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Molecular Mechanisms Of Poly [ADP-Ribose] Polymerase-1 In HIV-1 Infection

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ABSTRACT

Poly *(*ADP-ribose*)* polymerase-1 (PARP-1) is a cellular enzyme involved in genome stability and transcriptional regulation. The role of this protein in HIV-1 infection is largely controversial. Some reports indicated a fundamental role of PARP-1 in HIV-1 DNA integration and results from other laboratories do not support these conclusions. An important characteristic in all these experiments is that the HIV-1 target cells that were used express, in addition to PARP-1, the functional homologue PARP-2. We evaluated the role of PARP-1 in the chicken B lymphoblastoid cell line DT40. These cells naturally lack PARP-2 and support the early steps of HIV infection. We have observed that DT40 PARP-1 -/- cells was significantly more susceptible to infection with HIV- and murine leukemia virus (MLV)-derived viral vectors than their wild type counterpart. Expression of human PARP-1 in DT40 PARP-1 -/- cells decreased retroviral susceptibility to wild type levels, while expression of human PARP-2 has only a partial effect. Analysis of the retroviral life cycle by real time PCR indicated that levels of retroviral provirus were similar in DT40 wild type, PARP-1 -/-, and PARP-1 -/- cells expressing human PARP-1. These results, suggested that retroviral latency was established in cells expressing PARP-1. Treatment of HIV- or MLV-infected cells with the histone deacetylase inhibitor sodium butyrate or the DNA methyl transferase 1 inhibitor 5-azacytidine suppressed the differences in retroviral transgene expression observed in cells expressing or not PARP-1.

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Chapter 1

BACKGROUND AND SIGNIFICANCE

The HIV pandemic. Without a doubt, the Human Immunodeficiency Virus (HIV) pandemic is the most serious infectious disease challenge to public health today. According to the most recent AIDS epidemic updates (2010), 33.3 million people are living with HIV, whereas 2.7 million newly infection and 1.8 million deaths were reported just in this year. This trend in the infection determines that the daily rate of new infections worldwide surpasses 7,000 persons worldwide and the daily mortality rate is over 5,700 persons (15).

Though there has been significant progress in HIV research, treatment and prevention, HIV continued infection and mortality rates outpace that progress, and the disease remains at pandemic levels. Advances in antiretroviral drugs have significantly reduced in AIDS-related deaths, delayed the progression of the HIV infection to AIDS and diminished the rates of HIV transmission (15, 47). However, the current antiretroviral drugs do not target latent infection and therefore cannot eradicate HIV-1 infection. Therefore there is an important need of understanding the HIV latent infection to design drugs that specifically target this condition. This thesis focuses on the characterization of molecular mechanisms of HIV-1 latency.

HIV Life cycle. HIV is a retrovirus that is part of the *Lentivirus* genus, of the retroviridae family. HIV is able to enter the cell through a viral – cell membrane fusion mechanism. Fusion occurs through the interaction of viral glycoproteins that are present in the envelope and a receptor and co-receptors in the host cell membrane. The specific viral glycoproteins involved in this process are gp120 and gp41, and the cellular membrane receptors CD4 and coreceptors CCR5 or

CXCR4. Upon fusion, the virus uncoats and its single-stranded RNA genome is immediately reverse-transcribed into a double-stranded DNA copy. The viral DNA binds to host and viral proteins that are well define form the pre-integration complex. This complex is transported into the nucleus, where the viral DNA integrates into the host genome. Viral DNA integration is catalyzed by the viral enzyme integrase (IN), which performs two enzymatic activities essential for viral integration; 3' processing and DNA strand transfer of the DNA copy of the viral genome into the host genome. 3' processing of the linear viral genome occurs right after reverse transcription when a dinucleotide from each end of the HIV-1 genome end is cleaved off, leaving two-nucleotides long single-stranded regions on each end of the viral DNA (54,55). IN also catalyzes the transfer of the viral DNA strand into the host DNA. During viral DNA integration the host DNA is damaged and DNA repair is required for completion of integration (56). However, the repair pathway(s) is involved is not yet known. Only after viral DNA integration the viral genome is efficiently transcribed to produce new viral progeny. This determines that viral DNA integration is essential for retroviral infection. Transcription of the HIV genome is driven from the viral promoter located at the 5' long terminal repeat (LTR) (54).

Molecular mechanisms of HIV latency Long-term HIV-1 reservoirs are generated early during primary infection and have been estimated to be approximately 10^6 -10^7 cells (12) carrying an average of 3-4 proviruses per cell (28). This viral reservoir represents a major obstacle to the eradication of HIV-1 infection by the immune system or the most effective therapeutic interventions (14, 50). Although pre-integration and post-integration mechanisms of latency have been described, post-integration latency seems to be more relevant for persistent HIV-1 infection since the short-half life of non-integrated HIV-1 genomes (14). The main mechanisms maintaining HIV-1 latency ultimately regulates its transcription. Two main mechanisms for transcription to occur are for the chromatin microenvironment at the integration

site and the availability of transcription factors are important mechanisms (14, 48). The presence of strong host promoters at the integration site could lead to different types of transcriptional interference, whereas integration in transcriptionally repressed areas could lead to epigenetic modifications that suppress viral gene expression (48). These modifications include DNA CpG (Cytosine phosphate Guanine) methylation and specific histone post-translational modifications (14, 23, 24). The DNA methylation pattern is maintained during DNA replication by the activity of DNA methyltransferase 1. The process of DNA methylation occurs when the DNA base cytosine gets methylated and converted to a 5-Methylcytosine. Methylated cytosine at CpG islands are docking sites for methyl-binding protein that in turn tether chromatin remodeling complex (CS) that imprint modifications in the chromatin structure, thereby limiting the accessibility of the DNA to the transcriptional machinery. In addition to recruiting chromatin remodelers, DNA methylation can directly limit the access of the transcriptional machinery to the promoters (11, 44).

Molecular mechanisms of LEDGF/p75 in HIV infection LEDGF/p75 is a nuclear protein that belongs to the hepatoma-derived growth factor family, composed by other seven members (20, 40, 53). This protein is tightly bound to the chromatin, and this subcellular localization is essential for its cellular and virological roles. LEDGF/p75 is a cellular cofactor of HIV-1 DNA integration and therefore infection and also has a not very well characterized role in transcriptional regulation (41, 56). Nuclear localization of LEDGF/p75 is mediated by a nuclear localization signal during cellular interphase and during cell division through its chromatin binding domain (43, 44). This domain is composed by the PWWP domain and two AT hook motifs (43). All these regulatory regions are located in the N-terminal region of LEDGF/p75. In the C-terminal region of this protein is present the integrase binding domain that is involved in the interaction of LEDGF/p75 with different cellular proteins and with lentiviral integrases (7, 8,

14, 45). The ability of LEDGF/p75 to bind to chromatin during all the phases of the cell cycle and to interact with different proteins determines the chromatin tethering role of this protein. This role is central in both the HIV co-factor activity and the role in transcription of LEDGF/p75. In addition, other regions of the protein are involved in the HIV co-factor activity in a chromatin tethering-independent manner. These are two evolutionarily conserved regions in the N-terminus of LEDGF/p75, CR1 and a phosphorylated serine cluster in CR3. It has been postulated that this regions could serve as docking sites for other cellular proteins involved in HIV-1 DNA (23).

Role of PARP-1 in CpG DNA island methylation. Poly (ADP-ribose) polymerase (PARP)-1 is a nuclear enzyme that belongs to a family composed by seventeen members. These enzymes synthesize Poly (ADP) ribose (PAR) using Nicotinamide adenine dinucleotide (NAD^+) as a substrate. PARP-1 modular structure has three zinc fingers that form part of the DNA binding domain, a nuclear localization signal (NLS), an auto modification domain, and a binding domain for NAD^+ (catalytic site). The activity of DNA methyltransferase 1 is regulated by PARP-1. This enzyme catalyzes the synthesis and transfer of poly ADP ribose polymers (Parylation) to many different proteins in the cell including PARP-1 itself (at the automodification domain) (1, 32, 33). The level of parylated proteins is tightly regulated by the activity of poly (ADP-ribose) glycohydrolase (PARG) that degrades this polymer. Parylated PARP-1 has been demonstrated to bind DNA methyltransferase 1 inhibiting its activity (10), therefore PARP-1 negatively regulates DNA methylation at CpG islands.

Role of PARP-1 in HIV-1 infection. A role of PARP-1 in HIV DNA integration has been proposed, however this function is a matter of intense debate. Contradictory data have been

reported using either human- or mouse- derived primary or tumor cell lines in which functional PARP-1 has been depleted by siRNA expression, gene knockout, pharmacological inhibition, or the use of dominant mutants. In some of these experiments PARP-1 seems to be required for HIV-1 DNA integration, whereas in others it is reported to be dispensable or even to impair this viral process (3, 5, 17, 18, 21, 30, 51). However, an important caveat in all these experiments is that the HIV-1 target cells used express PARP-2 in addition to PARP-1. Due to the functional overlap between these enzymes (41), PARP-2 activities may mask a role of PARP-1 in HIV infection. The PARP-1 enzymatic activity is believed to be significantly augmented following DNA damage are cellular enzymes implicated in genome stability and transcriptional regulation (1, 2). These enzymes exhibit overlapping and specific functions (32-34). Functional redundancy between PARP-1 and -2 is illustrated by the fact that PARP-1 and PARP-2 double knockout mice exhibit early embryonic lethality (41). Although PARP-1 has the preponderant role, both enzymes participate in the detection, signaling, and repair of DNA strand breaks caused by genotoxic insults (20).

This thesis will focus on understanding the molecular mechanisms implicated in the novel activity of PARP-1 that we have discovered in our laboratory.

Significance. Advances in antiretroviral drugs have led to a significant reduction in AIDS-related deaths, delayed the progression of the HIV infection to AIDS and diminished the rates of HIV transmission (15, 47). However, the current antiretroviral drugs do not target latent infection and therefore can't eradicate HIV infection. Identification of the molecular mechanisms implicated in HIV latency, the focus of this thesis, is essential for the development of new drugs to eradicate or prevent the establishment of this viral reservoir. Long-term HIV-1 reservoirs are generated early during primary infection (28). This viral reservoir represents a major obstacle to eradication of HIV infection by the immune system or the most effective therapeutic

interventions (14, 50). Therefore understanding how latent infection is established will definitively impact our ability to eradicate HIV infection.

Specific aims.

This thesis focuses in understanding the role of PARP-1 in HIV infection. Our laboratory has identified PARP-1 as a LEDGF/p75-interacting protein in two independent proteomic analyses of LEDGF/p75. However, the implication of this interaction for HIV infection was unknown. Therefore, our research project focused in determining the role of PARP-1 in HIV infection. To uncover this role we have completed three different specific aims:

Specific aim 1. *To define the regions of LEDGF/p75 required for the interaction with PARP-1*. We evaluated a panel of LEDGF/p75 mutants by immunoprecipation to establish the regions interacting with PARP-1

Specific aim 2. *To determine the role of PARP-1 and PARP-2 in HIV and MLV infection*. In this specific aim we evaluated the susceptibility to retrovirus infection of cells lines lacking the expression of PARP-1 and/or PARP-2 or that were treated with a PARP pharmacological inhibitor. The step of the HIV viral life affected by PARP-1 was also determined.

