Assembly with the Cul4A-DDB1DCAF1 Ubiquitin Ligase Protects HIV-1 Vpr from Proteasomal Degradation

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Many viruses subvert the host ubiquitin-proteasome system to optimize their life cycle. We recently documented such a mechanism for the human immunodeficiency virus type 1 Vpr protein, which promotes cell cycle arrest by recruiting the DCAF1 adaptor of the Cul4A-DDB1 ubiquitin ligase, a finding now confirmed by several groups. Here we examined the impact of Cul4A-DDB1DCAF1 on Vpr stability. We show that the Vpr(Q65R) mutant, which is defective in DCAF1 binding, undergoes proteasome-mediated degradation at a higher rate than wild-type Vpr. DCAF1 overexpression stabilizes wild-type Vpr and leads to its cytoplasmic accumulation, whereas it has no effect on the Vpr(Q65R) mutant. Conversely, small interfering RNA-mediated silencing of DCAF1 decreases the steady state amount of the viral protein. Stabilization by DCAF1, which is conserved by Vpr species from human immunodeficiency virus type 2 and the SIVmac strain, results in increased G2 arrest and requires the presence of DDB1, indicating that it occurs through assembly of Vpr with a functional Cul4A-DDB1DCAF1 complex. Furthermore, in human immunodeficiency virus type 1-infected cells, the Vpr protein, issued from the incoming viral particle, is destabilized under DCAF1 or DDB1 silencing. Together with our previous findings, our data suggest that Cul4A-DDB1DCAF1 acts at a dual level by providing Vpr with the equipment for the degradation of specific host proteins and by counteracting its proteasome targeting by another cellular E3 ubiquitin ligase. This protection mechanism may represent an efficient way to optimize the activity of Vpr molecules that are delivered by the incoming virus before neosynthesis takes place. Targeting the Vpr-DCAF1 interaction might therefore present therapeutic interest.

Numerous proteins from various pathogenes have been shown to directly destruct the expression of specific host cell proteins, as first exemplified by the human papillomavirus E6 oncoprotein, which targets p53 to the proteasome (1). The ubiquitin-proteasome pathway plays a major role in the regulated turnover of cellular proteins (2). The covalent attachment of ubiquitin molecules to a target protein is a prerequisite for its recognition and proteolysis by the 26S proteasome. Ubiquitination involves three enzymatic activities: the E1 ubiquitin-activating enzyme, the E2 ubiquitin-conjugating enzyme, and the E3 ubiquitin ligase, which directly binds the cellular protein and therefore determines the specificity of the reaction. A major family of E3 ubiquitin ligases is represented by the cullin-ring ubiquitin ligase (CRUL) class. These multi-subunit enzymes share a modular organization in which a catalytic core containing the invariant Roc1 ring finger protein and a cullin family member is associated with various adaptor proteins responsible for substrate recognition (3). For example, cullin 1 (Cul1) associates with Skp1 and a member of the F-box protein family to form an SCF (Skp1-cullin 1-F-box) complex (3), whereas cullin 4A (Cul4A) assembles with DDB1 (damaged DNA-binding protein 1) and a member of the DCAF (DDB1-cullin 4-associated-factor) family (4).

When usurping the proteasome-ubiquitin pathway, pathogens most often target the E3 ubiquitin ligase component to trigger the abnormal degradation of specific cellular proteins (5, 6). In this process, the viral protein acts as an adaptor between an E3 ubiquitin ligase and a cellular protein, which is subsequently ubiquitinated and targeted for degradation by the proteasome. The HIV virus extensively uses this strategy because three of its auxiliary proteins have been shown to target a CRUL. Vpu induces the degradation of the CD4 viral receptor by recruiting βTrCP, the F-box component of the SCFβTrCP complex (7). Vif triggers the degradation of APOBEC3G via hijacking of the cullin 5-ElonginB-ElonginC ubiquitin ligase (8), and as recently shown by us (11) and others (9, 10, 12–14), Vpr (viral protein R) targets the DCAF1 adaptor of the Cul4A-DDB1 ligase to induce cell cycle arrest in G2.

