HIV-1 Vpr degrades the HLTF DNA translocase in T cells and macrophages

Hichem Lahouassa[a,b,c,1,2], Marie-Lise Blondot[a,b,c,1,3], Lise Chauveau[d,e], Ghina Chougui[a,b,c], Marina Morel[a,b,c], Marjorie Leduc[a,b,c,f], François Guillonneau[a,b,c,f], Bertha Cecilia Ramirez[a,b,c], Olivier Schwartz[d,e,g], and Florence Margottin-Goguet[a,b,c,4]

[a]INSERM, U1016, Institut Cochin, Paris 75014, France; [b]CNRS, UMR8104, Paris 75014, France; [c]Department of Infectiology and Microbiology, Université Paris Descartes, Sorbonne Paris Cité, Paris 75014, France; [d]Department of Virology, Virus & Immunity Unit, Institut Pasteur, Paris 75015, France; [e]CNRS Unité de Recherche Associée 3015, Paris 75015, France; [f]3P5 Proteomic Facility, Paris Descartes University, Paris 75014, France; and [g]Vaccine Research Institute, Creteil 94010, France

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Viruses often interfere with the DNA damage response to better replicate in their hosts. The human immunodeficiency virus 1 (HIV-1) viral protein R (Vpr) protein has been reported to modulate the activity of the DNA repair structure-specific endonuclease subunit (SLX4) complex and to promote cell cycle arrest. Vpr also interferes with the base-excision repair pathway by antagonizing the uracil DNA glycosylase (Ung2) enzyme. Using an unbiased quantitative proteomic screen, we report that Vpr down-regulates helicase-like transcription factor (HLTF), a DNA translocase involved in the repair of damaged replication forks. Vpr subverts the DDB1–cullin4-associated-factor 1 (DCAF1) adaptor of the Cul4A ubiquitin ligase to trigger proteasomal degradation of HLTF. This event takes place rapidly after Vpr delivery to cells, before and independently of Vpr-mediated G2 arrest. HLTF is degraded in lymphocytic cells and macrophages infected with Vpr-expressing HIV-1. Our results reveal a previously unidentified strategy for HIV-1 to antagonize DNA repair in host cells.

HIV | restriction factor | DNA repair | Vpr target | SILAC

In addition to their role in maintaining genome integrity, DNA repair proteins participate in other cellular processes including innate immune signaling (1, 2). Immunodeficiency diseases may arise from defects in DNA helicases or translocases involved in the repair of DNA replication forks, as demonstrated, for instance, in Schimke immune-osseous dysplasia (SIOD) (2). Another example is provided by Aicardi–Goutières syndrome (AGS), in which the overproduction of type I interferon (IFN) is associated with mutations of proteins involved in DNA synthesis and repair, namely SAM domain and HD domain-containing protein 1 (SAMHD1), ribonuclease H2 (RNase H2), and three prime repair exonuclease 1 (Trex1) (3).

Cross-talk between the HIV and DNA repair pathways occurs at different steps of the virus life cycle, including reverse transcription, integration, and sensing of viral nucleic acids (4). In this context, the Vpr protein, expressed by both HIV-1 and HIV-2/simian immunodeficiency virus (SIV) sooty mangabey (smm) lineages, has drawn much attention. Vpr is not required for infection of most cell lines or primary $CD4^+$ T cells (5–7). A replication defect for *vpr*-deleted viruses has been reported in dendritic cells and macrophages, with important donor-to-donor variability (6, 8–11). It was recently suggested that Vpr favors infection of macrophages by counteracting a restriction factor targeting Env expression and viral release (12). Vpr is also necessary for efficient cell-to-cell spread of HIV-1 from macrophages to $CD4^+$ T lymphocytes (13). Vpr plays an important role in vivo. $SIV_{MAC}\Delta vpr$ viruses rapidly revert to a WT version when injected in rhesus macaques (14). A similar reversion has been observed in a laboratory worker accidentally contaminated with a *vpr*-deficient strain of HIV-1 (15, 16). Several studies also reported mutations in the *vpr* gene in long-term nonprogressor (LTNP) patients (17–20). Several lines of evidence indicate that Vpr interferes with DNA repair pathways (21). First, the best renowned activity of Vpr, its ability to mediate a G2 arrest of the cell cycle, depends on the activation of the ATR-mediated (ATR: ataxia telangiectasia mutated and Rad3 related) DNA damage response (22). G2 arrest requires Vpr binding to DCAF1, an adaptor of the Cul4A-DDB1 ubiquitin ligase, which is involved in DNA repair in noninfected cells (23–29). More recently, Vpr has been shown to activate the SLX4 complex (SLX4com) with the help of DCAF1 (21, 30). SLX4com associates with several endonucleases, including Mus81, to coordinate the repair of specific replication-born double strand breaks (DSBs) and collapsed replication forks (31–33). It has been proposed that Vpr triggers replication stress and G2 arrest through inappropriate activation of SLX4com (30). This activation would lead to the elimination of viral DNA and, subsequently, virus escape from immune sensing. Vpr also recruits uracil DNA glycosylase (Ung2), an enzyme that prevents mutagenesis by eliminating uracil from DNA molecules, thereby initiating the base-excision repair (BER) pathway (34, 35). Vpr targets Ung2 for degradation through hijacking Cul4A-DDB1 (36). Ubiquitin ligases act on several substrates, which led us to speculate that Vpr may target additional unknown host proteins for proteasomal degradation.