Specific aim 3. *To establish the role of epigenetic mechanism in the effect of PARP-1 on retroviral infection.* Our results in specific aim 2 indicated that PARP-1 induced retroviral latency. Therefore, we determined the implication of DNA methyltransferase 1 and Histone Deacetylase in the viral activity of PARP-1 using pharmacological inhibitors of these enzymes.

Chapter 2

MATERIALS AND METHODS

Cell lines and Viruses. DT40-derived cell lines: DT40-derived cells were kindly provided by Shunichi Takeda (Crest Laboratory, Department of Radiation Genetics, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto, Japan). DT40 is a chicken B lymphocyte cell line (wild type cells, WT). KO cells are DT40 PARP-1 -/- cells. KO cells were engineered to express human PARP-1 (h-1) or human PARP-2 (h-2) (22) .

Human cells: Jurkat and Supt1 cells are $CD4^+$ T cell lines. HEK 293T are epithelial cells. LEDGF/p75-deficient cell line T_{L3} derives from the Supt1 cell line (37) and were engineered to expresses Flag-tagged LEDGF/p75 WT or mutants, or LEDGF/p52 (19).

Retroviral Vectors: MLV luc and HIV Trip luc (or eGFP) derived from murine leukemia virus (MLV) and HIV, respectively, are single-round infection viruses that express luciferase (or eGFP) from an internal CMV promoter (**Fig. 5a**), these vectors were used to challenge DT40-derived cells. Human-derived cell lines were infected with single-round infection viruses that express luciferase from the HIV LTR promoter (Hluc) (37). Production of these vectors has been previously described (19). Briefly, HEK 293T cells were transfected with a plasmid expressing the genome of the viral vector (pTrip luc or eGFP), the packaging plasmid pCMVΔR8.91, and the envelope plasmid pMD.G encoding the vesicular stomatitis virus glycoprotein G. Production of Hluc required transfection of HEK 293T cells with pHluc and the envelope plasmid pMD.G. For the production of MLV-derived vectors the packaging cell line Phoenix A was transfected with a plasmid expressing the genome of the viral vector (pMLV luc) and the envelope plasmid pMD.G. Seventy-two hours after transfection the viral supernatant was collected, filtered and concentrated by ultracentrifugation on a sucrose cushion. Different doses of each viral vector

were assayed in single-round infectivity assays to select a viral dose that produce relevant levels of the transgene expression without causing cellular toxicity.

Immunoblotting. Cellular lysates were resolved by SDS-PAGE and transferred overnight to PVDF membranes at 100 mAmp at 4°C. Membranes were blocked for one hour in TBS containing 10% milk and then incubated with the corresponding primary antibody that was diluted in TBS-5%, milk-0.05%, and Tween 20 (antibody dilution buffer). PARP-1: 2C10 (dilution 1/1000), PARylation: sc-56198, Santa Cruz (dilution 1/250), PARP-2: sc-133886, Santa Cruz (dilution 1/500), FLAG: M2, Sigma (Diluted 1/500), alpha tubulin: clone B-5-1-2, Sigma (dilution 1/4000). Membranes were incubated overnight at 4°C with the corresponding antibodies. Primary antibody-bound membranes were washed in TBS-0.1% Tween 20 three times for 5 minutes and bound antibodies were detected by chemoluminescence with specific secondary antibodies coupled to HRP.

DNase extraction of chromatin-bound proteins (S2 fraction). Previously described procedures (39) were followed with minor modifications. Figure 3 of reference (39) shows a validation experiment where multiple proteins located in different cellular compartments were evaluated. Briefly, $3x10^6$ T_{L3}-derived cells expressing different LEDGF/p75 WT and mutants, or LEDGF/p52 were washed in PBS and lysed for 15 mins on ice in 100ul of CSK I buffer (10mM Pipes pH6.8, 100mM NaCl, 1mM EDTA, 300mM sucrose, 1mM $MgCl_2$, 1mM DTT, 0.5% Triton X-100) containing protease inhibitors (final concentration: leupeptine 2ug/ml, aprotinin 5ug/ul, PMSF1mM, pepstatin A 1ug/ml). Cells lysed in CSKI buffer were centrifuged at 1000g for 6 mins at 4°C and the pellet resuspended in 100ul of CSK II buffer supplemented with protease inhibitors, 4 units of turbo DNase (Ambion) and 11ul of 10X turbo DNase reaction buffer. DNase treatment of this pellet was conducted at 37°C for 30 mins and then followed by extraction with

$(NH_4)_2SO_4$ 250mM for 15 mins at 37°C. The DNase/ $(NH_4)_2SO_4$ treated sample was centrifuged at 22,000g for 3 mins and the supernatant saved for analysis (chromatin-bound fraction, S2).

Immunoprecipitation. The interaction of the LEDGF/p75 with PARP-1 was evaluated by immunoprecipitation using the DNase extracted chromatin-bound proteins (S2 fraction). S2 fractions were clarified by centrifugation at 22,000g for 3 mins and the supernatant adsorbed with goat anti mouse Igs-coated magnetic beads (Pierce) for 30 mins at 4ºC. Goat anti mouse Igs-coated magnetic beads (100ul) were also incubated for 20 mins on ice with 3ug of anti-Flag Mab, or LEDGF/p75 (BD Transduction Laboratories), or anti-Myc MAb (negative control, clone 9E10, Covance) diluted in CSKI buffer. Then, the primary antibody-loaded beads were separated from the unbound antibodies, mixed with the preadsorbed S2 fraction and rotated for 3 hrs at 4°C. After this incubation, beads were washed three times in CSKI buffer and bound proteins eluted by boiling in 30ul of Laemmli sample buffer. Immunoprecipitated proteins were analyzed by immunoblotting with anti-PARP-1 or anti-LEDGF/p75 Mabs. To evaluate the strength of the interaction LEDGF/p75-PARP-1, the S2 fraction used in the LEDGF/p75-immunoprecipitation were supplemented with different concentrations of NaCl.

HIV-1 DNA integration analysis. Viral integration was quantified by detection of total HIV-1 cDNA (gag DNA, **Fig. 6a**) by real-time PCR in DT40-derived cells ten days after HIV infection. Three completely independent experiments using two different viral preparations were performed. In these experiments, 10^5 cells were infected with DNase-treated HIV Trip luc viral supernatant and cultured for ten days. Then DNA was extracted (High pure PCR template preparation kit, Roche) from 10^6 infected cells and 20ng of DNA were used for the detection of total HIV-1 cDNA, Histone 4 DNA and 2LTR circles All these real time PCRs were performed in

a MiniOpticon system (Bio-rad) with primers and conditions previously described (37). Fold change was calculated using the ΔCt method as recommended in the thermo-cycler manual and differences were expressed in percentage, considering the value for DT40 KO as 100%.

Single-round viral infectivity assay. Cells were plated at $1x10^5$ cells in 500µl of culture medium in 24-well plates and infected with different MOI's of the different HIV- or MLV-derived single-round infection viral vectors. Two, four, or ten days post-infection, cells were collected by centrifugation at 1000g for six minutes and the pellet lysed in 200µl of PBS-1% on ice for ten mins. 100ul of the cell lysate were mixed with 100µl of substrate (*Bright-Glow*™ Luciferase Assay System, Promega), incubated for 10 minutes in the dark and then the luciferase activity was quantified using a microplate luminometer. Simultaneously, cellular ATP levels were measured in these samples by mixing 100 ul of the cellular lysate with 100µl of substrate (*Bright-Glow*™ Cell Viability Assay System, Promega), incubating for 10 minutes in the dark and then the quantifying the luminescence levels with a microplate reader luminometer. eGFP expression in cells infected with HIV Trip eGFP were measured four or ten days after infection by FACS.

Pharmacological Treatments. Three days after retroviral infection, 10^5 cells were plated in 500 ul of culture medium in a 24-well plate and treated for 24 hrs with Sodium Butyrate (5uM) or 5-Azacytidine (60uM). Then, cells were used to determine luciferase and ATP levels as described above. 10^5 cells were plated in 500 ul of culture medium in a 24-well plate and treated for 24 hrs with 3,4-Dihydro-5[4-(1-piperindinyl)butoxy]-1(2H)-isoquinoline (DPQ), a PARP inhibitor, then cells were infected with different single-round replication retroviruses or used to determine the cellular levels of poly ADP ribose polymers by immunoblotting as described above. Infection was determined three days after infection by measuring the cellular levels of luciferase and ATP, as described above.

Chapter 3

RESULTS

Specific aim 1.

PARP-1 specifically interacts with LEDGF/p75. In two independent proteomic analyses carried out as collaboration with two other laboratories, PARP-1 was identified as a LEDGF/p75 interacting protein (Data not shown). To verify this interaction, chromatin-bound proteins were extracted using extensive DNase treatment [S2 fractions (39)] from $CD4^+$ T cells that express endogenous levels of LEDGF/p75 and PARP-1, and LEDGF/p75 was immunoprecipitated in the presence of DNase from this DNA-free extract. An anti-Myc antibody was used as negative control in these experiments. Then, the presence of PARP-1 in the inmunoprecipitated proteins was evaluated by immunoblotting with specific antibodies. Using this method we demonstrated the interaction of LEDGF/p75 with PARP-1 in the absence of DNA (**Fig**. **1**). During apoptosis PARP-1 is cleaved by the caspase-3,-6,-7 complex at a DEVD motif in the NLS. This cleavage produces two protein fragments p24 and p89 that contain the N-terminal DNA binding domain and the C-terminal catalytic region of this protein, respectively. LEDGF/p75 co-immunoprecipitated with the full-length (116 kDa) and the C-terminal (89 kDa) fragment of PARP-1 protein. These results suggested that the N-terminal region of PARP-1 is dispensable for the interaction with LEDGF/p75.

To map the LEDGF/p75 region interacting with PARP-1 $CD4^+$ T cells severely depleted of LEDGF/p75 (T_{L3} cells (37)) engineered to express different LEDGF/p75 mutants (19) or the LEDGF/p52 splicing product were evaluated by immunoprecipitation as described above. Using this method we determined that LEDGF/p75, but not p52, interacts with PARP-1 (**Fig. 2a**). Since these splicing products share the same N-terminal region but carry a complete different C-terminus, these data suggest that LEDGF/p75 interacts with PARP-1 through the C-terminal

region. Therefore, a panel of LEDGF/p75 mutants lacking different structural regions in the C-terminus was evaluated (**Fig. 2b**).