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† The abbreviations used are: E3, ubiquitin-protein isopeptide ligase; siRNA, small interfering RNA; SV, simian immunodeficiency virus; HIV-1, human immunodeficiency virus, type 1; HIV-2, human immunodeficiency virus, type 2; CRUL, cullin-ring ubiquitin ligase; Cull1, cullin 1; Cull4A, cullin 4A; HA, hemagglutinin; PBS, phosphate-buffered saline; GFP, green fluorescent protein; agm, African green monkey; iso, isoform; DN, dominant-negative; WT, wild-type; WD, tryptophan-aspartate repeat.
The HIV-1 Vpr gene has orthologs in the genomes of HIV-2 and the related SIVmac viruses (15). The Vpr protein, which is about 100 amino acids long, is encapsidated in the produced virions (16). This strongly suggests a role of Vpr in the early steps of the viral life cycle, which occur in the absence of viral protein synthesis and which eventually lead to integration into the host genome of the proviral DNA competent for transcription (17). Whether Vpr-induced G2 arrest provides a direct advantage for the virus or is merely the consequence of a more upstream event has not yet been clarified. In any event, recruitment by Vpr of the Cul4A-DDB1DCAF1 ubiquitin ligase is most likely functionally significant.

Remarkably, little is known as to the fate of the viral protein itself in the documented examples of viral proteins that operate through the recruitment of an E3 ligase. To address this question, we took advantage of our previous isolation of a Vpr mutant, which is defective in DCAF1 binding (11). Our results indicate that the Cul4A-DDB1DCAF1 ligase does not sense Vpr as a substrate and instead prevents its recognition by another E3 ligase responsible for its constitutive targeting to the proteasome.

EXPERIMENTAL PROCEDURES

Plasmid Constructs—The pCS2-Myc construct expressing Cul1 was kindly provided by Peter Jackson. The sequence coding for a dominant-negative form of Cul1 (the N-terminal region of Cul1 from residues 1 to 328) was subcloned into the same vector. pAS1B constructs encoding HA-tagged Vpr from HIV-1 Lai and HA-Vpr-Q65R, the vector expressing FLAG-tagged DCAF1-iso2 (GenBank™ accession number BC022792), and pCS2 vector expressing Myc-tagged DCAF1-WD domain have been described previously (11). DCAF1-iso1 (GenBank™ accession number NP_055518) was subcloned into the vector used to express FLAG-tagged DCAF1-iso2. The pAS1B as well as the pLex constructs of Vpr from HIV-2, SIVmac, and SIVagm have been described previously (18).

Cell Culture and Transfection Procedures—HeLa cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 2 mM glutamine and 10% fetal calf serum. Plasmid and siRNA transfections were performed using FuGENE 6 reagent (Roche Applied Science) and DharmaFECT reagent (Dharmacon), respectively. When indicated, cells were treated with 20 μM MG132 (Sigma) for 6 h.

siRNA—Control siRNA and siRNAs used to target the DCAF1 large isoform have been described previously (11). The following siRNAs purchased as a pool from Dharmacon were used to target DDB1: CACUAGAUCGCAUAAUAAUU/GAAGGUUCUUUGCGGAUCAUU/CAUCGACGGUGACUGAUUU/CAUCUCCGCGUCAUCUGUU. Yeast Methods—Two-hybrid experiments were performed in the L40 yeast strain as described previously (18).

