5399.
3. Pauza C (1990) Two bases are deleted from the termini of HIV-1 linear DNA during integrative recombination. Virology 179: 836–839.
4. Engelman A, Mizuchi K, Craige R (1991) HIV-1 DNA integration: Mechanism of viral DNA cleavage and DNA strand transfer. Cell 67: 1211–1221.
5. Turhune F, Devroe E, Sillier PA, Engelman A (2004) Human cell proteins and human immunodeficiency virus DNA integration. Front Biosci 9: 3107–3208.
6. Katzman M, Katz RA, Skalka AM, Leis J (1989) The avian retrovirus integration protein cleaves the terminal sequences of linear viral DNA at the in vivo sites of integration. J Virol 63: 5319–5327.
7. Bushman FD, Fujisawa T, Craige R (1990) Retroviral DNA integration directed by HIV integration protein in vitro. Science 249: 1555–1558.
8. Craige R, Fujisawa T, Bushman F (1990) The IN protein of Moloney murine leukemia virus processes the viral DNA ends and accomplishes their integration in vitro. Cell 62: 829–837.
35. Sutherland HG, Newton K, Brownstein DG, Holmes MC, Kress C, et al. (2006) The role of LEDGF/p75 in chromosomal tethering of HIV. PLoS Pathogens 2: e47. doi:10.1371/journal.ppat.0020047.

34. Fatma N, Singh DP, Shinohara T, Chylack LT Jr (2001) Transcriptional regulation of the antioxidant protein 2 gene, a thiol-specific antioxidant, by lens epithelium-derived growth factor/p75 integrase binding domain inhibits human immunodeficiency virus replication. J Biol Chem 280: 11498–11509.

33. Singh DP, Fatma N, Kimura A, Chylack J, Leo T, Shinohara T (2001) LEDGF/p75 form stable tetramers and associates with LEDGF/p75 protein in human cells. J Biol Chem 276: 372–381.

32. Llano M, Saenz DT, Meehan A, Wongthida P, Peretz M, et al. (2006) An essential role for LEDGF/p75 in HIV infection. Science 314: 461–464.

31. Hombrouck A, De Rijck J, Hendrix J, Vandekerckhove L, Voet A, et al. (2007) The interaction of LEDGF/p75 with integrase is lentivirus-specific and modulates their enzymatic activity in vitro. Nucleic Acids Res 35: 113–124.

30. Bouyac-Bertoia M, Dvorin JD, Fouchier RAM, Jenkins Y, Meyer BE, et al. (2005) The interaction of LEDGF/p75 with integrase is lentivirus-specific and modulates their enzymatic activity in vitro. Nucleic Acids Res 33: 113–124.

29. Cherepanov P, Maertens G, Proost P, Devreese B, Van Beumen J, et al. (2003) LEDGF/p75 functions downstream from preintegration complex formation to effect gene-specific HIV-1 integration. Genes Dev 17: 1676–1778.

28. Cherepanov P, Maertens G, Proost P, DeVreese B, Van Beumen J, et al. (2003) LEDGF/p75 form stable tetramers and associates with LEDGF/p75 protein in human cells. J Biol Chem 278: 372–381.

27. Vanegas M, Llano M, Delgado S, Thompson D, Peretz M, et al. (2005) Integrase mutants defective for interaction with LEDGF/p75 are impaired in chromosomal tethering and HIV-1 replication. J Biol Chem 280: 25517–25525.

26. Vanegas M, Llano M, Delgado S, Thompson D, Peretz M, et al. (2005) The interaction of LEDGF/p75 with integrase is lentivirus-specific and modulates their enzymatic activity in vitro. Nucleic Acids Res 33: 113–124.

25. Cherepanov P, Maertens G, Proost P, DeVreese B, Van Beumen J, et al. (2003) LEDGF/p75 form stable tetramers and associates with LEDGF/p75 protein in human cells. J Biol Chem 278: 372–381.

24. Ciuffi A, Llano M, Poeschla E, Hoffmann C, Leipzig J, et al. (2005) A role for LEDGF/p75 in targeting HIV DNA integration. Nat Med 11: 1287–1293.

