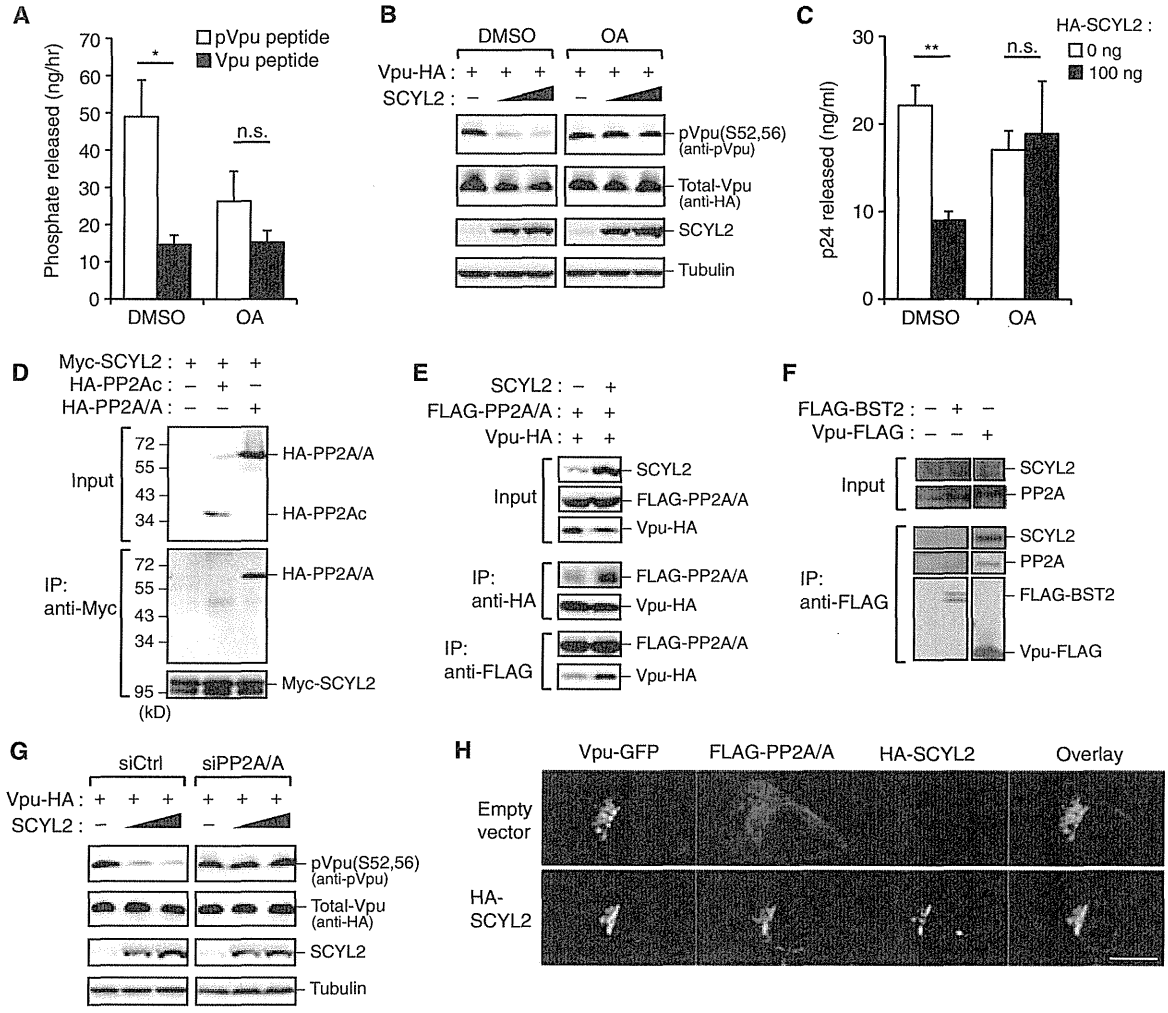

Fig. 6. SCYL2 promotes PP2A-mediated dephosphorylation of Vpu. (A) SCYL2 immunoprecipitates have phosphatase activity toward Ser^{52} and Ser^{56} on Vpu. HEK 293T cells expressing HA-SCYL2 were lysed and subjected to immunoprecipitation with an anti-HA antibody in buffer containing dimethyl sulfoxide (DMSO) or 10 nM OA. The immunoprecipitates were incubated with either pVpu peptide (white bar) or the nonphosphorylated equivalent (black bar). After 8 hours, the amounts of released phosphates in the reaction mixture were measured. n.s., not significant; $^*P = 0.0410$; $n = 3$ experiments. (B) OA inhibits the SCYL2-mediated dephosphorylation of Vpu. HEK 293T cells were transfected with plasmids expressing Vpu-HA and SCYL2 at a molar ratio of 1:5 or 1:10. Cells were treated with DMSO or 10 nM OA for 18 hours before being harvested. Forty-eight hours after transfection, cell lysates were subjected to Western blotting with the indicated antibodies. (C) OA blocks SCYL2-mediated restriction of viral release. HeLa cells were cotransfected with pNL4-3 (100 ng) with or without plasmid encoding SCYL2. Cells were then treated with DMSO control or 10 nM OA for 18 hours before being harvested. Forty-eight hours after transfection, culture supernatants were subjected to p24 ELISA. n.s., not significant; $^{**}P = 0.0039$; $n = 3$ experiments. (D) SCYL2 interacts with the scaffold