The DNA damage tolerance pathway (DDT) allows stalled replication forks to bypass DNA lesions, such as gaps or DSBs, both in S and G2 phases (37, 38). In response to DNA damage or replicative stress, proliferating cell nuclear antigen (PCNA) is monoubiquitylated by Rad6/Rad18, leading to the recruitment of

Significance

Human immunodeficiency viruses (HIV) have developed strategies to interfere with DNA repair in host cells. Some DNA repair pathways represent restriction mechanisms that counteract the virus as soon as it penetrates into the host cell, before the establishment of an interferon response. Here we identify helicase-like transcription factor (HLTF) as a new protein degraded by the viral protein R (Vpr) from HIV-1. HLTF mediates the repair of stalled replication forks to bypass DNA lesions and ensure genome integrity. HLTF is degraded early after Vpr delivery to T lymphocytes or macrophages that represent relevant target cells for HIV. The discovery of HLTF as a DNA repair protein degraded by Vpr in infected cells paves the way for novel unexpected restriction mechanisms.

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[1]H.L. and M.-L.B. contributed equally to the work.

[2]Present address: Ecole Nationale Supérieure Vétérinaire, Alger 16000, Algeria.

[3]Present address: Laboratoire Microbiologie Fondamentale et Pathogénicité, Université de Bordeaux, Unité Mixte de Recherche 5234, Bordeaux 33000, France.

[4]To whom correspondence should be addressed. Email: florence.margottin-goguet@inserm.fr.

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translesion synthesis polymerases. PCNA can be further polyubiquitinated by the budding yeast Rad5 ubiquitin ligase or its human orthologs helicase-like transcription factor (HLTF) and SNF2 histone linker PHD finger RING finger helicase (SHPRH) (37, 38). By ensuring the completion of S-phase following DNA damage, Rad5/HLTF/SHPRH contribute to genome integrity. HLTF is a DNA-binding protein (39–41) first described as a transcription factor (42, 43), but later authenticated as a protein involved in DNA repair, in tumor suppression, and in the early stages of carcinogenesis (44–47). Although both HLTF and SHPRH are able to polyubiquitinate PCNA, they do not act redundantly (48). HLTF facilitates fork reversal activity but also DNA strand invasion and formation of a D-loop structure in an ATP-independent manner (49–51). Furthermore, HLTF and SHPRH might not be only dedicated to the DDT pathway but more generally involved in DNA repair (48).

Here, we undertook a quantitative proteomic approach, based on a stable-isotope labeling by amino acids in cell culture (SILAC) strategy coupled with LC-MS/MS, to describe global changes in the cellular landscape under Vpr treatment (52). We used the property of Vpr to be incorporated into virions and virus-like particles (VLPs) (53, 54) to deliver the protein to target cells. We report that HIV-1 Vpr induces the early degradation of HLTF in primary cellular targets of HIV and analyzed the underlying molecular mechanisms.