23. Vandegraaff N, Engelman A (2007) Molecular mechanism of HIV integration and therapeutic intervention. Expert Rev Mol Med 9: 1–19.

22. Ciuffi A, Llano M, Poeschla E, Hoffmann C, Leipzig J, et al. (2005) A role for LEDGF/p75 in targeting HIV DNA integration. Nat Med 11: 1287–1293.

21. De Rijck J, Vandekerckhove L, Gijbers R, Hombrouck A, Hendrix J, et al. (2006) Overexpression of the lens epithelium-derived growth factor/p75 integrase binding domain inhibits human immunodeficiency virus replication. J Biol Chem 280: 11498–11509.

20. Llano M, Saenz DT, Meehan A, Wonghida P, Peretz M, et al. (2006) An essential role for LEDGF/p75 in HIV infection. Science 314: 461–464.

19. Hombrouck A, De Rijck J, Hendrix J, Vandekerckhove L, Voet A, et al. (2007) The interaction of LEDGF/p75 with integrase is lentivirus-specific and modulates their enzymatic activity in vitro. Nucleic Acids Res 35: 113–124.

18. Shun M-C, Raghavendra NK, Vandegraff N, Daigle JE, Hughes S, et al. (2007) LEDGF/p75 binds and tethers the c-Myc-interacting protein JPO2 to chromatin. J Cell Biol 177: 839–849.

17. Hombrouck A, De Rijck J, Hendrix J, Vandekerckhove L, Voet A, et al. (2007) The interaction of LEDGF/p75 with integrase is lentivirus-specific and modulates their enzymatic activity in vitro. Nucleic Acids Res 35: 113–124.

16. Llano M, Saenz DT, Meehan A, Wongthida P, Peretz M, et al. (2006) An essential role for LEDGF/p75 in HIV infection. Science 314: 461–464.

15. De Rijck J, Vandekerckhove L, Gijbers R, Hombrouck A, Hendrix J, et al. (2006) Overexpression of the lens epithelium-derived growth factor/p75 integrase binding domain inhibits human immunodeficiency virus replication. J Biol Chem 280: 11498–11509.

14. Ciuffi A, Llano M, Poeschla E, Hoffmann C, Leipzig J, et al. (2005) A role for LEDGF/p75 in targeting HIV DNA integration. Nat Med 11: 1287–1293.

13. Vandegraaff N, Engelman A (2006) Transcriptional corepressor p52 binds and tethered the e-Myc-interacting protein JPO2 to chromatin. J Cell Biol 177: 839–849.

12. Van Maele B, Busschots K, Vandekerckhove L, Christ F, Debyser Z (2006) Differential interaction of HIV-1 integrase and JPO2 with the C-terminal domain of LEDGF/p75. J Mol Biol 360: 760–773.

11. Ciuffi A, Bushman FD (2006) Retroviral DNA integration: HIV and the role of the HIV-1 integrase cofactor LEDGF/p75. Trends Biochem Sci 31: 98–105.

10. Sherman PA, Fyfe JA (1990) Human immunodeficiency virus integration protein expressed in Escherichia coli holds possesses selective DNA cleaving activity. Proc Natl Acad Sci USA 87: 5119–5123.

9. Katz RA, Merkel G, Kulkosky J, Les J, Slalka AM (1990) The avian retrovirus IN protein is both necessary and sufficient for integrative recombination in vivo. Cell 63: 87–95.

8. Sherman PA, Fyfe JA (1990) Human immunodeficiency virus integration protein expressed in Escherichia coli holds possesses selective DNA cleaving activity. Proc Natl Acad Sci USA 87: 5119–5123.

7. Ciuffi A, Bushman FD (2006) Retroviral DNA integration: HIV and the role of the HIV-1 integrase cofactor LEDGF/p75. Trends Biochem Sci 31: 98–105.