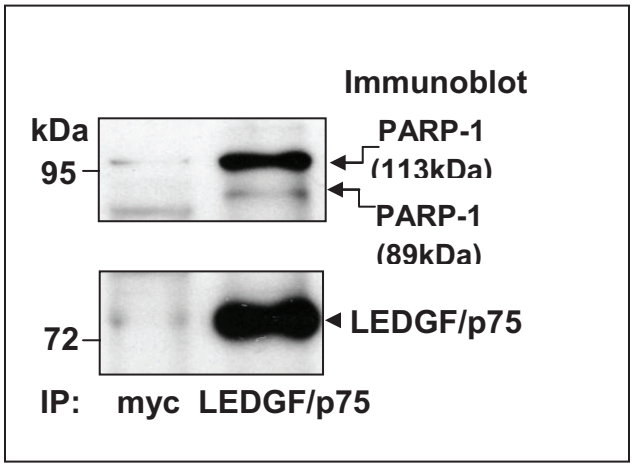


Figure 1. Interaction of LEDGF/p75 with PARP-1*. Chromatin-bound proteins were extracted from CD4$^+$T cells (Supt1 cell line) expressing endogenous levels of LEDGF/p75 and PARP-1 by extensive DNase treatment [S2 fraction (39)]. LEDGF/p75 was immunoprecipitated in the presence of DNase with a specific monoclonal antibody and immunoprecipitated proteins analyzed for the presence of PARP-1 and LEDGF/p75 by immunoblotting. An anti-Myc monoclonal antibody was used as negative control in the immunoprecipitation reaction. Full-length PARP-1 (113 kDa) and a caspase-generated proteolytic fragment corresponding to the C-terminal region (89 kDa) of the protein were specifically co-immunoprecipitated with LEDGF/p75.*

Importantly, only a mutant lacking the IN biding domain (IBD) was unable to interact with PARP-1, indicating that PARP-1 binds to this region of LEDGF/p75. We further determined whether other regions in the N-terminus of LEDGF/p75 were also implicated in the binding to PARP-1 (**Fig. 2a and b**). Interestingly, LEDGF/p75 mutants lacking the PWWP domain did not interact with PARP-1. These results then establish that interaction of LEDGF/p75 with PARP-1 require two independent protein regions of LEDGF/p75, mainly the IBD and PWWP domains. Since the PWWP domain is part of the chromatin-binding domain of LEDGF/p75 and PWWP mutants are defective in chromatin binding (39) we postulate that the defective PARP-1 interaction of mutants lacking the PWWP domain could be because the interaction of LEDGF/p75 with PARP-1 requires that LEDGF/p75 is tightly bound to the chromatin. Alternatively, the IBD and the PWWP domain could form a surface of PARP-1 interaction in the LEDGF/p75 folded protein. In support of this possibility our lab has reported that both PWWP and IBD contain residues targeted for post-translational modification by the small ubiquitous-like modifier (SUMO) (9), therefore these two protein domains could be in the folded protein closer than expected by their

location in the primary structure of the protein. In further support of the interaction between the N-terminus and the C-terminus of LEDGF/p75 it has been observed that antibodies binding to the N-terminus of denature LEDGF proteins (immunoblotting) can immunoprecipitate native LEDGF/p52 but not native LEDGF/p75 (36).

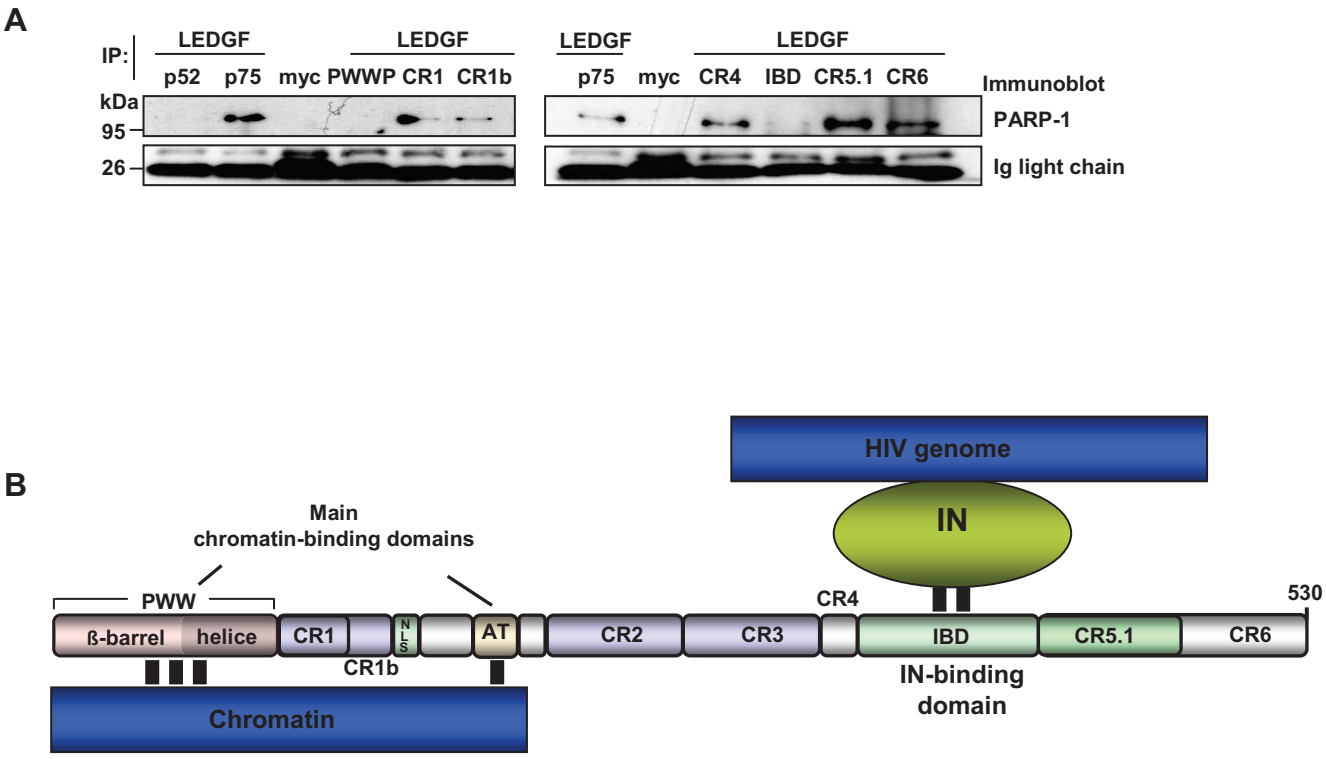


***Figure 2. LEDGF/p75 regions involved in the interaction with PARP-1**. **(a)** LEDGF/p75-deficient CD4$^+$ T cells (Supt1 cell line) engineered to express different C-terminally Flag-tagged LEDGF/p75 mutants or the LEDGF/p52 splicing variant were used in these experiments. Chromatin-bound proteins were extracted from these cells by DNase treatment (S2 fraction) and LEDGF/p75 was immunoprecipitated in the presence of DNase with anti-Flag monoclonal antibody. Immunoprecipitated proteins were analyzed for the presence of PARP-1 using a specific monoclonal antibody by immunoblotting. An anti-Myc monoclonal antibody was used as negative control in the immunoprecipitation reactions. LEDGF/p75 but not LEDGF/p52 interacts with PARP-1. LEDGF/p75 mutants lacking the IN binding domain (IBD) or the PWWP domain failed to interact with PARP-1. Detection of the Ig light chain was used to demonstrate that similar amount of antibodies were immunoprecipitated in each of the experiments. **(b)**. LEDGF/p75 protein regions. CRs represent charged regions in the LEDGF/p75 molecule that are also evolutionarily conserved.*

The interaction of LEDGF/p75 with HIV IN also requires the IBD (**Fig. 2b**). This interaction is very strong occurring even in the presence of 500 mM NaCl (4). To determine the strength of the interaction of LEDGF/p75 with PARP-1, immunoprecipitations were carried out with DNA-free chromatin-bound proteins in the presence of different NaCl concentrations. Similarly to LEDGF/p75-IN interaction the interaction of LEDGF/p75 was very strong (**Fig. 3**). These data also demonstrated the specificity of this protein interaction.

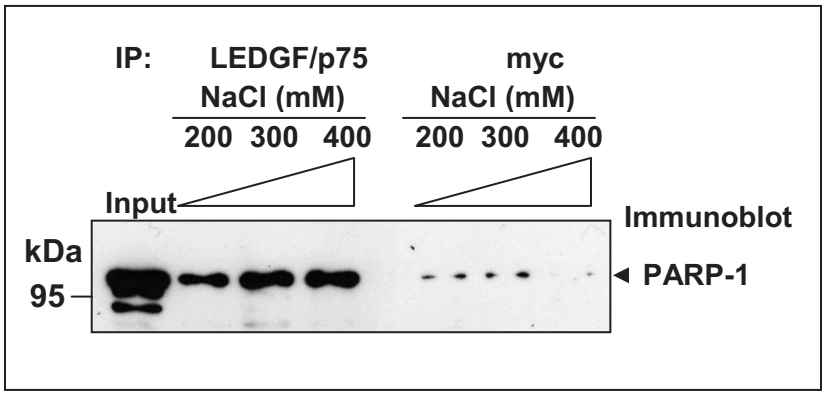


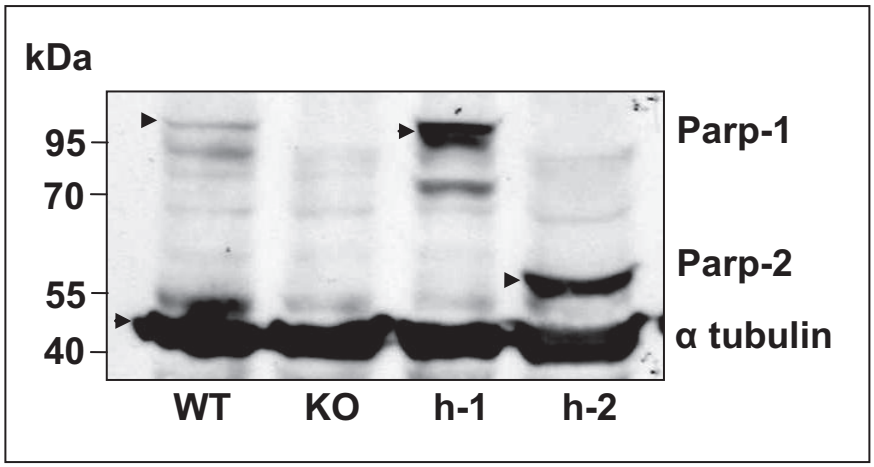
Figure 3. Effect of the ionic strength on the LEDGF/p75-PARP-1 interaction*. Chromatin-bound proteins were extracted from CD4$^+$ T cells (Supt1 cell line) expressing endogenous levels of LEDGF/p75 and PARP-1 by extensive DNase treatment (S2 fraction). LEDGF/p75 was immunoprecipitated in the presence of DNase and different concentrations of NaCl using a specific monoclonal antibody. Immunoprecipitated proteins were analyzed for the presence of PARP-1 by immunoblotting. An anti-Myc monoclonal antibody was used as negative control in the immunoprecipitation. LEDGF/p75 strongly interacts with PARP-1.*

Specific aim 2.

Role of PARP-1 and PARP-2 in retroviral infection.

As described previously, the exact role of PARP-1 and PARP-2 in HIV integration remains unclear. . Because the functional overlap between these enzymes (41), PARP-2 activities could mask a role of PARP-1 in HIV infection. In order to solve this limitation, we analyzed the function of PARP-1 in HIV infection in the chicken B lymphocyte cell line DT40. These cells naturally lack PARP-2 expression and are viable after PARP-1 knockout (22) (**Fig. 4**), in addition they are a reliable model to study the early events of the HIV life cycle, including uncoating, reverse transcription, DNA integration and HIV transgene expression (6, 42, 43).