Results

HLTF Is Down-Regulated by HIV-1 Vpr in a Proteasome-Dependent Manner. We first examined which cellular proteins are modulated on HIV-1 Vpr delivery in HeLa cells. These cells represent a convenient model to perform a SILAC analysis, and Vpr is known to be active in HeLa cells, promoting, for instance, cell cycle arrest (26). To this aim, VLPs containing WT Vpr or a G2 arrest-defective Vpr mutant (Vpr S79A), both tagged with an HA epitope, or Vpr-negative (empty) VLPs were delivered to HeLa cells stably labeled respectively with light, medium, or heavy isotopes (Fig. S1*A*) (55). Using VLPs enabled us to focus on cellular changes that may occur without de novo *Vpr* expression. The experiment was performed in pseudoduplicate (S1 and S2) by switching VLPs and isotopes (Fig. S1*A*). The presence of Vpr in target cells was confirmed by Western blot, with similar levels of WT and Vpr S79A being detected (Fig. S1*B*). As expected, WT Vpr, but not the Vpr S79A mutant, induced G2 arrest (Fig. S1*C*) (55). The efficiency of the cell cycle block was modest because cells were harvested 12 h after Vpr delivery, to focus on early events (Fig. S1*C*). A total of 2,196 and 2,272 proteins were detected by MS, respectively, in S1 and S2 nuclear fractions; among them, 1,572 and 1,601 proteins could be reliably quantified. The levels of most of these proteins (82% in S1; 92% in S2) were not affected by WT Vpr relative to the control (empty VLPs). About 10% of the proteins showed variations in quantity of at least 20% with only 33 proteins reproducibly found in S1 and S2 (Fig. S1*D*). Most of these proteins were stabilized or enhanced by Vpr (Fig. S1*D*) and only eight had reduced levels. HLTF, detected with 13 peptides and with a reduction of more than 60% of its relative quantity, appeared as a preferential cellular target of Vpr. Vpr S79A also affected HLTF levels but less than WT Vpr (Fig. S1*E*). Interestingly, most of the proteins stabilized by WT but not by S79A Vpr are known to be up-regulated in the G2 phase of the cell cycle (Cyclin B/CCNB1, Aurora kinases A/AURKA and B/AURKB, inner centromere protein (INCENP), Annilin/ANLN, or Polo kinase 1/PLK1, for example; Fig. S1*E*). The presence of this G2 arrest protein signature indirectly validates our strategy. Taken together, these data indicate that SILAC represents a powerful tool to study rapid large-scale proteomic changes in the cellular environment induced by HIV-1 Vpr, as has been recently demonstrated for other HIV proteins (56). Of note, we also validated our SILAC procedure with the observation that SAMHD1 levels were strongly decreased on delivery of HIV-2/SIVsmm Vpx protein as expected (57, 58).

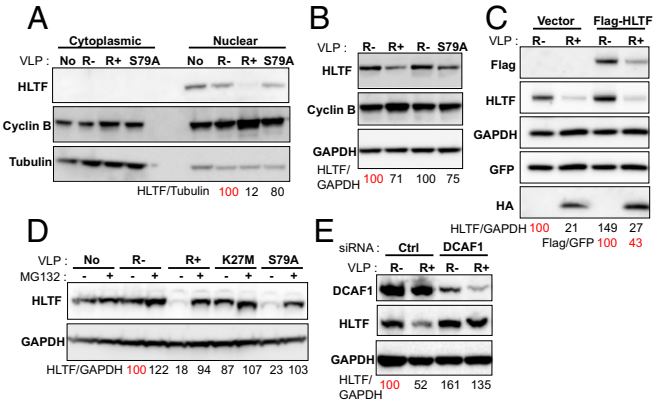

Fig. 1. HIV-1 Vpr down-regulates HLTF in a DCAF1-dependent manner. (*A*) HLTF expression in HeLa cells is down-regulated by VLP-encapsidated WT HIV-1 Vpr. Cells were transduced with the VLP used for SILAC. After 24 h, cells were harvested and fractioned. Protein expression was analyzed by Western blotting. (*B*) HLTF expression in Jurkat T cells is down-regulated by VLP-encapsidated WT HIV-1 Vpr. Cells were treated 48 h with VLP and lysed, and whole cell extracts were analyzed by Western blot. (*C*) Endogenous and exogenous HLTF expression levels are reduced by VLP-encapsidated WT HIV-1 Vpr. HeLa cells were cotransfected with a vector expressing Flag-HLTF or an empty vector together with a transfection control vector encoding the GFP (ratio10:1). Twenty-four hours after transfection, cells were transduced with the VLP and were harvested 24 h later. Protein expression was analyzed by Western blotting. (*D*) HLTF depletion induced by HIV-1 Vpr is proteasome-dependent. HeLa cells were treated with or without MG132 during 6 h after VLP incubation. Cells were transduced with the different VLP: empty VLP (R–), VLP containing wt Vpr (R+), or VLP containing Vpr K27M or Vpr S79A. (*E*) Vpr hijacks DCAF1 to mediate HLTF degradation. HeLa cells were treated with siRNA Control or siRNA against DCAF1 for 24 h. Cells were then transduced with the VLP and harvested 24 h later. Protein expression was analyzed by Western blotting. In each panel, quantification was performed: ratios between signals were calculated relative to a 100% reference indicated in red. All Western blots are representative of three independent experiments except *B*, which corresponds to a direct analysis of cells used for the SILAC.

Western blot analysis of the nuclear fraction of HeLa cells that had been incubated with VLPs containing WT Vpr confirmed the HLTF steady-state amount reduction together with cyclin B/CCNB1 stabilization (Fig. 1*A*). HLTF was not detected in the cytoplasmic fraction (Fig. 1*A*). HLTF down-regulation was also observed in Jurkat T cells similarly treated with VLPs (Fig. 1*B*). The G2 arrest-defective Vpr S79A mutant also decreased HLTF levels, but to a lesser extent than WT Vpr (Fig. 1 *A*, *B*, and *D*), supporting our SILAC results. A Flag-tagged HLTF expressed under the control of a CMV promoter was also down-regulated by Vpr, suggesting that Vpr does not reduce HLTF expression through transcriptional modulation of the HLTF promoter (Fig. 1*C*, Flag panel for exogenous HLTF and HLTF panel for total HLTF).