6. Van Maele B, Buschots K, Vandekerckhove L, Christ F, Debyser Z (2006) Cellular co-factors of HIV-1 integration. Trends Biochem Sci 31: 98–105.
63. Zielke SP, Stevenson M (2006) Modest but reproducible inhibition of human immunodeficiency virus type 1 infection in macrophages following LEDGF/p75 silencing. J Virol 80: 7275–7280.

64. Lewinski MK, Bugrove D, Shin P, Chen H, Hoffmann C, et al. (2003) Genome-wide analysis of chromosomal features repressing human immunodeficiency virus transcription. J Virol 79: 6610–6619.

65. Schroder ARW, Shin P, Chen H, Berry C, Ecker JR, et al. (2002) HIV-1 integration in the human genome favors active genes and local hotspots. Cell 110: 521–529.

66. MacNeil A, Sankale JL, Meloni ST, Sarr AD, Mboob S, et al. (2006) Genomic sites of human immunodeficiency virus type 2 (HIV-2) integration: Similarities to HIV-1 in vivo and possible differences in vivo. J Virol 80: 7316–7321.

67. Hematti P, Hong BK, Ferguson C, Ander R, Hanawa H, et al. (2004) Distinct genomic integration of MLV and SIV vectors in primate hematopoietic stem and progenitor cells. PLoS Biol 2: e423. doi:10.1371/journal.pbio.0020423.

68. Crise B, Li Y, Yuan C, Morroco DR, Whity D, et al. (2003) Simian immunodeficiency virus integration preference is similar to that of human immunodeficiency virus type 1. J Virol 79: 12199–12204.

69. Kang Y, Morelli CJ, Scherfa TE, Xie L, Tran DJ, et al. (2006) Integration site choice of a feline immunodeficiency virus vector. J Virol 80: 8120–8125.

70. Hacker CV, Vink GA, Wardell TW, Lee S, Treasure P, et al. (2006) The integration profile of ELAV-based vectors. Mol Ther 14: 536–545.

71. Wu X, Li Y, Crise B, Burgess SM (2003) Transcription start regions in the human genome are favored targets for MLV integration. Science 300: 1749–1751.

72. Nowrouzi A, Dittrich M, Klanke C, Heinkelein M, Rammling M, et al. (2006) Genome-wide mapping of foamy virus vector integrations into a human cell line. J Gen Virol 87: 1339–1347.

73. Trobridge GD, Miller DG, Jacobs MA, Allen JM, Kiern H-P, et al. (2006) Foamy virus vector integration sites in normal human cells. Proc Natl Acad Sci U S A 103: 1498–1503.

74. Mclntosh B, Beitel BF, Schroder ARW, Shin P, Chen H, et al. (2004) Retroviral DNA integration: ASLV, HIV, and MLV show distinct target site preferences. PLoS Biol 2: e234. doi:10.1371/journal.pbio.0020234.

75. Narezkina A, Taganov KD, Litwin S, Stoyanova R, Hayashi J, et al. (2004) Genome-wide analysis of avian sarcoma virus integration sites. J Virol 78: 11056–11063.

76. Derse D, Crise B, Li Y, Princler G, Lum N, et al. (2007) HTLV-1 integration target sites in the human genome: Comparison with other retroviruses. J Virol 81: 6731–6741.

77. Pandey KK, Saha S, Grandgenett DP (2007) Transcriptional coactivator LEDGF/p75 modulates human immunodeficiency virus type 1 integrase-mediated concerted integration. J Virol 81: 3969–3979.

78. Yu F, Jones GS, Hong M, Wagner AH, MacArthur HL, et al. (2007) HIV-1 integrase preassembled on donor DNA is refractory to activity stimulation by LEDGF/p75. Biochemistry 46: 2899–2908.

79. Schwartz O, Marclach V, Frigerat B, Arenzana-Seisdedos F, Heared JM (1998) Antiviral activity of the proteasome on incoming human immunodeficiency virus type 1. J Virol 72: 3845–3850.

80. Butler SL, Johnson EP, Bushman FD (2002) Human immunodeficiency virus cDNA metabolism: Notable stability of two-long terminal repeat circles. J Virol 76: 3739–3747.