Figure 4. Expression of PARP-1 and PARP-2 in DT40-derived cells*. Cells (0.6 x 10^6/lane) were lysed in Laemli buffer and analyzed by immunoblotting for the expression of PARP-1 and PARP-2. Alpha tubulin was detected as a loading control. h-1 cells express substantially higher amounts of PARP-1 as compared to WT cells. Arrow heads indicate the different proteins detected.*



DT40 PARP-1 +/+ (WT), DT40 PARP-1 -/- (KO), (22) were challenged with single-round infection HIV reporter viruses expressing eGFP or luciferase from an internal cytomegalovirus immediate-early (CMV) promoter [HIV Trip eGFP or Luc, respectively (37) (**Fig. 5a**)] and infection was evaluated two, four, and ten days later by measuring eGFP or luciferase expression in the challenge cells. As a control, luciferase levels were normalized to cellular ATP levels to exclude any contribution of a differential cell number or cellular viability in the challenged cells at the time of the evaluation of HIV infection. This control was included in **all** the experiments described in this thesis that involve determination of luciferase. Two days after infection, HIV-derived transgene expression was readily detectable in the challenged cells and no differences, in transgene expression were observed between the cell lines analyzed (Data not shown) At this early time point transgene expression from non-integrated and integrated viruses could be detected. Therefore to focus our analysis only on integrated viruses, infection was also evaluated four and ten days postinfection. Importantly, at these later time points, KO cells showed between 4-5 times more susceptibility to HIV infection than WT cells (**Fig. 5b and 5c**).

We extended our studies to KO cells engineered to express human PARP-1 (h1) or human PARP-2 (h2) and to the retrovirus murine leukemia virus (MLV), the MLV was used as a test to understand if PARP-1 and 2 are susceptible to HIV or other retroviruses. The MLV reporter virus used expresses luciferase from an internal CMV promoter (MLV luc, **Fig. 5a**) (37). WT, KO, h1, and h2 cells were challenged with HIV Trip luc or MLV luc and infection was evaluated four days after challenge by measuring luciferase. Data in **fig 5c i**ndicate that KO cells were between five (HIV) to 18 (MLV) fold more susceptible to retroviral infection than cells expressing chicken PARP-1 (WT cells). This increased susceptibility was completely reduced to wild type levels after reexpression of human PARP-1. However, the effect of human PARP-2 was only partial (**fig 5c**). These results clearly indicate that PARP-1, and to a lesser extent PARP-2, impairs HIV and MLV infection, suggesting a functional redundancy of these two human enzymes in retroviral infection. These results also indicated that the effect of PARP-1 on retroviral infection is unlikely to be dependent only on its interaction with LEDGF/p75 (**Fig. 1-3**) since this cellular cofactor does not interact with MLV IN (38) or influence MLV infection (37).

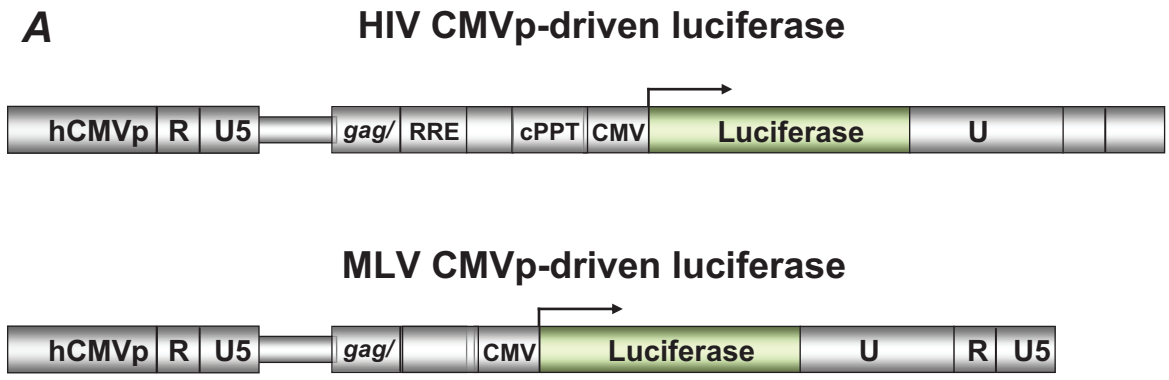


Figure 5A. Role of PARP-1 and PARP-2 in retroviral infection. (a) *Structure of the plasmids expressing HIV- and MLV-derived single-round infection viral vectors used in the experiments described in this thesis.*

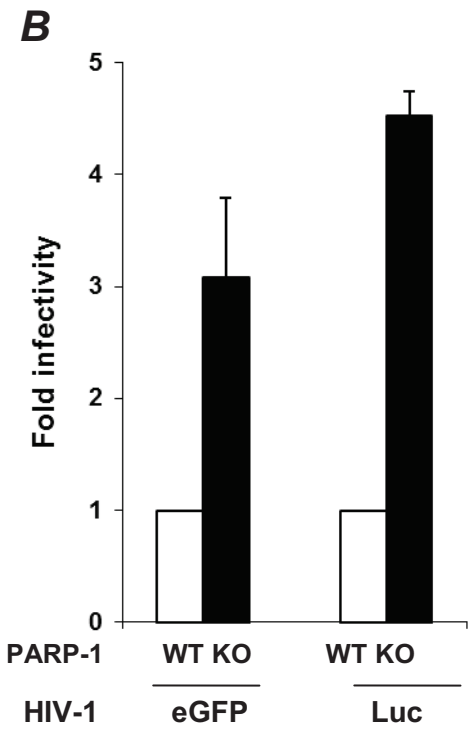


Figure 5B. Role of PARP-1 and PARP-2 in retroviral infection. (b) *Single-round infection experiments of DT40 wild type (WT) and PARP-1 -/- (KO) cells with HIV-derived viral vectors expressing eGFP or luciferase. Infectivity was determined ten days after challenge by measuring transgene expression. Transgene expression levels found in WT cells were used for comparison.*

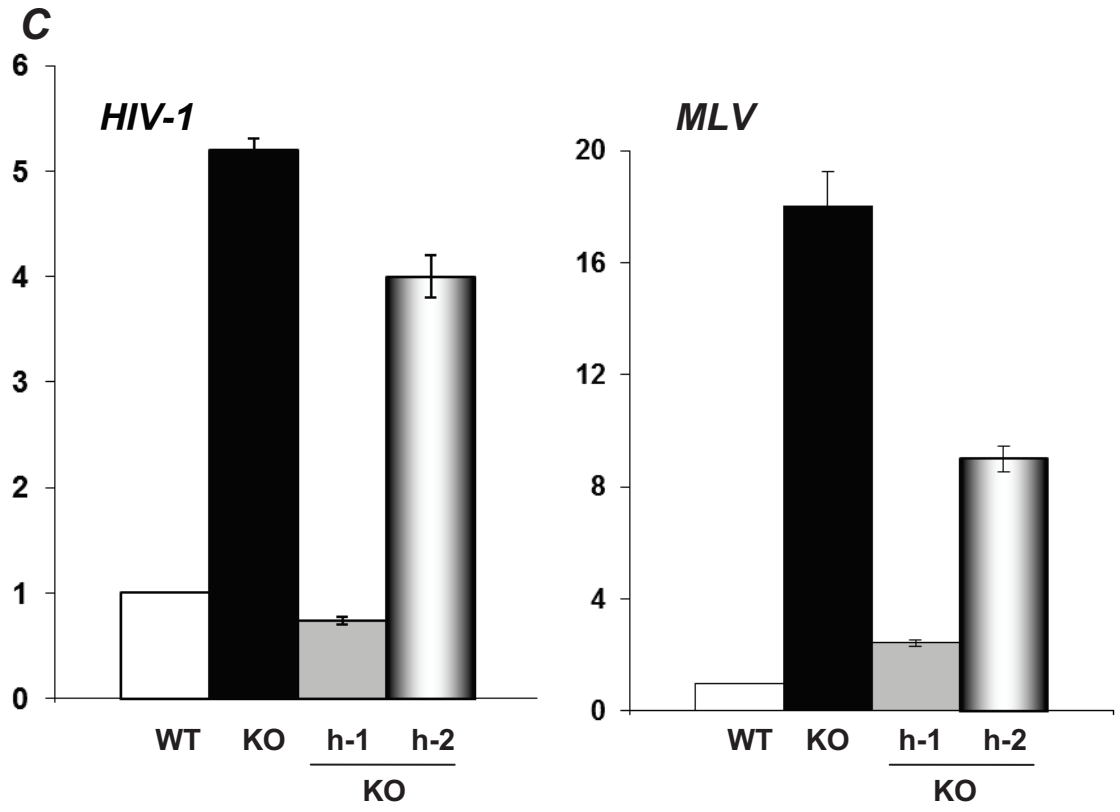


Figure 5C. Role of PARP-1 and PARP-2 in retroviral infection. (c) *Fold infectivity observed in single-round infection experiments of DT40 wild type (WT) and PARP-1 -/- (KO) cells and KO cells engineered to express human PARP-1 (h-1) or human PARP-2 (h-2) with HIV- or MLV-derived viral vectors expressing luciferase. Infectivity was determined four days after infection by measuring transgene expression. Luciferase activity is expressed relative to the levels found in WT cells. In the experiments represented in* ***Fig. 5 a-c****, ATP cellular levels were measured in parallel to luciferase measurements to normalize the luciferase activity to the cell number and viability of the infected cells. Standard deviations in the experiments included in* ***fig 5*** *represent triplicate measurements of transgene expression. Results in* ***fig. 5*** *are representative of more than five independent experiments performed with different viral preps.*

Effect of PARP-1 on the HIV life cycle. The fact that the differences in susceptibility to HIV infection between cells expressing or not PARP-1 were not observed after forty-eight hours of infection suggests that the effect of PARP-1 on retroviral infection occurs at or after viral DNA integration. In order to evaluate the step of the viral life cycle affected by PARP-1 we measured by real time PCR the levels of different forms of the HIV genome. Following entry the single-stranded RNA genome is reverse transcribed to a DNA copy. This DNA copy associates with viral and cellular proteins, forming the pre-integration complex that is imported into the nucleus where the viral DNA integrates into the host genome. The nuclear viral DNA that fails to integrate is then circularized or degraded. Eventually circular forms are also degraded or diluted during cell division. Therefore, after several days postinfection only the integrated viral DNA persists. To measure the viral genome by quantitative real time PCR we used a set of primers that hybridize on the gag DNA in the HIV genome (**Fig**. **6a**). Because this DNA fragment is present in both the integrated and the non-integrated forms of the viral genome, we will determine the gag DNA levels ten days after infection to favor detection of integrated viral DNA. In addition, 2LTR circles, the predominant non-integrated form of the viral genome, were also measured (**Fig. 6b**). To calculate the integrated fraction of gag DNA we normalized the levels of gag DNA to the levels of 2LTR circles that were previously normalized for the levels of histone 4

DNA, a loading control. Using this strategy, we determined that the levels of integrated HIV DNA were slightly higher in cells expressing PARP-1 than in knockout cells (**Fig. 6b**). These data indicated that PARP-1 impairs HIV infection at a postintegration step.