We then asked whether HLTF down-regulation was proteasome dependent. To this aim, we incubated cells with MG132, a proteasome inhibitor. HLTF down-regulation in the presence of WT or S79A Vpr was inhibited by MG132 (Fig. 1*D*). Of note, a second G2 arrest-defective Vpr mutant, Vpr K27M, was unable to decrease HLTF levels (Fig. 1*D*). Furthermore, knockdown by siRNA of the DCAF1 ubiquitin ligase adaptor, previously shown to be hijacked by Vpr, inhibited Vpr-mediated HLTF down-regulation (Fig. 1*E*). Altogether, these results support a model in which HIV-1 Vpr uses the Cul4A-DDB1 ubiquitin ligase through DCAF1 binding to induce the proteasomal degradation of HLTF.

HLTF Degradation Occurs Independently from Vpr-Mediated G2 Arrest in HeLa Cells. HLTF is an enzyme that stabilizes and repairs stalled replication forks and controls G2/M transition (49, 59, 60). Considering HLTF activities led us to investigate its role in Vpr-mediated G2 arrest. In an experiment in which HLTF levels

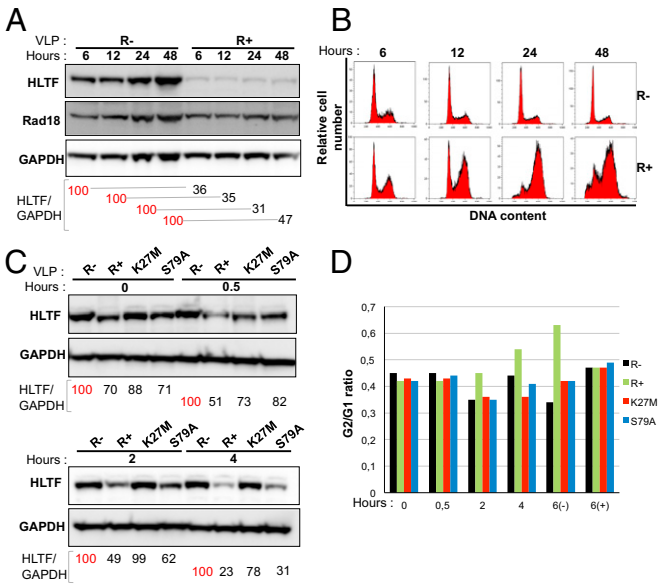

Fig. 2. HLTF degradation induced by HIV-1 Vpr precedes G2/M arrest. (*A*) Kinetic of HLTF disappearance. HeLa cells were transduced with empty VLP or WT Vpr VLP for 2 h. Cells were harvested 6, 12, 24, and 48 h after VLP treatment, and whole cell lysates were analyzed by Western blot. Quantification was performed: ratios between the HLTF and the GAPDH signals were calculated relative to a 100% reference indicated in red. The Western blot is representative of two independent experiments. (*B*) Kinetic of Vpr-mediated G2 arrest. Cell cycle analysis after VLP treatment, same time points as in *A*. (*C*) Short time kinetic of HLTF disappearance. HeLa cells were transduced with empty VLP (R–), VLP containing WT Vpr (R+), or Vpr mutants K27M or S79A. Cells were harvested at time 0 min, 30 min, 2 h, and 4 h after the 2-h VLP treatment, and whole cell lysates were analyzed by Western blot as in *A*. Quantification was performed as in *A*. (*D*) HLTF degradation precedes Vpr-mediated cell cycle arrest. Cell cycle analysis performed after VLP treatment at the indicated time points (same as in *C*) for the 6-h time point: 6(–) indicates with no MG132 treatment and 6(+) with MG132 all along the kinetic.

were monitored over time, we observed its down-regulation 6 h after VLP treatment (Fig. 2*A*). The effect of Vpr on G2 cell cycle arrest was modest at 6 h and increased over time (Fig. 2*B*). Rad18, a protein that interacts with HLTF at the replication fork, was not affected by Vpr (Fig. 2*A*) (48). We then examined the effect of Vpr at earlier time points. Strikingly, down-regulation of HLTF was detected as soon as 30 min after VLP treatment (Fig. 2*C, Upper*) and intensified over time from 0 to 4 h (Fig. 2*C*). An increase in G2 arrested cell number also started to be detected at 4 h after VLP treatment (Fig. 2*D*). Vpr S79A induced HLTF degradation, but not as efficiently as Vpr WT as previously mentioned (Figs. 1 and 2*C*), with 23% and 31% HLTF remaining with the WT and mutant protein, respectively, 4 h after VLP addition. HLTF levels did not significantly changed from 0 to 4 h in the presence of Vpr K27M, as already observed in Fig. 1*E*. Thus, Vpr-mediated HLTF degradation is unlikely a consequence of Vpr-mediated G2 arrest but rather precedes this arrest. Furthermore, the partial ability of the G2 arrest-defective Vpr S79A mutant to induce HLTF degradation suggests that a block in the cell cycle is not a prerequisite for the modulation of HLTF expression.