Western Blot Analysis—Cells grown in 6-well plates were lysed in 200 μl/well M-PER buffer (Pierce) containing 150 mM NaCl and protease inhibitors (Sigma). Proteins from cell extracts were separated by SDS-PAGE and transferred onto polyvinylidene difluoride membranes and revealed by immunoblot analysis using a chemiluminescent procedure (ECL, GE Healthcare). Where indicated, signals were acquired by a LAS-3000 apparatus (Fuji) for further quantification using the provided software. Monoclonal antibodies directed against HA, Myc (9E10), and GFP were obtained from Roche, and anti-FLAG M2 monoclonal antibody was purchased from Sigma. Anti-DCAF1/VprBP rabbit polyclonal antibody was dis-
submitted by Gentaur and anti-β-catenin monoclonal antibody by BD Transduction Laboratories. The p24 HIV-1 capsid protein was revealed using a rabbit anti-p24 polyclonal antibody provided by the National Institute for Biological Standards and Control, Hertfordshire, United Kingdom.

Cell Cycle Analysis—Cells were scraped and fixed with 70% ethanol 48 h post-transfection. Following RNase A treatment and propidium iodide staining, cells expressing the internal membrane-anchored GFP were analyzed for their DNA content using a Cytomics FC500 cell analyzer (Beckman Coulter). At least 10,000 GFP-positive cells were analyzed for their distribution in the different phases of cell cycle using the MultiCycle software.

Immunofluorescence Assays—HeLa cells were seeded onto glass coverslips in 6-well plates at a density of 2 × 10⁵ cells/well and transfected with 100 ng of HA-Vpr-expressing vector and 400 ng of FLAG-DCAF1 plasmid per well. Forty-eight h post-transfection, cells were washed twice with PBS, fixed for 15 min in 4% paraformaldehyde, and washed again in PBS. Cells were permeabilized for 15 min in PBS containing 1% bovine serum albumin and 0.2% Tween 20 prior to incubation for 1 h with rabbit anti-FLAG antibodies (Sigma) diluted to 1:500. Following three washes in PBS/Tween buffer (PBS containing 0.2% Tween 20), cells were incubated for 30 min with Alexa Fluor 488-labeled anti-rabbit immunoglobulin antibodies (1:300) and Alexa Fluor 488-coupled anti-HA antibodies (1:300). After three washes in PBS/Tween buffer and mounting in medium containing 4’,6-diamidino-2-phenylindole (Vectashield), cells were examined by direct fluorescence microscopy.

Generation of HIV-1 Virus Particles—293T cells (1.5 × 10⁶ cells) were cotransfected with HIV-1 proviral DNA deficient for Vpr expression (pNL4-3ΔVpr) along with two plasmids coding for the vesicular stomatitis virus glycoprotein and HA-tagged Vpr in a ratio of 4:1:4. The culture supernatants were collected 48 h after transfection, and released viral particles were filtered through 0.45-μm pore filters. Viral titers were determined using P4R5 Magi indicator cells.

Viral Entry Assays—Forty-eight h after siRNA treatment, P4R5 cells (2 × 10⁵ cells) were incubated for 2 h with virus particles (multiplicity of infection ~1). Cells were then washed with PBS and treated once with 0.5% trypsin/EDTA to remove virus from the cell surface and further incubated for 6 h in culture medium. Cells were then harvested, lysed in M-PER buffer, and analyzed by Western blotting.