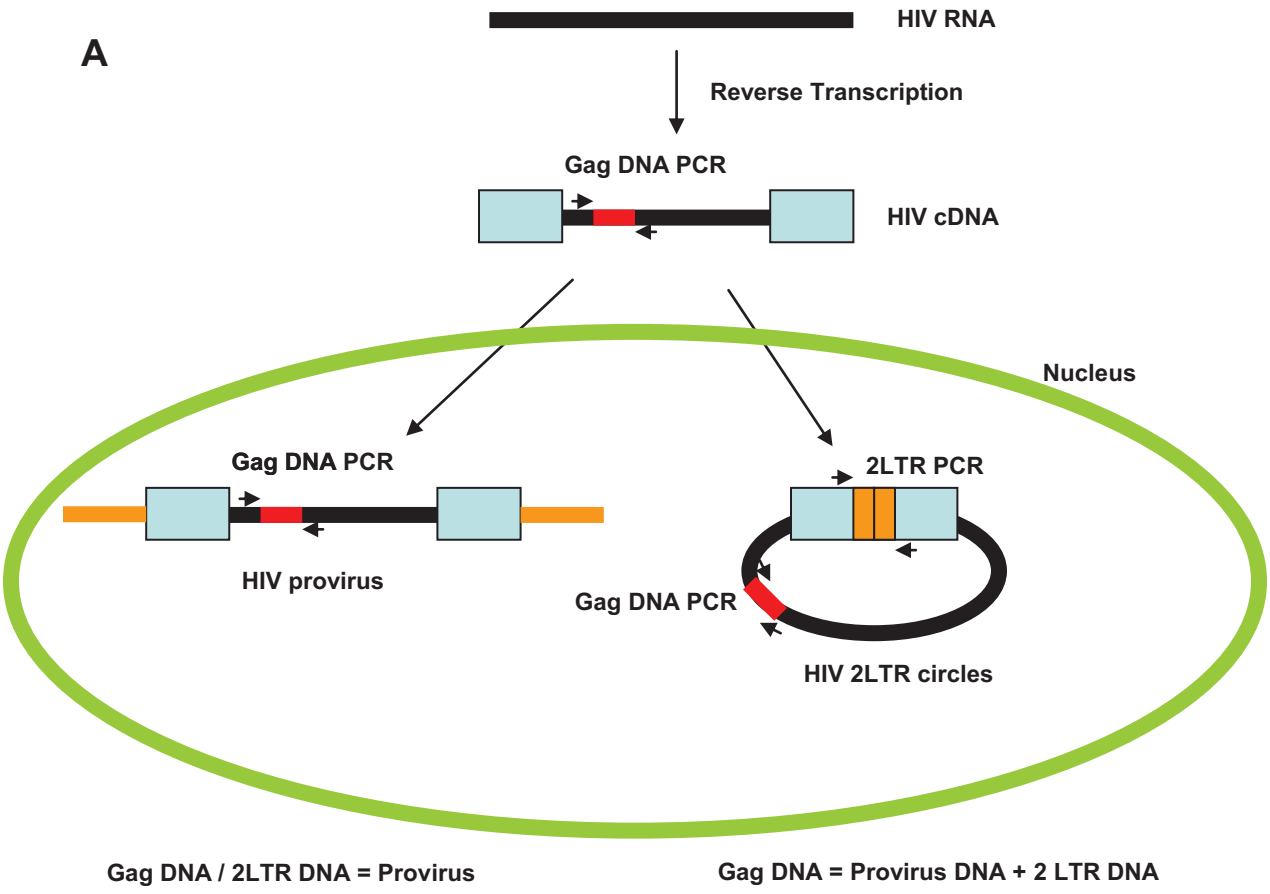


Figure 6A. Levels of HIV proviral DNA in cells differing in PARP-1 expression (a) *Real time PCR strategy used to calculate HIV provirus levels. Notice that the gag DNA PCR used can detect integrated and non-integrated forms of the viral genome. The extranuclear linear viral genome is very transient with a half-life of less than 24 hrs, the 2LTR circles can persist longer (48 -72 hrs) whereas the integrated viral genome is permanent.*

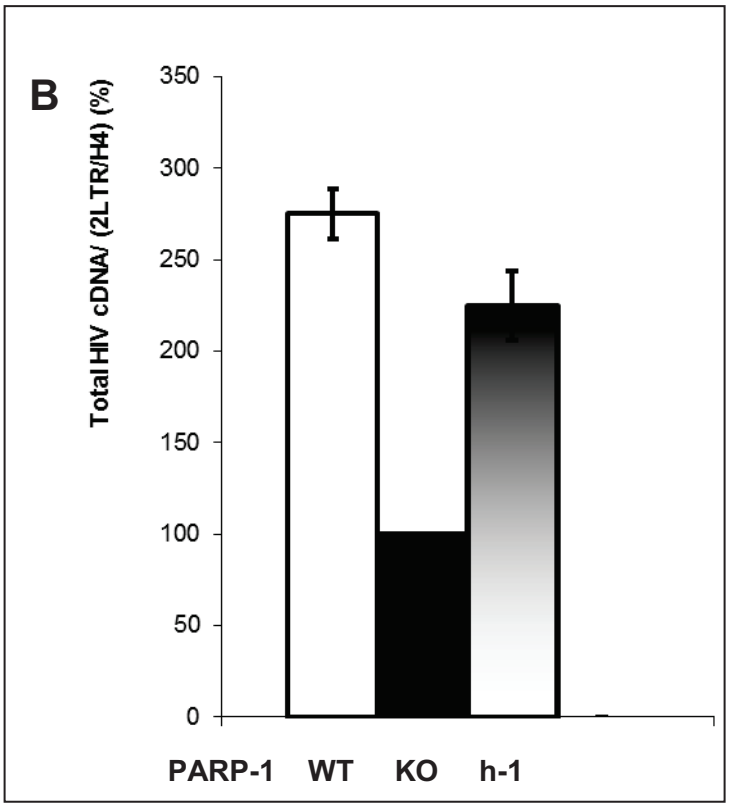


Figure 6B. Levels of HIV proviral DNA in cells differing in PARP-1 expression (b). *Quantification of the HIV provirus levels. DT40 wild type (**WT**), PARP-1-/- (**KO**), and PARP-1-/- expressing human PARP-1 (**h-1**) cells were infected with HIV Trip luc and ten days later the levels of total HIV cDNA, 2LTR circles, and histone 4 were quantified by real-time PCR. Total HIV cDNA levels were normalized to histone 4-normalized 2LTRs levels. Standard deviations were calculated using triplicate PCR experiments. The data are representative of three independent infections.*

Effect of pharmacological inhibition of PARP-1 on HIV infection. PARP-1 catalyzes the synthesis of poly ADP ribose from NAD^+, in addition, other non-enzymatic activities have been described in this enzyme (20, 45). To determine whether or not the enzymatic activity of PARP-1 mediates the effects of this protein on retroviral infection, we treated the cells with 3,4-Dihydro-5[4-(1-piperindinyl)butoxy]-1(2H)-isoquinoline (DPQ), a PARP inhibitor, for 24 hrs before viral challenge. DPQ was kept in the culture medium during infection and until infectivity was analyzed. Using this approach we studied Jurkat and 293T cells, as well as the DT40-

derived cells WT, KO, and h-1. In all these experiments we observe no differences between the treated and the non-treated cells (**Fig. 7a**).

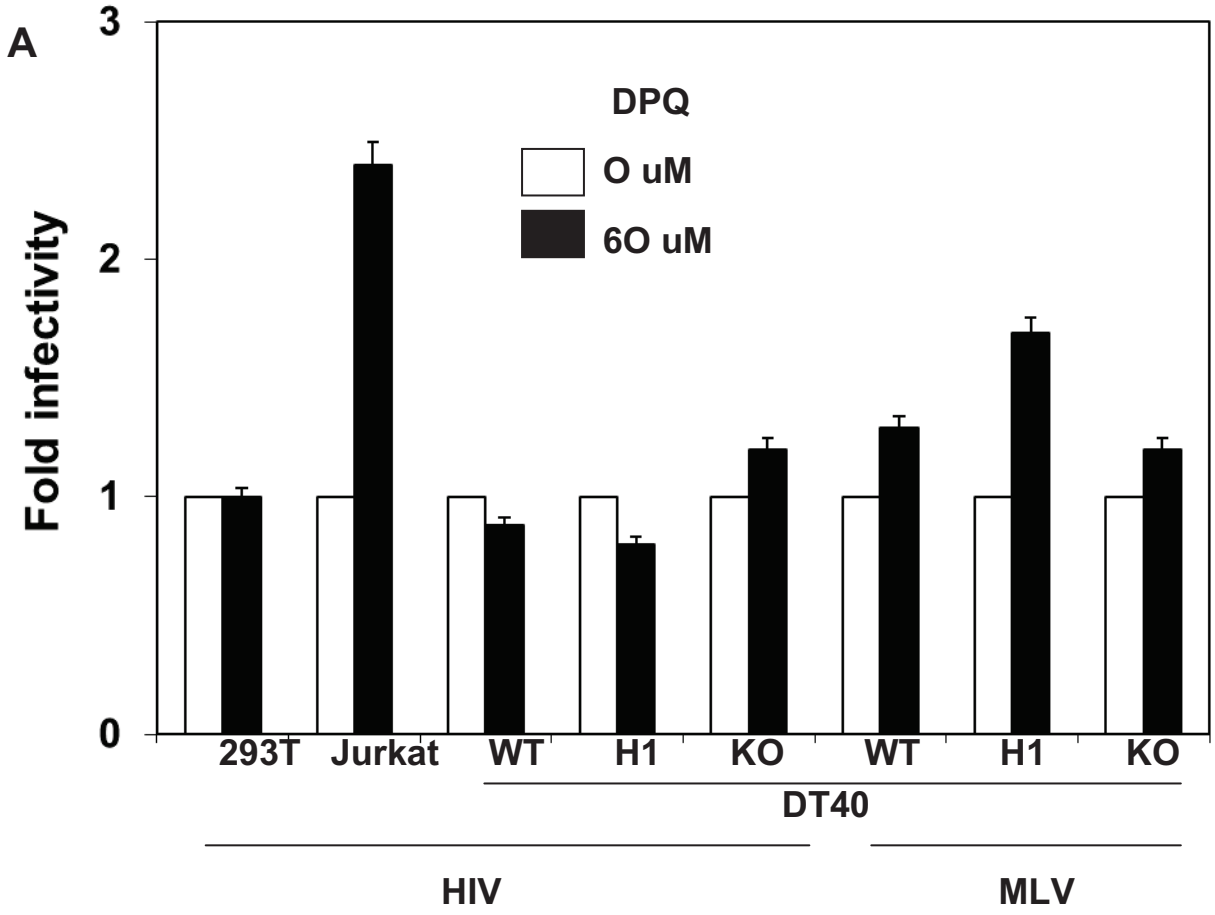


***Figure 7A**. **Effect of pharmacological inhibition of PARP-1 on its viral activity. (a)** DT40 WT, KO, and h-1 cells were treated or not with DPQ (60 uM) for 24 hrs and then infected with HIV- or MLV-derived viral vectors. 72 hrs after infection the luciferase levels were measured in the treated and not treated cells. Luciferase levels were normalized to cellular ATP levels measured in the same samples.*

The studied cells were treated or not with DPQ for 24 hrs and the basal levels of poly ADP ribose were measure by immunoblotting with an specific antibody (**Fig. 7b**). Under these conditions only a partial reduction of PARP activity was reached at the highest dose of DPQ. Cellular cofactors interacting with the incoming HIV virions are required at extremely low levels (37); therefore the partial PARP-1 activity present in the DPQ-treated cells could be sufficient for affecting HIV infection. Therefore, we can not firmly exclude a role of the enzymatic activity of PARP-1 on its viral activity.