Vpr triggers the degradation of the SLX4-associated Mus81 endonuclease, although conflicting results have been reported regarding the link between Vpr-mediated Mus81 degradation and G2 arrest (30, 61). We confirmed that Vpr degrades both Mus81 and HLTF and that this degradation required DCAF1 (Fig. 3*A*). In addition, silencing of HLTF did not inhibit Mus81 degradation and, inversely, silencing of Mus81 did not prevent HLTF degradation, suggesting that the two events occur independently of each other (Fig. 3*A*). Although DCAF1 siRNA inhibited Vpr-mediated G2 arrest as expected, four distinct HLTF siRNA did not affect Vpr-mediated cell cycle arrest (Fig. 3*B* and Fig. S2 *A* and *B*). Furthermore, HLTF siRNA alone did not perturb the cell cycle (Fig. 3*B* and Fig. S2 *A* and *B*).

We further studied the links that may exist between HLTF degradation and G2 arrest. To this aim, we analyzed the ability of a panel of Vpr mutants to block the cell cycle and to degrade HLTF (Fig. S2*C*). The proteins were expressed in HeLa cells by transfection. We identified mutants that displayed various abilities to degrade HLTF (Fig. S2*C*). We then selected three mutants (G56A, K27M, and S79A) that we delivered in HeLa cells through VLPs (Fig. 3 *C* and *D*). The K27M mutant was inactive in both assays, whereas S79A partly degraded HLTF (47% HLTF remaining, compared with 18% with the WT protein) without altering the cell cycle (Fig. 3 *C* and *D*). In contrast, the Vpr G56A mutant induced G2 arrest but did not degrade HLTF (Fig. 3 *C* and *D*). Altogether our results strongly suggest that HLTF degradation and G2 arrest can be genetically uncoupled.

Vpr Down-Regulates HLTF in Infected T Cells and Primary Macrophages. We then investigated whether HLTF is degraded in HIV-1–infected cells. MT4 and Jurkat T cells, as well as HeLa cells, were infected with WT or Δ*Vpr* HIV-1, and HLTF levels were measured after 3 d. The levels of HLTF were strongly decreased in the presence of Vpr in the three cell types (Fig. 4*A*; 1%, 16%, and 38% HLTF remaining after infection with WT HIV-1 in comparison with the Δ*Vpr* virus in MT4, Jurkat, and HeLa cells, respectively). Nonetheless, as expected from the literature, Vpr had no effect on viral replication in these cells. WT or Δ*Vpr* HIV similarly infected MT4 cells, as assessed by measuring Gag expression by flow cytometry at different time points (Fig. S3). Moreover,