A subunit of PP2A (PP2A/A). HEK 293T cells were transfected with expression plasmids for the indicated proteins. Cell lysates were immunoprecipitated with an anti-Myc antibody, and bound proteins were visualized by Western blotting. (E) SCYL2 enhances the interaction between Vpu and PP2A/A. HEK 293T cells were transfected with vectors encoding the indicated proteins. Cell lysates were immunoprecipitated with anti-HA or anti-FLAG antibodies, and bound proteins were visualized by Western blotting analysis. (F) Vpu interacts with endogenous SCYL2 and PP2A. HEK 293T cells were transfected with vectors encoding the indicated proteins. Cell lysates were immunoprecipitated with anti-FLAG antibody, and bound proteins were visualized by Western blotting analysis. (G) Depletion of PP2A/A inhibits SCYL2-mediated dephosphorylation of Vpu. HEK 293T cells were treated with either control (siCtrl) or PP2A/A-specific siRNA (siPP2A/A) for 24 hours before being transfected with plasmids expressing Vpu-HA and SCYL2 at molar ratios of 1:5 and 1:10. Forty-eight hours after transfection, cell lysates were subjected to Western blotting with antibodies against the indicated proteins. (H) Immunofluorescence analysis of HeLa cells transiently coexpressing Vpu-GFP (green), FLAG-PP2A/A (red), and either HA-SCYL2 (blue) or empty vector. Scale bar, 10 μm. Data shown in (B) and (D) to (H) are representative of three experiments.