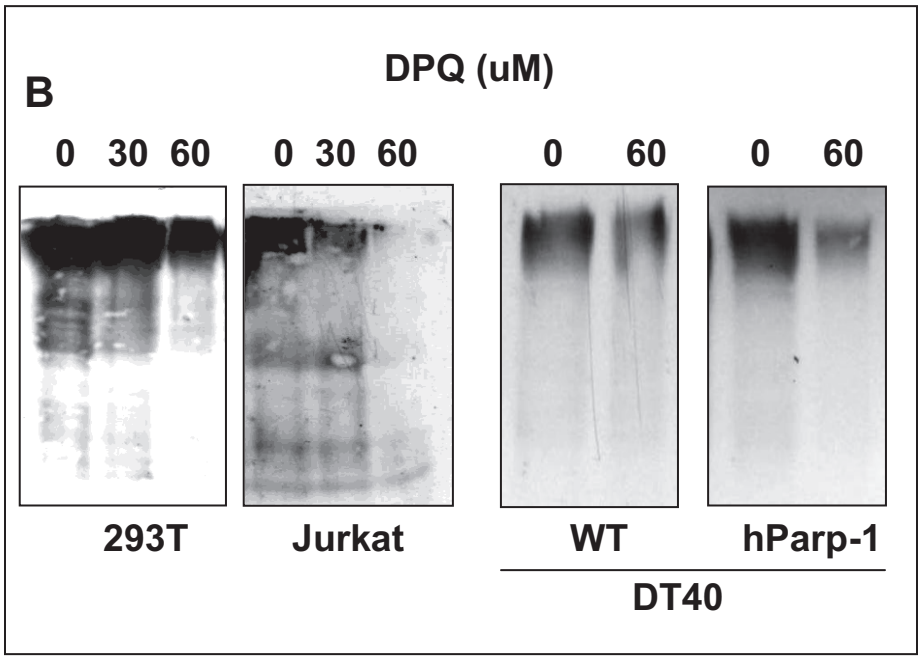


Figure 7. Effect of pharmacological inhibition of PARP-1 on its effect on its viral activity. (b) *Effect of DPQ pretreatment on basal levels of cellular poly ADP ribose. Cells were treated or not with DPQ (60 uM) for 24 hrs and then lysed for immunoblotting analysis of cellular poly ADP ribose levels.*

Because DPQ is unstable, we performed an additional experiment (**Fig. 7c**) in which we pretreated the cells before infection and then we added fresh DPQ to the cells every 24 hrs until infectivity was measured 72 hrs after infection (intensive DPQ treatment). As described above, ATP levels in the treated and un-treated cells were determined and luciferase activity was normalized to that. In this experiment we observed that DPQ treatment increased by 4 fold the susceptibility of Jurkat cells to HIV infection (**Fig. 7c**). These results suggest that inhibition of PARP-1 activity could increase the susceptibility of human cells to HIV infection, additionally

supporting our previous observations in chicken cells. However, this experiment was done only once and therefore needs to be considered as a preliminary observation. We will repeat this experiment with a panel of human $CD4^+$ T cells and a battery of PARP inhibitors.

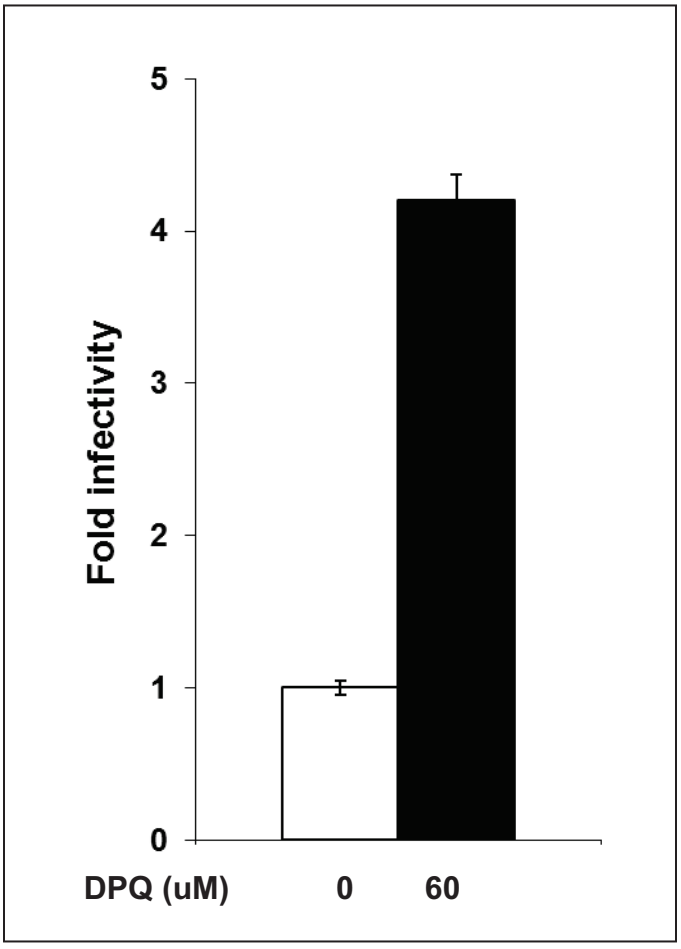


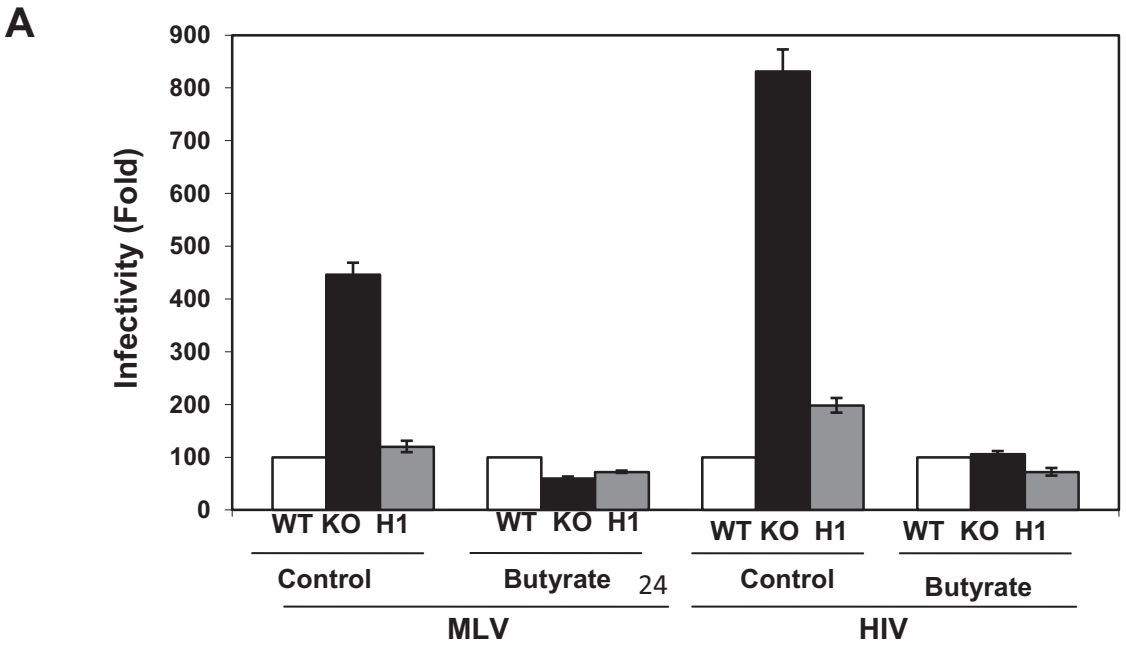
Figure 7. Effect of pharmacological inhibition of PARP-1 on its viral activity. ***(c)*** *Cells were treated as described in panel* ***a*** *but fresh DPQ was added to the culture every 24 hrs until infectivity was determine 72 hrs after viral challenged. Standard deviations represent triplicate measurement of luciferase levels. Data in panel (****a and b****) are representative of two independent experiments, whereas data in panel* ***c*** *represent only one experiment.*

Specific aim 3.

Effect of PARP-1 on HIV silencing. PARP-1 affects HIV transgene expression without affecting proviral formation, a hallmark of HIV latency. The most common cause of HIV latency is transcriptional silencing of the integrated provirus by epigenetic mechanisms (8, 14, 27). Among the different epigenetic mechanisms that regulate gene expression, CpG island DNA methylation (8, 14) and histone deacetylation (27) are considered the leading causes of HIV latency. In order to evaluate the involvement of these mechanisms in the effect of PARP-1 on

retroviral infection, cells infected with HIV Trip luc or MLV luc were treated for 24 hours with sodium butyrate, a histone deacetylase (HDAC) inhibitor, or 5-azacytidine, a DNA methyltransferase 1 inhibitor, and the levels of luciferase and ATP were measured. Importantly, pharmacological inhibition of HDAC dramatically eliminated the differences in transgene expression in HIV- or MLV-infected cells that express or not PARP-1 (**Fig. 8a**), indicating a role of HDAC in PARP-1-induced retroviral silencing. Since HDAC are recruited to methylated DNA, we also investigated the role of DNA methyltransferase 1 in the PARP-1 viral activity. Interestingly, although treatment with 5-azacytidine removed the differences of transgene expression between cells expressing or not PARP-1 that has been infected with HIV or MLV (**Fig. 8b and c**), the intensity of this effect was variable among different experiments. In addition, the effect of 5-azacytidine was stronger in PARP-1 -/- (KO) cells expressing human PARP-1 (h-1 cells) than in the DT40 wild type cells. A possible explanation for this differential effect in WT and h-1 cells is the dissimilar levels of PARP-1 expressed by these cells, h-1 expresses substantially higher levels of PARP-1 than WT cells (**Fig. 4**).

In summary, these data indicated that PARP-1 induces retroviral silencing by epigenetic mechanisms that involve the activity of HDAC and DNA methyltransferase 1.