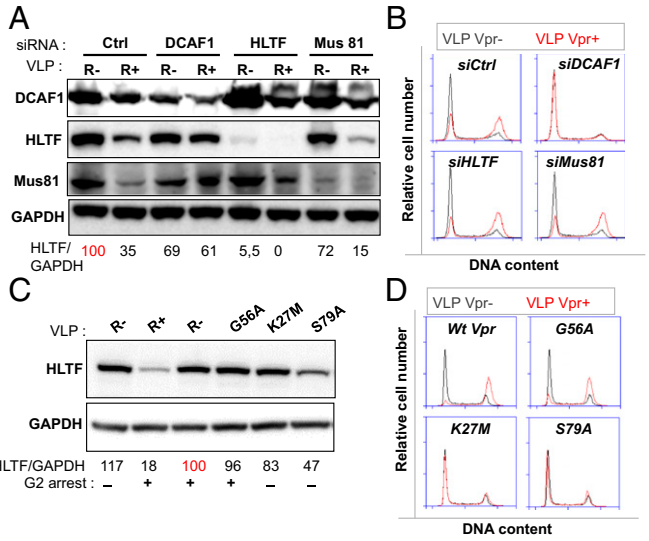

Fig. 3. HLTF degradation and G2 arrest are two independent activities of HIV-1 Vpr. (*A*) Mus81 depletion does not trigger HLTF degradation and, conversely, HLTF depletion does not trigger Mus81 degradation. HeLa cells were treated with siRNA control or siRNA against DCAF1, HLTF, or Mus81 for 24 h. Cells were then transduced with the VLP and harvested 24 h later. Protein expression was analyzed by Western blotting. Quantification was performed: ratios between the HLTF and the GAPDH signals were calculated relative to a 100% reference indicated in red. The Western blot is representative of three independent experiments. (*B*) Depletion of HLTF by siRNA does not perturb cell cycle and does not inhibit Vpr-mediated G2 arrest. HeLa cells were treated as in *A*, and cell cycle was analyzed 24 h after VLP treatment. (*C*) The Vpr G56A mutant, defective for HLTF degradation, is still able to arrest the cell cycle. HeLa cell were treated with VLP [empty VLP (R–), VLP containing WT Vpr (R+), or mutants G56A, K27M, or S79A] for 24 h. Protein expression was then assessed by Western blot and quantification performed as in *A* and the cell cycle analyzed (*D*).

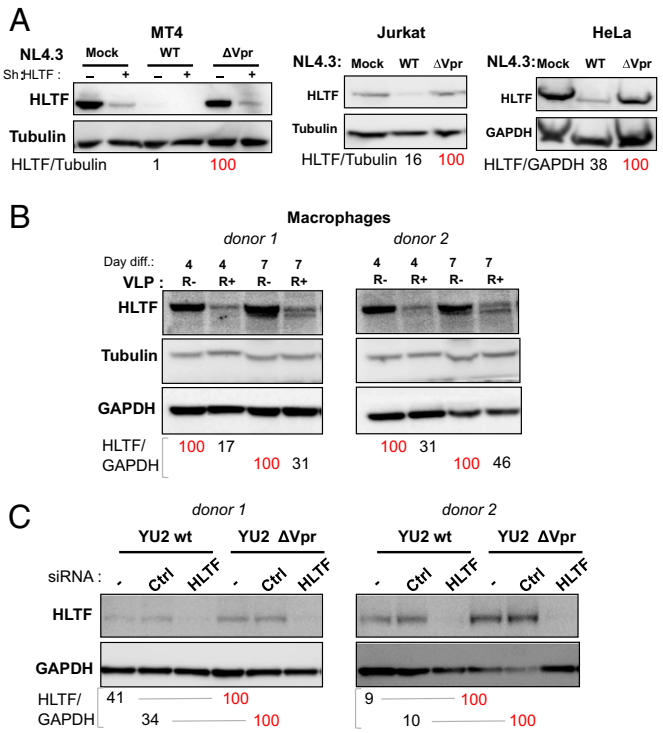

Fig. 4. HLTF degradation is induced by HIV-1 Vpr in infected T cells and HeLa cells. (*A*) HLTF is degraded in MT4 cells by HIV-1 viruses expressing WT Vpr (*Left*), in Jurkat T cells (*Center*), and in HeLa cells (*Right*). MT4 cells were transduced with lentiviruses expressing shRNA against HLTF and cultured in the presence of doxycycline for 3 d. Cells were then either not infected or infected with HIV-1 NL4.3 WT or *Vpr*-deleted viruses (ΔVpr) for 48 h. Jurkat and HeLa cells were either not infected or infected with NL4.3 WT or *Vpr*-deleted viruses (ΔVpr). Cells were lysed 48 h after infection, and cell lysates were analyzed by Western blot. (*B*) Vpr from HIV-1 VLP triggers HLTF degradation in macrophages. Monocyte-derived macrophages were differentiated for 4 or 7 d and then exposed to HIV-1 VLP containing or not Vpr for 24 h [empty VLP (R−), VLP containing WT Vpr (R+)]. Cells were then lysed, and HLTF expression was analyzed by Western blot. In each panel, quantification was performed: ratios between signals were calculated relative to a 100% reference indicated in red. All Western blots are representative of three independent experiments. (*C*) HLTF levels are reduced in macrophages following infection with the YU2 WT virus, in comparison with the ΔVpr virus. Monocyte-derived macrophages (MDMs) were treated with siRNA control or siRNA against HLTF for 24 h. Cells were then infected with either YU2 WT virus or YU2 ΔVpr virus (ΔVpr). Analysis of HLTF and GADPH expression was done in extracts collected at day 16 after infection. The kinetic of replication is shown Fig. S4.

HLTF was silenced in MT4 cells by using a doxycycline-inducible shRNA (Fig. 4*A*, *Left*). HLTF silencing did not alter infection levels of WT or Δ*Vpr* HIV (Fig. S3).