RESULTS

DCAF1 Binding Regulates Vpr Stability—In the course of our previous study of Vpr-mediated G2 arrest (11), we reproducibly observed that the Vpr(Q65R) mutant, which is defective in DCAF1 binding, was expressed at a slightly lower steady state level than its wild-type counterpart. To accurately assess their relative stabilities, Vpr(Q65R) and Vpr WT were coexpressed with GFP as an internal control in cells treated or not with the proteasome inhibitor MG132. Western blot analysis followed by quantification of the Vpr/GFP ratios confirmed that Vpr(Q65R) accumulated at a lower level than Vpr WT (Fig. 1A, compare lanes 3 and 1 and Fig. 1B). MG132 treatment increased the amount of both Vpr WT and Vpr(Q65R), the latter being stabilized three times more than wild-type Vpr (Fig. 1C). These observations indicate that Vpr is degraded via the proteasome pathway and that the Q65R mutation leads to enhanced targeting of the protein to the proteasome. This raised the issue that the previously shown defect of Vpr(Q65R) in G2 arrest activity reflects its enhanced turnover rather than its inability to recruit DCAF1. To address this point, the amounts of transfected expression vectors were adjusted to achieve equivalent expression of Vpr(Q65R) and Vpr WT (Fig. 1D, see Western blot and quantification panels). Under such conditions, Vpr(Q65R) still failed to induce G2 arrest in contrast to Vpr WT(Fig. 1E). Altogether, these data suggested that both the activity and the stability of Vpr depend on its ability to bind DCAF1 and that its degradation depends on a different ubiquitin ligase. Schröflauer et al. (19) reported that besides Cul4A, Cul1 could coimmunoprecipitate Vpr, which prompted us to examine the potential role of the Cul1-based E3 ligases in Vpr degradation. We used a truncated form of Cul1 (Cul1-DN), which acts as a dominant-negative mutant by competing with full-length Cul1 for the binding of its adaptors and substrates. As expected, expression of Cul1-DN led to the stabilization of endogenous β-catenin, a well known substrate of the Cul1-Skp1-βTrCP E3 ligase, which acts as a dominant-negative mutant by competing with full-length Cul1 for the binding of its adaptors and substrates. As expected, expression of Cul1-DN led to the stabilization of endogenous β-catenin, a well known substrate of the Cul1-Skp1-βTrCP complex (20) (Fig. 2, compare lanes 5 and 1). In contrast, Cul1-DN did not enhance the pool of the unstable Vpr(Q65R) mutant, ruling out a role of Cul1-based CRULs in targeting Vpr for proteasome-mediated degradation.

Supporting our hypothesis that Vpr stability depends on its ability to bind DCAF1, overexpression of full-length DCAF1 (DCAF1-iso1) led to increased accumulation of Vpr WT (Fig. 3B, lanes 1 and 3) but did not affect the pool of Vpr(Q65R) (Fig. 3B,
FIGURE 3. Overexpression of DCAF1 protects wild-type Vpr from proteasome-mediated degradation, whereas silencing of DCAF1 decreases steady state expression of Vpr. A, a schematic representation of the used derivatives of DCAF1 is shown. Isoform 1 is the major product of the dcaf1 gene, whereas iso2 represents a naturally occurring minor variant. The previously described DCAF1-WD domain encompasses the Vpr- and DDB1-binding regions. The WD repeat motifs are depicted by filled circles. In B, C, and D, Vpr WT or its DCAF1-binding defective counterpart was expressed either alone or with the indicated DCAF1 derivative. A vector encoding GFP was included as an internal expression control, and the amount of transfected DNA was kept constant by the addition of the relevant empty vector. Transfection of HeLa cells, treatment with MG132, and Western blot analysis of cell lysates were carried out as described in the Fig. 1 legend. E, HeLa cells were transfected with 10 ng DCAF1-specific or control (Ctrl) siRNAs. One day later, cells were transfected with the indicated Vpr construct along with a GFP-expressing vector. In this particular experiment, comparable expression of the two Vpr species was achieved by using 1 μg of the Vpr(Q65R) versus 200 ng of the Vpr WT expression vectors. Two days after this second transfection round, HeLa cells were processed for Western blot analysis as described in the Fig. 1 legend. F, the signals shown in the left panel were quantified, and the calculated HA/GFP ratios were represented in the bar graph shown in the right panel.