81. Mulder LC, Nieuwen MA (2000) Degradation of HIV-1 integrase by the N-end rule pathway. J Biol Chem 275: 29749–29753.

82. Cateau S, Hoffmann C, Bushman F (1996) Chromosome structure and human immunodeficiency virus type 1 cDNA integration: Centromeric alphaoid repeats are a disfavored target. J Virol 72: 4005–4014.

83. Holman AG, Coffin JM (2005) Symmetrical base preferences surrounding HIV-1, avian sarcoma/leukosis virus, and murine leukemia virus integration sites. Proc Natl Acad Sci U S A 102: 6103–6107.

84. Wu X, Li Y, Crise B, Burgess SM, Munroe DJ (2005) Weak palindromic consensus sequences are a common feature found at the integration target sites of many retroviruses. J Virol 79: 5211–5213.

85. Wang GP, Ciuffi A, Leopaj J, Berry CC, Bushman FD (2007) HIV integration site selection: Analysis by massively parallel pyrosequencing reveals association with epigenetic modifications. Genome Res 17: 1186–1194.

86. Bugrove D, Lewinski M, Bushman F, Verlin F (2005) Molecular mechanisms of HIV-1 proviral latency. Expert Rev Anti Infect Ther 3: 805–814.

87. Ciuiff A, Diamond TL, Huang Y, Marshall HM, Bushman FD (2006) Modulating target site selection during human immunodeficiency virus DNA integration in vitro with an engineered tethering factor. Hum Gene Ther 17: 960–967.

88. Gonzalez-Ruiz D, Godlike H (2006) Targeting protein–protein interactions with small molecules: Challenges and perspectives for computational binding epitope detection and ligand finding. Curr Med Chem 13: 2607–2625.

89. Molteni V, Greenwood J, Rhodes D, Huang Y, Kwaikowski W, et al. (2001) Identification of a small-molecule binding site at the dimer interface of the HIV integrase catalytic domain. Acta Crystallogr D 57: 536–544.

90. Hayouka Z, Rosenbluh J, Levin A, Loya S, Leebelder M, et al. (2007) Inhibiting HIV-1 integrase by shifting its oligomerization equilibrium. Proc Natl Acad Sci U S A 104: 8316–8321.

91. Marshall HM, Ronen K, Berry C, Llanos M, Sutherland H, et al. (2007) Role of PSIP1/LEDGF/p75 in lentiviral infectivity and integration targeting. PLoS ONE 2: e1540. doi:10.1371/journal.pone.0001540.

92. Faure A, Calneds C, Desgbert C, Casonvijo M, Caunmow-Sarco C, et al. (2005) HIV-1 integrase crosslinked oligomers are active in vitro. Nucleic Acids Res 33: 977–986.

93. Guiot E, Carayon K, Deletis O, Simon F, Taar P, et al. (2006) Relationship between the oligomeric status of HIV-1 integrase on DNA and enzymatic activity. J Biol Chem 281: 22707–22719.

94. Li M, Mizutani C, Burke TRJ, Graig R (2006) Retroviral DNA integration: reaction pathway and critical intermediates. EMBO J 25: 1295–1304.

95. Fletcher III TM, Soares MA, McPherson S, Hui H, Wickeren M, et al. (1997) Complementation of integrase function in HIV-1 virions. EMBO J 16: 5123–5138.

96. Wu X, Liu H, Xiao H, Conway JA, Hunter E, et al. (1997) Functional RT and IN incorporated into HIV-1 particles independently of the Gag/Pol precursor protein. EMBO J 16: 5113–5122.

97. Katzman M, Katz RA (1999) Substrate recognition by retroviral integrases. Adv Virus Res 52: 371–393.

98. Raghavendra NK, Engelhan A (2007) LEDGF/p75 interferes with the formation of synaptic nucleoprotein complexes that catalyze full-site HIV-1 DNA integration in vitro: implications for the mechanism of viral cDNA integration. Virology 360: 1–5.

99. DeLano WL (2002) The PyMOL molecular graphics system. Available: http://www.pymol.org/. Accessed 7 December 2007.