We then examined the activity of Vpr in primary human macrophages, in which Vpr has been reported to give an advantage to the virus in multicycle infection assays (6, 8–11). The role of HLTF has not yet been examined in such nondividing cells. We found that HLTF is expressed at day 4 or day 7 following differentiation of freshly isolated monocytes into macrophages (MDMs) (Fig. 4*B*; two donors). HLTF levels were decreased by two- to sixfold following macrophage incubation for 24 h with Vpr-containing HIV-1 VLPs compared with Vpr-negative VLP (Fig. 4*B*). This decrease was similarly observed with macrophages at day 4 or 7 after differentiation (Fig. 4*B*). This experiment demonstrated that incoming Vpr is sufficient to decrease endogenous levels of HLTF on viral entry. HLTF levels were also reduced following productive infection with the YU2 macrophage-tropic strain expressing Vpr compared with the isogenic ΔVpr virus (Fig. 4*C*; two donors, Western blot at day 16 after infection). As expected, viral replication was delayed and less efficient in the absence of Vpr (Fig. S4) (6, 8–11). However, depletion of HLTF by siRNA did not impact viral replication (Fig. S4).

Altogether, our results indicate that Vpr degrades HLTF in natural cell targets of HIV-1.

Discussion

HLTF, a Bona Fide Cellular Substrate of HIV-1 Vpr. SILAC provides an unbiased view of global changes in protein levels under different parallel conditions. Surprisingly, HLTF was the sole cellular target that was clearly down-regulated in the presence of HIV-1 Vpr, among more than 2,000 proteins quantified. Several other host proteins have been proposed to be targeted by Vpr, but previous results were mostly based on candidate-based approaches and with often modest down-regulation (62–64). Importantly, HLTF is down-regulated by Vpr in infected T cells. The effect of Vpr on HLTF is reminiscent of Vpx-induced SAMHD1 degradation (57, 58). Both Vpr and Vpx act rapidly, when incoming virions enter the cell, to trigger proteasomal degradation of their target proteins by hijacking ubiquitin ligases. Vpr is closely related to Vpx. These two proteins have a common evolutionary origin (65, 66), share similar amino acid sequences, and are incorporated into virions (67, 68). Despite these similarities, the two proteins display different activities (69). HIV-1 Vpr does not degrade SAMHD1. It will be worth examining the effect of a large panel of Vpr proteins from HIV-1, HIV-2, and SIV strains on HLTF, to understand the evolutionary pressures associated with this novel activity. It will also be of interest to determine whether this degradation involves a direct interaction between HLTF and HIV-1 Vpr.

Why Does Vpr Induce HLTF Degradation? How could HLTF degradation impact HIV-1 replication and pathogenicity? As part of the repair of damaged replication forks, one obvious possibility was that HLTF was involved in Vpr-mediated G2 arrest. This hypothesis was attractive because both Vpr and depletion of some DNA translocases having apparent redundant function with HLTF interfere with SLX4com. For example, SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A-like protein 1 (SMARCAL1) was reported to interfere with MUS81 structure-specific endonuclease subunit (Mus81) or the SLX4 endonuclease complex and the repair of damaged replication forks (30, 70, 71). However, several lines of evidence suggest that HLTF is not involved in the cytostatic activity of Vpr. Silencing of HLTF by siRNA did not perturb the cell cycle, nor did it affect Vpr-mediated G2 arrest. In addition, some Vpr mutants that do not measurably degrade HLTF were still able to arrest the cell cycle. Thus, HLTF degradation represents a new activity of Vpr apparently distinct from G2 arrest, at least in HeLa cells. Further work will help determine how the removal of HLTF by Vpr may impact DNA repair on HIV-1 infection of macrophages, dendritic cells, and T cells.

HLTF is a DNA translocase involved in postreplication DNA repair occurring in the S phase of the cycle (49, 72–74). As such, it has been mostly studied in dividing cells, in which DNA repair processes are important to avoid aberrant DNA synthesis that would be a source of mutations or DSBs. It is tempting to speculate that HLTF could exert additional functions, because we report here that the protein is expressed in nondividing macrophages. Cell cycle-related proteins, for instance, the cyclin/Cdk inhibitor p21, which was first thought to be only dedicated to the control of cell cycle progression, were later on identified in macrophages as a repressor of HIV-1 replication (75, 76). Our experiments did not demonstrate a rescue of Vpr-deleted HIV-1 replication in HLTF-silenced macrophages. It will be interesting to further explore a potential antiviral role of HLTF in other nondividing cells such as dendritic cells and in primary lymphocytes. One alternative and non-mutually exclusive possibility is that HLTF degradation is related to the ability of Vpr to interfere with the immune response and the cooperation between immune cells (77–81). For example, whether HLTF degradation

is related to the ability of Vpr to escape or promote immune detection in primary cells should be investigated. A role of HLTF in DNA sensing could be a consequence of its capacity to bind ssDNA ends (82). It is also possible that HLTF is not a direct target of Vpr and that its degradation results from the inactivation of another cellular protein that was not detected in our SILAC experiments. Looking for interacting partners of HLTF and their potential antiviral role could help addressing this issue.