lanes 5 and 7). As a control, we checked that DCAF1 overexpression increases the bulk level of DCAF1 (supplementary Fig. S2). MG132 treatment restored identical levels of Vpr WT and Vpr(Q65R), in the absence or in the presence of DCAF1 (Fig. 3B, lanes 2, 4, 6, and 8), indicating that differences in Vpr and Vpr(Q65R) expression levels result from differences in protein stability. These differences are abolished when the proteasome is inactivated. We next conducted similar experiments using two other DCAF1 derivatives previously shown to bind Vpr (11), namely DCAF1 iso2 that lacks a 460-amino acid region as compared with DCAF1 iso1 and DCAF1-WD, which encompasses the WD40 repeat domain of the protein (Fig. 3A). Both DCAF1 iso2 and DCAF1 WD stabilized Vpr but not Vpr(Q65R) (Fig. 3C and D, compare lanes 3 and 1 and lanes 7 and 5). These results suggested that the abundance of DCAF1 molecules available for Vpr binding impacts on the viral protein pool. In support of this conclusion, siRNA-mediated depletion of DCAF1 decreased steady state levels of Vpr WT but not of Vpr(Q65R) (Fig. 3E, compare lanes 2 and 1 and lanes 4 and 3, and Fig. 3F).

DCAF1-mediated Stabilization of Vpr Is Conserved in Other Primate Lentiviral Lineages—The region surrounding the Gln65 residue in Vpr from HIV-1, which is critical for DCAF1 binding, is highly conserved in Vpr proteins from other primate lentiviruses (11), and we hypothesized that DCAF1-Vpr interaction would be conserved across these lentiviral species. Confirming this prediction, yeast two-hybrid assays revealed a strong interaction between DCAF1 and Vpr from SIVmac, HIV-2, and SIVagm (supplementary Fig. S1). We next investigated whether DCAF1 also modulated the stability of these Vpr species. The results shown in Fig. 4, A and B, indicate that the HIV-2 and SIVmac Vpr proteins behave similarly to the HIV-1 Vpr protein. Their turnover is dependent on the proteasome pathway as shown by their increased accumulation following a 6-h treatment with MG132, and overexpression of DCAF1 enhanced their steady state levels by decreasing their degradation rate (see quantification in Fig. 4B). By contrast, Vpr from SIVagm shows a higher intrinsic stability because its accumulation was affected neither by MG132 treatment nor by overexpression of DCAF1. In this respect, SIVagm Vpr was more similar to the related Vpx protein than to the other Vpr species (data not shown). We also note that Vpr from SIVagm shows no detectable interaction with uracil-N-glycosylase 2, in contrast to Vpr species from HIV-1, HIV-2, and SIVmac (supplementary Fig. S1), and that it fails to induce G2 arrest in HeLa cells (data not shown and Ref. 21).

Vpr Redistributes into the Cytoplasm upon Stabilization by DCAF1—To confirm DCAF1-mediated stabilization of Vpr at the cellular level, we performed immunofluorescence studies (Fig. 5). In cells expressing exogenous FLAG-DCAF1, a stron-
ger staining was observed for HA-Vpr WT and the viral protein accumulated in the cytoplasm (Fig. 5A). By contrast, the Vpr(Q65R) mutant was detected with similar intensity regardless of DCAF1 coexpression and remained nuclear (Fig. 5B). Our finding that overexpression of DCAF1 leads to cytoplasmic accumulation of Vpr WT is consistent with previous observations reported by Zhang et al. (22). In light of the currently proposed mechanism of Vpr-induced cell cycle arrest (11), it might suggest that ubiquitination of Vpr targets occurs in the cytoplasm following recruitment of the Cul4A-DDB1-DCAF1 complex.

**Vpr Is Stabilized within an Active Cul4A-DDB1-DCAF1 Complex**—Stabilization of Vpr might merely result from an association with free DCAF1 that could mask the recognition site of another E3 ubiquitin ligase. Alternatively, it might require Vpr assembly with a functional Cul4A-DDB1-DCAF1 ubiquitin ligase. To address this question, we coexpressed Vpr and DCAF1 in cells where DDB1 was depleted by siRNA treatment. As expected, DCAF1-mediated Vpr stabilization still occurred in cells transfected with control siRNA (Fig. 6A, lanes 1 and 2). In contrast, DDB1 silencing decreased steady levels of Vpr WT and prevented Vpr stabilization by DCAF1 overexpression (Fig. 6A, lanes 3 and 4). That Vpr stabilization was dependent on DDB1 suggested that the whole Cul4A-DDB1-DCAF1 ubiquitin ligase is required to protect Vpr from proteasomal degradation. In agreement with this hypothesis, DCAF1 overexpression significantly enhanced Vpr-mediated G2 arrest (Fig. 6B).