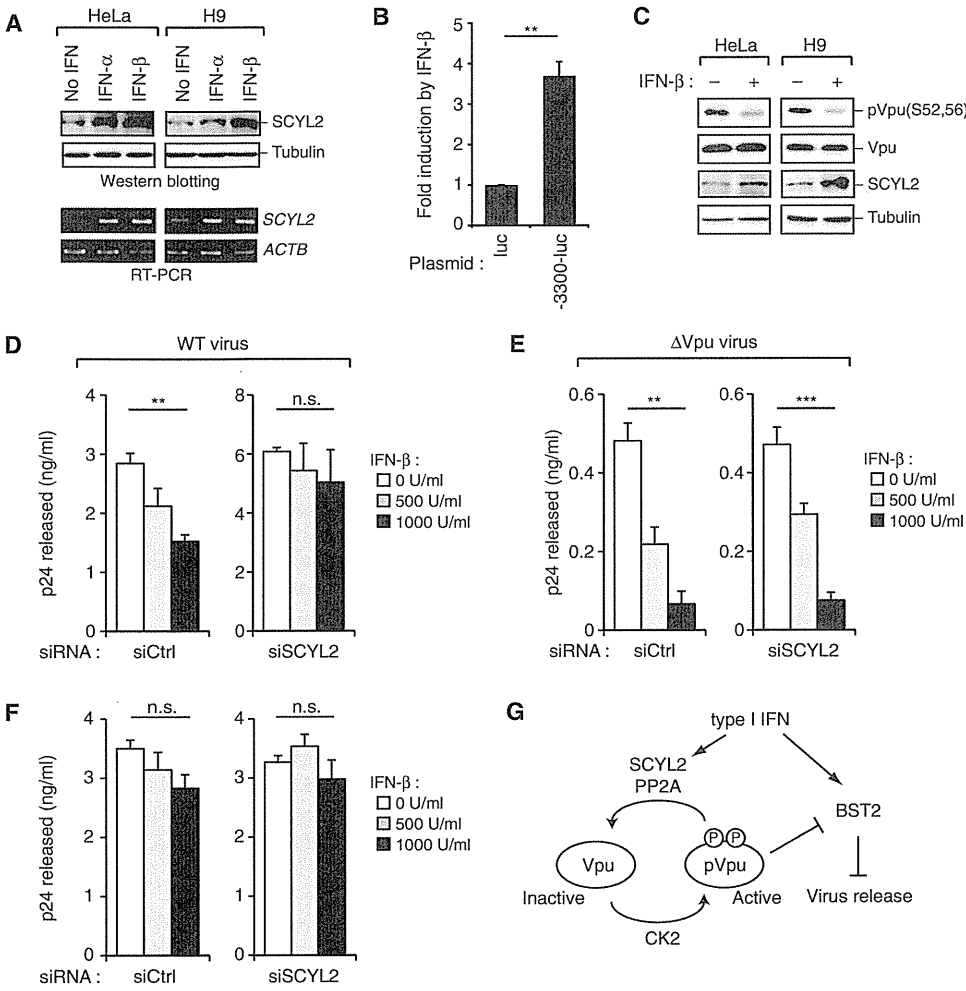

Fig. 7. SCYL2 mediates the type I IFN–induced antiviral response. (A) Type I IFN increases the abundance of SCYL2. Western blotting analysis (top) and RT-PCR analysis (bottom) of the indicated cells treated with IFN-α or IFN-β (1000 U/ml) for 6 hours before harvesting. Representative data from three experiments are shown. (B) The SCYL2 promoter is transactivated by IFN-β. HeLa cells were cotransfected with the pGL4-luc vector encoding SCYL2 promoter (-3300-luc) together with pGL4-TK-Rluc as a transfection control. Forty-eight hours after transfection, cells were treated with IFN-β (1000 U/ml) for 6 hours and cell lysates were subjected to a luciferase reporter assay. The fold induction in IFN-treated cells was determined. $^{**}P = 0.0036$; $n = 3$ experiments. (C) IFN-β induces Vpu dephosphorylation in infected T cells. The indicated cells were infected with VSVG-pseudotyped HIV-1_{NL4-3} [at a multiplicity of infection (MOI) of 0.2] and treated with IFN-β (1000 U/ml) for 8 hours before being harvested. Cell lysates were subjected to Western blotting with antibodies against the indicated proteins. Data are representative of three experiments. (D to F) SCYL2 depletion partially blocks the antiviral effect of IFN-β. (D and E) HeLa cells or (F) BST2-knockdown HeLa cells were treated with either control or SCYL2-specific siRNA for 24 hours before being infected with (D and F) VSVG-pseudotyped HIV-1_{NL4-3} or (E) its Vpu-deficient derivative at an MOI of 0.01. Forty-eight hours after infection, the cells were washed and treated with IFN-β (0, 500, or 1000 U/ml) for 8 hours. Culture supernatants and cell lysates were subjected to p24 ELISA. n.s., not significant; $^{**}P = 0.0079$ for (D); $^{**}P = 0.0012$ and $^{***}P = 0.0007$ for (E); $n = 3$ experiments. (G) A model depicting the proposed signaling cascade in type I IFN–mediated viral release restriction. Type I IFN induces the production of both BST2 and SCYL2 in infected cells. SCYL2 binds to Vpu, recruits PP2A, and inhibits the anti-BST2 activity of Vpu, thereby facilitating the BST2-mediated restriction of viral release.

these observations indicated that SCYL2 is itself IFN-inducible, and that by antagonizing Vpu function and consequently facilitating BST2-mediated restriction, SCYL2 stimulates the anti-HIV activity of type I IFN (Fig. 7G).