HLTF belongs to a small family of DNA translocases, including SMARCAL1 and zinc finger Ran-binding domain-containing protein 3 (ZRANB3)/AH2, that catalyze fork regression activity in vitro (83). SMARCAL1 deficiency causes the SIOD human disease, which is associated with immune deficiency (84). This observation, together with our finding that HLTF is antagonized by a viral protein, may suggest that these DNA translocases could play a role in the immune defense against pathogens.

HIV-1 and HIV-2/SIV Interfere with Distinct DNA Repair Pathways. HIV-1 and HIV-2/SIV have likely developed distinct strategies to interfere with DNA repair pathways. Vpx from HIV-2/SIV targets SAMHD1, an enzyme that hydrolyses nucleotides potentially required for DNA repair and that prevents viral reverse transcription (85–87), whereas we show here that HIV-1 Vpr triggers HLTF degradation and thus manipulates the DDT pathway. Vpr also likely interferes with the BER pathway through Ung2 recruitment/degradation and prematurely activates the SLX4 complex. The use of distinct strategies to impact the DNA repair pathway may underline the different pathological outcomes associated with HIV-1 and HIV-2/SIV. Future studies should aim to analyze the ability of divergent lentiviruses to induce the degradation of human and simian HLTF, to evaluate its importance in pathology and in cross-species transmission (88). It will also be worthwhile to search for signatures of positive selection in HLTF sequences originating from various species. Such analysis will complement functional studies and will help evaluate the impact of HLTF at the host–virus interface.

Experimental Procedures

SILAC. Full details for the SILAC procedure are provided in the *SI Experimental Procedures*.

Viruses and VLP Production. VLPs and viruses were produced in 293 T cells cotransfected by the calcium-phosphate method. The Δ-Env HIV-1 viruses (DHIV NL4.3 viruses) were produced by using pNL4.3 deltaEnv HIV-1 constructs lacking the gene encoding Vpr (DHIVΔVpr) or encoding WT Vpr (DHIV WT) along with a plasmid encoding the vesicular stomatitis virus glycoprotein G (VSV-G). The proviral plasmids were a kind gift from Vincente Planelles, University of Utah, Salt Lake City (89). HIV-1 VLPs were produced by using psPAX2 lentiviral packaging plasmid along with the plasmid encoding VSV-G and a plasmid encoding either HA-tagged WT or mutant Vpr. Briefly, 48 h after transfection, the culture supernatants were collected and filtered through 0.45-μm pore filters. The viral particles were then concentrated in 10% (wt/vol) polyethylene glycol 6000 (Sigma) containing 300 mM NaCl. Viral and VLP productions were measured by quantification of p24 (HIV) levels by using an ELISA (Innotest; Fujirebio, or ZeptoMetrix Corporation). Viruses were titrated by using the reporter cells TZM-bl obtained through the National Institutes of Health (NIH) AIDS Reagent Program, Division of AIDS, NIH (TZM-bl from John C. Kappes, Xiaoyun Wu, and Tranzyme Inc., catalog number 8129).

Transduction and Infection. Cells transduced by VLPs were incubated for 2 h in DMEM or RPMI supplemented with 5 μg/mL Dextran (Sigma) and an equivalent of 50–100 ng p24 or p27 of HIV-1 or SIV VLP, respectively, for 2×10^5 cells, and then complete medium was added. The proteasome inhibitor Mg132 (Sigma) was used at a final concentration of 20 μM for 6 h from the beginning of VLP incubation or with DMSO as a control. Jurkat cells were infected for 2 h in complete RPMI medium supplemented with 5 mM Hepes and 5 μg/mL Dextran.

Peripheral blood mononuclear cells (PBMCs) from the blood of anonymous donors (obtained in accordance with the ethical guidelines of the Institut Cochin) were isolated by Ficoll (Sigma) density-gradient separation. Monocytes were isolated by positive selection with CD14 magnetic MicroBeads (Miltenyi Biotec). MDMs were obtained by culturing the monocytes for 7 d in RPMI containing 10 ng/mL granulocyte-macrophage colony stimulating factor and 20 ng/mL macrophage colony-stimulating factor. YU2 WT and YU2 ΔVpr viruses were kind gifts from Serge Benichou, Institut Cochin, Paris.

MDM cells were infected (at a multiplicity of infection of 0.1) for 3 h in complete RPMI medium without serum at 37 °C. Cells were washed twice with PBS to remove inoculum and cultured in RPMI complete medium. Viral production was measured by quantification of p24 levels in the cellular supernatants collected at times 0, 1, 2, 5, 9, 12, and 16 d after infection by using an ELISA (Innotest; Fujirebio).

Cells, plasmid constructs, siRNA, shRNA, and the procedures for cell cycle analysis and Western blots are described in the *SI Experimental Procedures*.

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