**Virion-associated Vpr Is Protected from Degradation within the Cul4A-DDB1-DCAF1 Complex**—In this system, the detected Vpr protein could only come from the incoming viral particle. In agreement with our hypothesis, both DDB1 and DCAF1 silencing decreased the levels of the incoming Vpr protein (Fig. 7B, compare lanes 2 and 1 and lanes 3 and 1).

**DISCUSSION**

The main finding of our study is that the Cul4A-DDB1-DCAF1 CRUL, which is usurped by HIV-1 Vpr to promote cell cycle arrest, protects the viral protein from proteasomal degradation by another E3 ubiquitin ligase. This mechanism can take place in the early steps of the viral cell cycle when the virion-associ-
ated protein has just been delivered to the infected cell. To the best of our knowledge, this is the first documented example of a mechanism in which a viral protein exploits a host E3 ligase both to mediate its activity and to extend its half-life, thus creating a positive feedback loop.

A more general feature of viral proteins that act by hijacking a host E3 ligase appears to be their escape from the ubiquitination process catalyzed by the recruited enzyme. Thus, we recently demonstrated that HIV-1 Vpu is not targeted for degradation by the CRUL that it recruits although it binds to the βTrCP adaptor through the very motif, which normally labels proteins as substrates. Instead another as yet non-identified ligase ensures proteasome targeting of the viral protein (23). A similar process is likely to occur for the HPV E6 and E7 proteins, whose fate as adaptors or substrates involves separable domains of the viral protein (24, 25). Thus, E7 targets the Rb protein for degradation through the use of a cullin 2 CRUL (26) and is itself degraded by a Skp2-containing Cul1 CRUL (27). Another example documented by Precious et al. (28) is the SV5 V protein, which induces STAT1 degradation through the use of the Cul4A-DDB1 core ligase. These authors elegantly underscore a catalytic process, where a small pool of V protein is recycled to degrade a large excess of STAT1. How an E3 ligase discriminates substrates from adaptors regardless of their viral or host origin is an intriguing question that is still a matter of debate (for a review, see Ref. 29). In any event, protection of its own constituents is a requisite for an E3 ligase to achieve a catalytic ubiquitination process as opposed to a rather inefficient stoichiometric process.

Only a few studies have addressed the stability of Vpr WT protein. Kewalramani and Emerman (30) reported a half-life of ~20 h for HIV-1 Vpr, in marked contrast with a much shorter half-life of HIV-2 Vpr, estimated to 90 min. We did not observe

![FIGURE 6. DCAF1-mediated stabilization of Vpr occurs within a functional Cul4-DDB1^DCAF1^ E3 ligase. A, silencing of DDB1 prevents Vpr stabilization by DCAF1. HeLa cells were transfected with 10 nM DDB1-specific or control (ctrl) siRNAs. One day later, cells were transfected with Vpr WT construct together with DCAF1-WD vector or the corresponding empty vector. A GFP-expressing vector was included to standardize the transfection efficiencies. Two days after this second transfection round, HeLa cells were processed for Western blot analysis as described in the Fig. 1 legend. The signals shown in the left panel were quantified, and the calculated HA/GFP ratios are represented in the bar graph shown in the right panel. B, Vpr-induced G2 arrest is enhanced by overexpression of DCAF1. HeLa cells were transfected with a GFP expression vector along with vectors expressing Vpr WT, DCAF1, or both as indicated. The total amount of DNA was kept constant by the addition of empty expression vectors. Cell cycle analysis was performed as described in the legend to Fig. 1D.](image)