DISCUSSION

Here, we demonstrated that SCYL2 is an IFN-inducible gene that regulates the phosphorylation of HIV-1 Vpu by recruiting PP2A to the viral protein, thereby inhibiting Vpu function and enhancing the antiviral activity of BST2 in HIV-1–infected cells. SCYL2 was originally identified as a poly-L-lysine–stimulated kinase that phosphorylates the β2 subunit of the adaptor protein AP-2 (*50*). Although the idea of whether SCYL2 has kinase activity is still controversial (*52*), a subsequent study suggested that SCYL2 is a multifunctional protein involved in membrane trafficking between the *trans*-Golgi and endosomes (*51*). Other studies have reported a role for SCYL2 in the clathrin-mediated sorting of t-SNARE (target-soluble *N*-ethylmaleimide–sensitive factor attachment protein receptor) proteins (*52*) and in the Wnt signaling pathway (*59*). Here, we reveal an additional role for SCYL2 as an inhibitor of viral release in HIV-1–infected cells through its inhibition of the phosphorylation of Vpu.

A study predicted the existence of at least 4000 as yet unidentified phosphorylation sites on hundreds of viral proteins (*60*). Indeed, the phosphorylation of viral proteins is one of the most important processes for efficient viral replication and pathogenesis (*61*). Previous biochemical analyses have reported that the phosphorylation of Vpu on key serine residues (Ser^{52} and Ser^{56}) is catalyzed by CKII (*29–31*). Because CKII is a ubiquitously expressed serine and threonine (Ser/Thr) kinase (*62*), the Ser^{52} and Ser^{56} phospho-acceptor sites are likely to be constitutively phosphorylated in infected cells, suggesting that dephosphorylation could be a key step for exerting control over Vpu function. However, the sequence requirements of major cellular phosphatases are less specific than those of protein kinases (*63*), and additional cofactors are likely needed for phosphatases to target a specific substrate. Indeed, many transporter or adaptor proteins interact with subunits of phosphatases to promote substrate recognition by these dephosphorylating enzymes (*64*).

Although the phosphorylation of Vpu is required for optimal anti-BST2 activity, the precise molecular mechanism by which the phosphorylation status of Vpu is controlled has been elusive. The association of SCYL2 with both Vpu and PP2A suggests a previously unrecognized mechanism through which the activity of Vpu is regulated by SCYL2-mediated recruitment of PP2A and dephosphorylation. Our data suggest that SCYL2 induces the dephosphorylation

of Vpu, abrogating Vpu function and consequently inhibiting viral particle release through BST2. Our model of the SCYL2-PP2A-Vpu virus-host interaction is reminiscent of previously described interactions among cellular proteins; for example, cyclin G recruits PP2A to Mdm2 in a similar manner to facilitate the dephosphorylation and functional modification of Mdm2 (*65*).

Our model, however, does not directly address the original role of SCYL2 as a component of clathrin-coated vesicles. Although the role of clathrin during Vpu-induced BST2 inactivation is still uncertain, substantial data suggest that a clathrin-dependent pathway contributes to the antagonism of BST2 by Vpu (*19*, *24*, *66*, *67*). On the basis of our present findings that clathrin is required for SCYL2 function with respect to Vpu, SCYL2 could conceivably inhibit Vpu by modulating clathrin-mediated membrane trafficking. Of note in this regard, several studies have proposed that Vpu counteracts BST2 at least partly through a β-TrCP–independent (phosphorylation-independent) pathway (*54*, *55*). Indeed, we observed that SCYL2 interacted with a nonphosphorylated mutant of Vpu, as well as with wild-type Vpu, implying that SCYL2 might interfere with the anti-BST2 activity of Vpu irrespective of the phosphorylation status of Vpu. However, the phosphatase inhibitor OA almost completely abrogated the activity of SCYL2. Moreover, SCYL2 did not affect the virion release of an HIV-1 derivative encoding a nonphosphorylatable form of Vpu. Together, these results suggest that the function of SCYL2 as a Vpu inhibitor is likely dependent on the phosphorylation of the Vpu residues Ser^{52} and Ser^{56}.