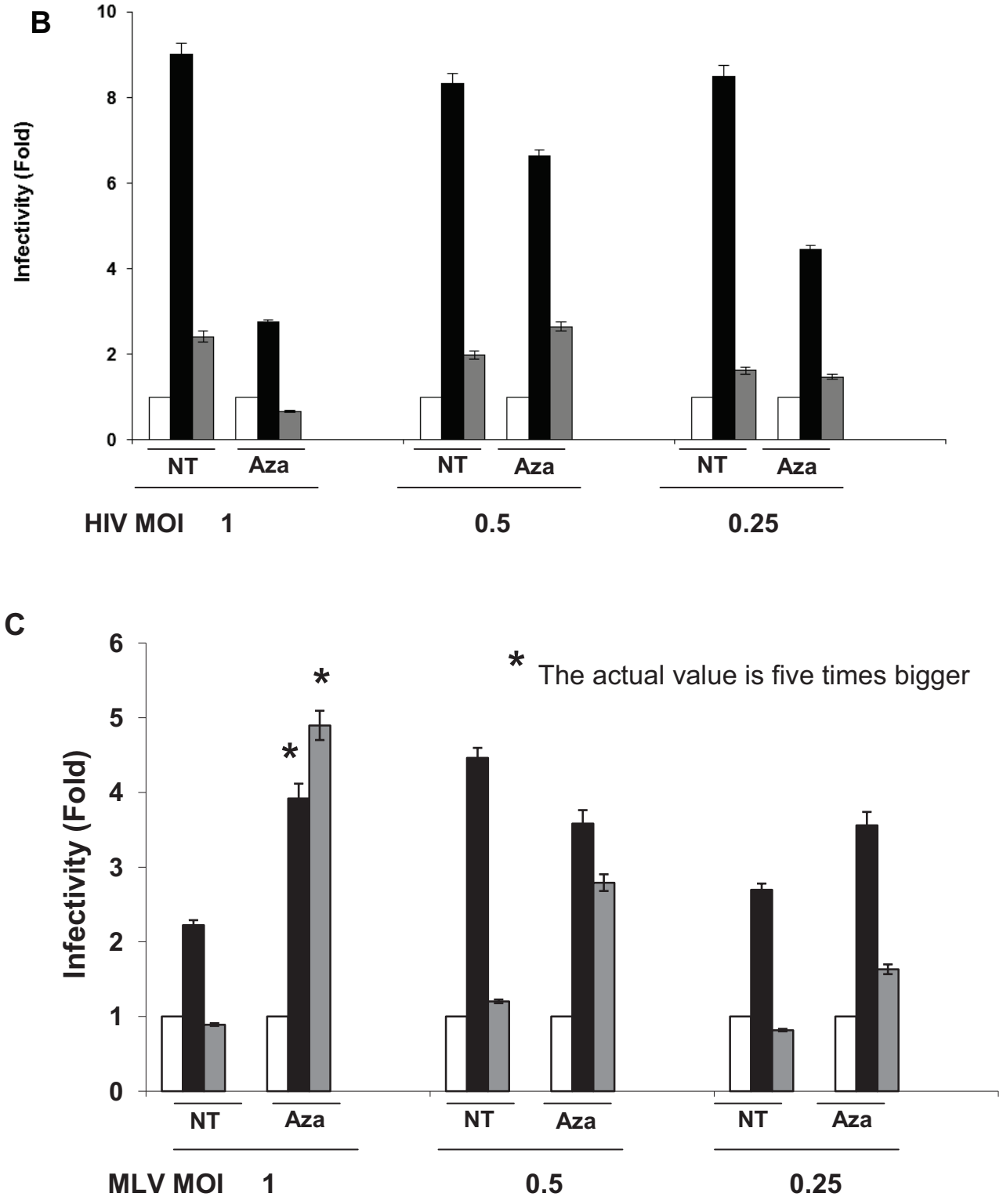


Figure 8. Effect of pharmacological inhibition of Histone Deacetylase (a) and DNA methyltransferase 1 (b and c) on the effect of PARP-1 on retroviral infection*. DT40 WT, KO, and h-1*

*cells were infected with different amounts of HIV- or MLV-derived viral vector and three days after infection the cells were treated or not with sodium butyrate (5uM) or 5-azacytidine (60uM) for 24 hrs and the luciferase levels measured in the treated and not treated cells. Luciferase levels were normalized to cellular ATP levels measured in the same samples. Standard deviations represent triplicate measurement of luciferase levels. Data in panel (**a**) are representative of three independent experiments; data in panel (**b and c**) represent only one experiment.*

Chapter 4

DISCUSSION

Our data indicate a role of PARP-1 in retroviral latent infection. In the presence of PARP-1 a significant fraction of the retroviral proviruses result silenced by epigenetic mechanisms that involve the activity of DNA methyl transferase 1 and HDACs. However, other proviruses escape this negative regulation. Because PARP-1 is very abundant in the cell, its cellular levels are unlikely to limit the effect of this enzyme on retroviral infection. These data suggest the existence of other cellular factor(s) that opposes to the silencing effect of PARP-1.

How PARP-1 triggers retroviral silencing is intriguing. Our data clearly indicate that the activity of DNA methyl transferase 1 and HDACs mediates the PARP-1 effect. DNA methyl transferase 1 methylates the DNA attracting DNA methyl-binding proteins that in turn recruits HDACs. HDACs remove acetyl groups from the tail of histones condensing the chromatin and as a consequence repressing transcription (7, 23, 25, 35, 46). However, it has also been shown that PARP-1 reduces DNA methylation by decreasing the activity of DNA methyltransferase 1, an effect that requires the enzymatic activity of PARP-1 (10, 53). Therefore, it is surprising that PARP-1 will directly induce retroviral silencing through a mechanism that involves DNA methyl transferase 1 activity. A possibility is that PARP-1 induces retroviral silencing by an indirect mechanism. For example, PARP-1 could promote the integration of retroviruses into heterochromatin and as a result the epigenetic marks of the neighbor chromatin could be extended to the proviruses. In this regards it has been reported a role of PARP-1 in promoting HIV-1 integration in centromeric regions (30). It is well established that retroviral integration into heterochromatin results in retroviral silencing (27, 31).

PARP-1 could promote retroviral DNA integration into heterochromatin by impairing the activity of cellular factors driving retroviral DNA integration into euchromatin. PARP-1 has been

reported to enzymatically modify over seventy different cellular proteins (26, 52) including, histones, chromatin remodeling proteins, transcription factors, and DNA repair proteins. In this regard, we have demonstrated the interaction of PARP-1 with LEDGF/p75 (**Fig**. **1-3**), a cellular factor that promotes HIV DNA integration into actively transcribed chromatin (13, 40). It is possible that PARP-1 could impair the HIV cofactor activity of a fraction of the LEDGF/p75 pool, therefore causing a reduction of integration into actively transcribed chromatin. In this case, the HIV preintegration complex would preferentially interact with cellular cofactors that drive HIV DNA integration into heterochromatin instead of with LEDGF/p75 that promotes integration into euchromatin. In support of this possibility it has been demonstrated at the genomic level that LEDGF/p75 is not amenable to HIV DNA integration at every location of the genome where this protein is present (16). Similarly, PARP-1 could modify the activity of cellular factor(s) involved in integration of MLV into euchromatin.

Conclusions.

In conclusion, we identified a novel role of PARP-1 in retroviral infection. Our data clearly indicate that PARP-1 promotes viral latency by epigenetic mechanisms that involve the activity of DNA methyltransferase 1 and Histone Deacetylases. We identified that the PWWP and the IN binding domain of LEDGF/p75 and the C-terminal region of PARP-1 are required for the LEDGF/p75-PARP-1 interaction. We also determined that PARP-1 and PARP-2 have an overlapping role in promoting retroviral latent infection. However, PARP-1 is more active than PARP-2 in this novel function. The implication of the enzymatic activity of PARP-1 or -2 in this function remains uncertain. Finally we established that PARP-1 induces retroviral latency by a mechanism that involves the enzymatic activity of DNA methyltransferase 1 and Histone Deacetylases.

BIBLIOGRAPHY

1. **Aguilar-Quesada, R., J. A. Munoz-Gamez, D. Martin-Oliva, A. Peralta-Leal, R. Quiles-Perez, J. M. Rodriguez-Vargas, M. Ruiz de Almodovar, C. Conde, A. Ruiz-Extremera, and F. J. Oliver.** 2007. Modulation of transcription by PARP-1: consequences in carcinogenesis and inflammation. Curr Med Chem **14:**1179-87.
2. **Aguilar-Quesada, R., J. A. Munoz-Gamez, D. Martin-Oliva, A. Peralta, M. T. Valenzuela, R. Matinez-Romero, R. Quiles-Perez, J. Menissier-de Murcia, G. de Murcia, M. Ruiz de Almodovar, and F. J. Oliver.** 2007. Interaction between ATM and PARP-1 in response to DNA damage and sensitization of ATM deficient cells through PARP inhibition. BMC Mol Biol **8:**29.
3. **Ariumi, Y., P. Turelli, M. Masutani, and D. Trono.** 2005. DNA damage sensors ATM, ATR, DNA-PKcs, and PARP-1 are dispensable for human immunodeficiency virus type 1 integration. J Virol **79:**2973-8.
4. **Astiazaran, P., M. T. Bueno, E. Morales, J. R. Kugelman, J. A. Garcia-Rivera, and M. Llano.** 2011
HIV-1 IN modulates the interaction of the HIV-1 cellular cofactor LEDGF/p75 with chromatin. Retrovirology **8:**27.
5. **Baekelandt, V., A. Claeys, P. Cherepanov, E. De Clercq, B. De Strooper, B. Nuttin, and Z. Debyser.** 2000. DNA-Dependent protein kinase is not required for efficient lentivirus integration. J Virol **74:**11278-85.
6. **Barr, S. D., J. Leipzig, P. Shinn, J. R. Ecker, and F. D. Bushman.** 2005. Integration targeting by avian sarcoma-leukosis virus and human immunodeficiency virus in the chicken genome. J Virol **79:**12035-44.
7. **Berger, S. L.** 2007. The complex language of chromatin regulation during transcription. Nature **447:**407-12.
8. **Blazkova, J., K. Trejbalova, F. Gondois-Rey, P. Halfon, P. Philibert, A. Guiguen, E. Verdin, D. Olive, C. Van Lint, J. Hejnar, and I. Hirsch.** 2009. CpG methylation controls reactivation of HIV from latency. PLoS Pathog **5:**e1000554.
9. **Bueno, M. T., J. A. Garcia-Rivera, J. R. Kugelman, E. Morales, G. Rosas-Acosta, and M. Llano.** 2010
SUMOylation of the lens epithelium-derived growth factor/p75 attenuates its transcriptional activity on the heat shock protein 27 promoter. J Mol Biol **399:**221-39.
10. **Caiafa, P., T. Guastafierro, and M. Zampieri.** 2009. Epigenetics: poly(ADP-ribosyl)ation of PARP-1 regulates genomic methylation patterns. Faseb J **23:**672-8.
11. **Cheng, X., and R. M. Blumenthal.** Coordinated chromatin control: structural and functional linkage of DNA and histone methylation. Biochemistry **49:**2999-3008.
12. **Chun, T. W., L. Carruth, D. Finzi, X. Shen, J. A. DiGiuseppe, H. Taylor, M. Hermankova, K. Chadwick, J. Margolick, T. C. Quinn, Y. H. Kuo, R. Brookmeyer, M. A. Zeiger, P. Barditch-Crovo, and R. F. Siliciano.** 1997. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. Nature **387:**183-8.
13. **Ciuffi, A., M. Llano, E. Poeschla, C. Hoffmann, J. Leipzig, P. Shinn, J. R. Ecker, and F. Bushman.** 2005. A role for LEDGF/p75 in targeting HIV DNA integration. Nat Med **11:**1287-9.
14. **Coiras, M., M. R. Lopez-Huertas, M. Perez-Olmeda, and J. Alcami.** 2009. Understanding HIV-1 latency provides clues for the eradication of long-term reservoirs. Nat Rev Microbiol **7:**798-812.
15. **De Clercq, E.** 2009. The next ten stories on antiviral drug discovery (part E): Advents, Advances, and Adventures. Med Res Rev.