![FIGURE 7. Vpr from the incoming HIV-1 particles is protected from degradation within the Cul4A-DDB1^DCAF1^ complex. P4R cells were transfected with 20 nM control (Ctrl), DDB1, or DCAF1-specific siRNAs. Two days later, cells were infected for 2 h with HIV-1 Vpr virus particles containing packaged HA-tagged Vpr. Cells were then trypsinized, washed, incubated for 6 h with medium culture, and processed for Western blot analysis as described in the Fig. 1 legend. The viral capsid (CA) was used to assess the quantity of incoming viral particles. The signals shown in the left panel were quantified, and the calculated HA/capsid protein ratios are represented in the bar graph shown in the right panel. A timeline of the experiment is presented at the top of the figure.](image)
such a difference between the turnover of HIV-1 Vpr and that of HIV-2 and SIVmac Vpr species. Moreover, based on the 2–3-fold stabilization of Vpr by a 6-h MG132 treatment, we estimate a Vpr half-life of ~3 h, close to the 4 h reported in the study of Schröflbauer et al. (19). The latter study also showed stabilization of Vpr by MG132 in agreement with a proteasome-mediated degradation process. That a relatively short half-life is conserved across Vpr species from HIV-1, HIV-2, and SIVmac despite the well known plasticity of retroviral genomes argues for a functional constrain. Among possible hypotheses, mutational inactivation of the Vpr degron might be incompatible for a functional constrain. Dual Role of Cul4A-DDB1-DCAF1 Ubiquitin Ligase toward Vpr

Stabilization of Vpr by the Cul4A-DDB1-DCAF1 is reminiscent of DDB1-mediated stabilization of the HBx protein encoded by human hepatitis B virus (31, 32). However, that HBx uses the DDB1-Cul4 ligase to induce the degradation of host proteins has not yet been addressed. In the case of Vpr, which is packaged in the virion (16), such a protection mechanism may represent an efficient way to optimize the activity of Vpr molecules that are delivered by the incoming virus before neo-synthesis takes place.

So far, no cell culture system has revealed a severe defect in HIV replication in the absence of Vpr. However, the functional importance of Vpr is strongly suggested by its conservation by HIV replication in the absence of Vpr. Nonetheless, the well known plasticity of retroviral genomes argues against an increased toxicity disadvantageous for the virus. The distinctive stability of SIVagm Vpr together with its lack of interaction with uracil-N-glycosylase 2 and of cell cycle arrest activity may raise questions as to its classification as a Vpr ortholog. In this respect, it is interesting to note that prior to the phylogenetic study of Tristem et al. (15), the Vpr-like protein encoded by SIVagm was actually classified as Vpx.