Our data indicate that human SCYL2 has no obvious efficacy against ancestral Vpu, which suggests that the SCYL2-Vpu interaction might have appeared during the evolution of primate lentiviruses. However, whether SCYL2 from GSN monkeys might interfere with SIV_{GSN} Vpu and whether primate SCYL2 has evolved to gain an antagonistic interaction with HIV-1 Vpu are currently unresolved questions. How can the acquisition of SCYL2-binding activity by HIV-1 Vpu be reconciled with the seemingly inhibitory function of SCYL2 on HIV-1 release? One possibility is that the SCYL2-Vpu interaction is associated with an unrecognized benefit in some aspect of the viral life cycle. For example, SCYL2 might increase the stability of Vpu through dephosphorylation. Indeed, a previous report suggested that nonphosphorylated Vpu is more stable than wild-type Vpu (*68*). Another possibility is that the cycle of phosphorylation and dephosphorylation is important for Vpu activity. Nonetheless, our observation that overexpression of SCYL2 inhibits the antagonism of BST2 by Vpu, whereas depletion of endogenous SCYL2 has the opposite effect, suggests that, at least with regard to this function, SCYL2 behaves as an inhibitor rather than a cofactor of Vpu.

In addition to BST2, several factors induced by type I IFN contribute to viral restriction during the late stages of virus assembly and release. For example, the ubiquitin-like protein ISG15 specifically inhibits the ubiquitination of Gag and its association with Tsg101, resulting in the restriction of HIV-1 budding (*40*). A tripartite motif (TRIM) protein, TRIM22, is another ISG that blocks HIV-1 assembly by disrupting the proper trafficking of Gag to the plasma membrane (*41*). These restrictions are Vpu-independent mechanisms because these factors either interact with Gag or modulate its function. Our data demonstrate that type I IFN can also trigger the dephosphorylation of Vpu through SCYL2 and thereby facilitate BST2-mediated viral restriction even in the presence of Vpu, implying a regulatory role for SCYL2 among the array of IFN-mediated antiviral host measures. In conclusion, our current study provides evidence that the IFN-mediated antiviral response targets the posttranslational modification of Vpu and contributes to the inhibition of HIV-1 release from infected cells. The molecular machinery underlying the posttranslational modification of Vpu may thus be an attractive therapeutic target for the treatment of HIV infection.

MATERIALS AND METHODS

In vitro protein production

A total of 412 complementary DNAs (cDNAs) encoding human protein kinases were constructed as described previously (*69*). The protein production method has been described previously (*42*, *70*, *71*). Briefly, DNA templates containing a biotin-ligating sequence were amplified by split-PCR with cDNAs and corresponding primers and then used with the GenDecoder protein production system (CellFree Science). For synthesis of HIV-1 Vpu protein, *vpu* genes derived from the pNL4-3 proviral plasmid (*72*) were generated by split-PCR and used as templates with the Wheat Germ Expression Kit (CellFree Science) in accordance with the manufacturer's instructions.

AlphaScreen-based protein-protein interaction assays

FLAG-tagged Vpu proteins were mixed with biotinylated kinases in 15 μl of reaction buffer [20 mM tris (pH 7.6), 5 mM $MgCl_2$, 1 mM dithiothreitol] in a well of 384-well optiplates (PerkinElmer) and incubated at 26°C for 1 hour. The mixture was then added to AlphaScreen buffer containing anti–immunoglobulin G (protein A) acceptor beads and streptavidin-coated donor beads (0.1 μl each; PerkinElmer) and the anti-FLAG M2 antibody (5 μg/ml; Sigma) and further incubated at 26°C. One hour later, AlphaScreen signals from the mixture were detected with an EnVision device (PerkinElmer) with the AlphaScreen signal detection program.