16. **De Rijck, J., K. Bartholomeeusen, H. Ceulemans, Z. Debyser, and R. Gijsbers.** 2010. High-resolution profiling of the LEDGF/p75 chromatin interaction in the ENCODE region. Nucleic Acids Res.
17. **Farzaneh, F., G. N. Panayotou, L. D. Bowler, B. D. Hardas, T. Broom, C. Walther, and S. Shall.** 1988. ADP-ribosylation is involved in the integration of foreign DNA into the mammalian cell genome. Nucleic Acids Res **16:**11319-26.
18. **Gaken, J. A., M. Tavassoli, S. U. Gan, S. Vallian, I. Giddings, D. C. Darling, J. Galea-Lauri, M. G. Thomas, H. Abedi, V. Schreiber, J. Menissier-de Murcia, M. K. Collins, S. Shall, and F. Farzaneh.** 1996. Efficient retroviral infection of mammalian cells is blocked by inhibition of poly(ADP-ribose) polymerase activity. J Virol **70:**3992-4000.
19. **Garcia-Rivera, J. A., M. T. Bueno, E. Morales, J. R. Kugelman, D. F. Rodriguez, and M. Llano.** 2010. Implication of serine residues 271, 273, and 275 in the human immunodeficiency virus type 1 cofactor activity of lens epithelium-derived growth factor/p75. J Virol **84:**740-52.
20. **Godon, C., F. P. Cordelieres, D. Biard, N. Giocanti, F. Megnin-Chanet, J. Hall, and V. Favaudon.** 2008. PARP inhibition versus PARP-1 silencing: different outcomes in terms of single-strand break repair and radiation susceptibility. Nucleic Acids Res **36:**4454-64.
21. **Ha, H. C., K. Juluri, Y. Zhou, S. Leung, M. Hermankova, and S. H. Snyder.** 2001. Poly(ADP-ribose) polymerase-1 is required for efficient HIV-1 integration. Proc Natl Acad Sci U S A **98:**3364-8.
22. **Hochegger, H., D. Dejsuphong, T. Fukushima, C. Morrison, E. Sonoda, V. Schreiber, G. Y. Zhao, A. Saberi, M. Masutani, N. Adachi, H. Koyama, G. de Murcia, and S. Takeda.** 2006. PARP-1 protects homologous recombination from interference by Ku and Ligase IV in vertebrate cells. Embo J **25:**1305-14.
23. **Imai, K., and K. Ochiai.** 2011. Role of histone modification on transcriptional regulation and HIV-1 gene expression: possible mechanisms of periodontal diseases in AIDS progression. J Oral Sci **53:**1-13.
24. **Imai, K., K. Ochiai, and T. Okamoto.** 2009. Reactivation of latent HIV-1 infection by the periodontopathic bacterium Porphyromonas gingivalis involves histone modification. J Immunol **182:**3688-95.
25. **Jenuwein, T., and C. D. Allis.** 2001. Translating the histone code. Science **293:**1074-80.
26. **Jiang, H., J. H. Kim, K. M. Frizzell, W. L. Kraus, and H. Lin.** 2010. Clickable NAD analogues for labeling substrate proteins of poly(ADP-ribose) polymerases. J Am Chem Soc **132:**9363-72.
27. **Jordan, A., D. Bisgrove, and E. Verdin.** 2003. HIV reproducibly establishes a latent infection after acute infection of T cells in vitro. Embo J **22:**1868-77.
28. **Jung, A., R. Maier, J. P. Vartanian, G. Bocharov, V. Jung, U. Fischer, E. Meese, S. Wain-Hobson, and A. Meyerhans.** 2002. Recombination: Multiply infected spleen cells in HIV patients. Nature **418:**144.
29. **Kameoka, M., S. Nukuzuma, A. Itaya, Y. Tanaka, K. Ota, K. Ikuta, and K. Yoshihara.** 2004. RNA interference directed against Poly(ADP-Ribose) polymerase 1 efficiently suppresses human immunodeficiency virus type 1 replication in human cells. J Virol **78:**8931-4.
30. **Kameoka, M., S. Nukuzuma, A. Itaya, Y. Tanaka, K. Ota, Y. Inada, K. Ikuta, and K. Yoshihara.** 2005. Poly(ADP-ribose)polymerase-1 is required for integration of the human immunodeficiency virus type 1 genome near centromeric alphoid DNA in human and murine cells. Biochem Biophys Res Commun **334:**412-7.

31. **Kauder, S. E., A. Bosque, A. Lindqvist, V. Planelles, and E. Verdin.** 2009. Epigenetic regulation of HIV-1 latency by cytosine methylation. PLoS Pathog **5:**e1000495.
32. **Kraus, W. L.** 2008. Transcriptional control by PARP-1: chromatin modulation, enhancer-binding, coregulation, and insulation. Curr Opin Cell Biol **20:**294-302.
33. **Kraus, W. L., and J. T. Lis.** 2003. PARP goes transcription. Cell **113:**677-83.
34. **Krishnakumar, R., and W. L. Kraus.** The PARP side of the nucleus: molecular actions, physiological outcomes, and clinical targets. Mol Cell **39:**8-24.
35. **Li, B., M. Carey, and J. L. Workman.** 2007. The role of chromatin during transcription. Cell **128:**707-19.
36. **Llano, M., J. Morrison, and E. M. Poeschla.** 2009. Virological and cellular roles of the transcriptional coactivator LEDGF/p75. Curr Top Microbiol Immunol **339:**125-46.
37. **Llano, M., D. T. Saenz, A. Meehan, P. Wongthida, M. Peretz, W. H. Walker, W. Teo, and E. M. Poeschla.** 2006. An essential role for LEDGF/p75 in HIV integration. Science **314:**461-4.
38. **Llano, M., M. Vanegas, O. Fregoso, D. Saenz, S. Chung, M. Peretz, and E. M. Poeschla.** 2004. LEDGF/p75 determines cellular trafficking of diverse lentiviral but not murine oncoretroviral IN proteins and is a component of functional lentiviral preintegration complexes. J Virol **78:**9524-37.
39. **Llano, M., M. Vanegas, N. Hutchins, D. Thompson, S. Delgado, and E. M. Poeschla.** 2006. Identification and characterization of the chromatin-binding domains of the HIV-1 IN interactor LEDGF/p75. J Mol Biol **360:**760-73.
40. **Marshall, H. M., K. Ronen, C. Berry, M. Llano, H. Sutherland, D. Saenz, W. Bickmore, E. Poeschla, and F. D. Bushman.** 2007. Role of PSIP1/LEDGF/p75 in lentiviral infectivity and integration targeting. PLoS ONE **2:**e1340.
41. **Menissier de Murcia, J., M. Ricoul, L. Tartier, C. Niedergang, A. Huber, F. Dantzer, V. Schreiber, J. C. Ame, A. Dierich, M. LeMeur, L. Sabatier, P. Chambon, and G. de Murcia.** 2003. Functional interaction between PARP-1 and PARP-2 in chromosome stability and embryonic development in mouse. Embo J **22:**2255-63.
42. **Mitta, B., C. C. Weber, and M. Fussenegger.** 2005. In vivo transduction of HIV-1-derived lentiviral particles engineered for macrolide-adjustable transgene expression. J Gene Med **7:**1400-8.
43. **Mitta, B., C. C. Weber, M. Rimann, and M. Fussenegger.** 2004. Design and in vivo characterization of self-inactivating human and non-human lentiviral expression vectors engineered for streptogramin-adjustable transgene expression. Nucleic Acids Res **32:**e106.
44. **Nugent, B. M., and M. M. McCarthy.** Epigenetic underpinnings of developmental sex differences in the brain. Neuroendocrinology **93:**150-8.
45. **Paddock, M. N., B. D. Buelow, S. Takeda, and A. M. Scharenberg.** The BRCT domain of PARP-1 is required for immunoglobulin gene conversion. PLoS Biol **8:**e1000428.
46. **Pedersen, M. T., and K. Helin.** 2011. Histone demethylases in development and disease. Trends Cell Biol **20:**662-71.
47. **Pomerantz, R. J., and D. L. Horn.** 2003. Twenty years of therapy for HIV-1 infection. Nat Med **9:**867-73.
48. **Shan, L., H. C. Yang, S. A. Rabi, H. C. Bravo, R. A. Irizarry, H. Zhang, J. B. Margolick, J. D. Siliciano, and R. F. Siliciano.** Influence of host gene transcription level and orientation on HIV-1 latency in a primary cell model. J Virol.
49. **Shun, M. C., N. K. Raghavendra, N. Vandegraaff, J. E. Daigle, S. Hughes, P. Kellam, P. Cherepanov, and A. Engelman.** 2007. LEDGF/p75 functions downstream from preintegration complex formation to effect gene-specific HIV-1 integration. Genes Dev **21:**1767-78.

50. **Siliciano, R. F.** What do we need to do to cure HIV infection. Top HIV Med **18:**104-8.
51. **Siva, A. C., and F. Bushman.** 2002. Poly(ADP-ribose) polymerase 1 is not strictly required for infection of murine cells by retroviruses. J Virol **76:**11904-10.
52. **Tao, Z., P. Gao, and H. W. Liu.** 2009. Studies of the expression of human poly(ADP-ribose) polymerase-1 in Saccharomyces cerevisiae and identification of PARP-1 substrates by yeast proteome microarray screening. Biochemistry **48:**11745-54.
53. **Zampieri, M., C. Passananti, R. Calabrese, M. Perilli, N. Corbi, F. De Cave, T. Guastafierro, M. G. Bacalini, A. Reale, G. Amicosante, L. Calabrese, J. Zlatanova, and P. Caiafa.** 2009. PARP1 localizes within the Dnmt1 promoter and protects its unmethylated state by its enzymatic activity. PLoS One **4:**e4717.
54. Pauza, C.D., Two bases are deleted from the termini of HIV-1 linear DNA during integrative recombination. Virology, 1990. 179(2): p. 886-9.
55. Miller, M.D., C.M. Farnet, and F.D. Bushman, Human immunodeficiency virus type 1 preintegration complexes: studies of organization and composition. J Virol, 1997. 71(7): p. 5382-90.
56. Engelman, A., K. Mizuuchi, and R. Craigie, HIV-1 DNA integration: mechanism of viral DNA cleavage and DNA strand transfer. Cell, 1991. 67(6): p. 1211-21.

CURRICULM VITA

Daniel Reyes was born at El Paso, TX, son of Daniel Reyes and Rita H. Esparza. Graduated from Americas High School in spring 2005, and then enrolled at the University of Texas at El Paso. While pursuing his bachelor's degree he was awarded a Howard Hughes Medical Institute (HHMI) fellowship to work under Dr.Llano's supervision as undergraduate. He obtained his bachelor's degree in Biological Sciences with a minor in chemistry in the spring of 2010 in the University of Texas at El Paso. The same year he entered the master's degree program in this university. He has presented his work in different national conferences as oral presentations or posters. In addition, he served as a teacher assistant during all his graduate studies in a national course organized by HHMI. He was selected by this HHMI program to train a group of teacher assistants from other universities at Janelia Farm laboratories. Daniel obtained his Master's degree in a year and a half and has a first-author publication in preparation. Now he will continue working in Dr. Llano lab as a Research Associate. His future goal is to work in the biotechnology industry or pursue the Ph.D. degree.