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REFERENCES
1. Scheffner, M., Huibregtse, J. M., Vierstra, R. D., and Howley, P. M. (1993) Cell 75, 495–505
2. Hershko, A., and Ciechanover, A. (1998) Annu. Rev. Biochem. 67, 425–479
3. Petroski, M. D., and Deshaies, R. J. (2005) Nat. Rev. Mol. Cell Biol. 6, 9–20
4. Lee, J., and Zhou, P. (2007) Mol. Cell 26, 775–780
5. Barry, M., and Fruh, K. (2006) Science’s STKE 335, pe21
6. Banks, L., Pim, D., and Thomas, M. (2003) Trends Biochem. Sci. 28, 452–459
7. Margottin, F., Bour, S. P., Durand, H., Selig, L., Becichou, S., Richard, V., Thomas, D., Strebel, K., and Benarous, R. (1998) Mol. Cell 1, 565–574
8. Yu, X., Yu, Y., Liu, B., Luo, K., Kong, W., Mao, P., and Yu, X. F. (2003) Science 302, 1056–1060
9. Hrecka, K., Gierszewskia, M., Srivastava, S., Kozaczkiewicz, L., Swanson, S. K., Flores, L., Washburn, M. P., and Skowronski, J. (2007) Proc. Natl. Acad. Sci. U. S. A. 104, 11778–11783
10. DeHart, J. L., Zimmerman, E. S., Ardon, O., Monteiro-Filho, C. M., Arganaraz, E. R., and Planelles, V. (2007) Virol. J. 4:57
11. Le Rouzi, E., Belaidouni, N., Estrabaud, E., Morel, M., Rain, J.-C., Transy, C., and Margottin-Goguet, F. (2007) Cell Cycle 6, 182–188
12. Tan, L., Ehrlich, E., and Yu, X. F. (2007) J. Virol. 81, 10822–10830
13. Wen, X., Duus, K. M., Friedrich, T. D., and de Noronha, C. M. (2007) J. Biol. Chem. 282, 27046–27057
14. Belzile, J. P., Duist, G., Rougeau, N., Mercier, J., Finzi, A., and Cohen, E. A. (2007) PLoS Pathog. 3, e85
15. Tristem, M., Marshall, C., Karpas, A., and Hill, F. (1992) EMBO J. 11, 3405–3412
16. Cohen, E. A., Dehni, G., Sodroski, J. G., and Haseltine, W. A. (1990) J. Virol. 64, 3097–3099
17. Poon, B., Grovit-Ferbas, K., Stewart, S. A., and Chen, I. S. (1998) Science 281, 266–269
18. Selig, L., Benichou, S., Rogel, M. E., Wu, L. I., Vodicka, M. A., Sire, J., Benarous, R., and Emerman, M. (1997) J. Virol. 71, 4842–4846
19. Schröflbauer, B., Yu, Q., Zeitlin, S. G., and Landau, N. R. (2005) J. Virol. 79, 10978–10987
20. Fuchs, S. Y., Spiegelman, V. S., and Kumar, K. G. (2004) Oncogene 23, 2028–2036
21. Zhu, Y., Gelbard, H. A., Roshal, M., Pursell, S., Jamieson, B. D., and Planelles, V. (2001) J. Virol. 75, 3791–3801
22. Zhang, S., Feng, Y., Narayan, O., and Zhao, L. J. (2001) Gene 263, 131–140
23. Estrabaud, E., Le Rouzi, E., Lopez-Verges, S., Morel, M., Belaidouni, N., Benarous, R., Transy, C., Berlioz-Torrent, C., and Margottin-Goguet, F. (2007) PLoS Pathog. 3, e104
24. Kehmeier, E., Ruhl, H., Voland, B., Stoppler, M. C., Androphy, E., and Stoppler, H. (2002) Virology 299, 72–87
25. Gonzalez, S. L., Stremmlau, M., He, X., Basile, J. R., and Munger, K. (2001) J. Virol. 75, 7583–7591
26. Huh, K., Zhou, X., Hayakawa, H., Cho, J. Y., Libermann, T. A., Jin, J., Harper, J. W., and Munger, K. (2007) J. Virol. 81, 9737–9747
27. Oh, K. J., Kalinka, A., Wang, J., Nakayama, K., Nakayama, K. I., and Bagchi, S. (2004) J. Virol. 78, 5338–5346
28. Precious, B. L., Carlos, T. S., Goodbourn, S., and Randall, R. E. (2007) Virology 368, 114–121
29. Wu, J. T., Chan, Y. R., and Chien, C. T. (2006) Trends Cell Biol. 16, 362–369
30. Kewalramani, V. N., and Emerman, M. (1996) Virology 218, 159–168
31. Bergametti, F., Bianchi, J., and Transy, C. (2002) J. Virol. 76, 706–715
32. Leupin, O., Bontron, S., and Subrin, M. (2003) J. Virol. 77, 6274–6283
33. Banks, L., Pim, D., and Thomas, M. (2003) Trends Biochem. Sci. 28, 452–459
34. Margottin, F., Bour, S. P., Durand, H., Selig, L., Benichou, S., Richard, V., Thomas, D., Strebel, K., and Benarous, R. (1998) Mol. Cell 1, 565–574
35. Yu, X., Yu, Y., Liu, B., Luo, K., Kong, W., Mao, P., and Yu, X. F. (2003) Science 302, 1056–1060