Cells and transfections

HEK 293T, HeLa, LLC-MK2, Jurkat, and H9 cells were cultured under standard conditions. Plasmid or siRNA transfection into adherent cells was performed with the Effectene or Hiperfectamine transfection reagent (Qiagen), respectively, in accordance with the manufacturer's instructions. Transfection of suspension cells with plasmids was performed with the Neon Transfection System (Invitrogen) according to the manufacturer's protocol.

Plasmids and viruses

Human SCYL2 (GenBank accession no. BC063798) full-length sequences (1 to 929 amino acid residues) and deletion inserts (1 to 375, 1 to 697, and 376 to 929 amino acids) were amplified from pCMV6-XL4-SCYL2 (Origene) with primer pairs containing restriction enzyme sites and a stop codon. These inserts were subcloned into pCMV-HA/pCMV-Myc vector (Clontech). Alternatively, on the basis of bioinformatic predictions, the 3.3-kb fragment upstream of the SCYL2-encoding gene was amplified from HEK 293T cDNA and subcloned into the pGL4.10 firefly luciferase reporter vector (Promega). A human codon-optimized HIV-1 Vpu expression vector (*73*) and a Vpu-deleted HIV-1 molecular clone (*74*) were provided by K. Strebel [National Institutes of Health (NIH), Bethesda, MD]. The Vpu(S52,56A) mutants were constructed with standard molecular cloning procedures. The construct encoding KSHV-K5 (*75*) was provided by P. Cannon (University of Southern California, Los Angeles, CA). The plasmids expressing PP2A/A and $PP2A_C$ (*76*) were provided by A. Yamashita (Yokohama City University, Kanagawa, Japan). The VSVG-pseudotyped virus stocks were produced by transient transfection of HEK 293T cells with the pNL4-3 proviral clone and vectors expressing VSVG at a molar ratio of 3:1. After 48 hours, the culture supernatants containing virus were collected, filtered through a 0.45-μm Millex-HV filter (Millipore), and immediately stored at −80°C until required.

siRNA, IFN treatments, and RT-PCR

An array of siRNAs targeting the genes shown in Fig. 1D, as well as BST2, CHC, and PP2A/A, was obtained from Qiagen and used as a mixture of three different targeting sequences. In experiments involving SCYL2 knockdown, we used at least two SCYL2-specific Stealth RNAi constructs (oligo ID

HSS124796 and HSS183194, Invitrogen) to avoid off-target effects. Stealth RNAi Luciferase Reporter Control (Invitrogen) was used as the negative control. For detection of SCYL2 mRNA in IFN-treated cells, total RNA was extracted with the RNeasy Mini Kit (Qiagen) from cells treated with or without recombinant human IFN-α (1000 U/ml; Sigma) or IFN-β (1000 U/ml; PBL Biomedical Laboratories) for 6 hours before harvesting. The cDNA was generated with ReverTra Ace (Toyobo) and an oligo$(dT)_{20}$ primer, and then PCR was performed with Ex Taq (Takara Bio). The PCR primers used were as follows: *SCYL2*, 5′-gggaatcagcaaattgacaaagttt-3′ (forward) and 5′-agtccttagtctgtttaacagtagc-3′ (reverse); *ACTB*, 5′-ggacttcgagcaagagatgg-3′ (forward) and 5′-agcactgtgttggcgtacag-3′ (reverse).

Single-cycle virus release assays

For transfection-based assays, cells in 12-well plates were cotransfected with pNL4-3 or pNL4-3ΔVpu (100 ng) and either an SCYL2 expression vector or an empty vector (0, 50, and 100 ng) and cultured for 2 days. For infection-based assays, cells were infected with the VSVG-pseudotyped HIV-1 at an MOI of 0.01 or 0.2 for 8 hours and cultured for 2 days (HeLa cells) or 4 days (H9 cells). In experiments with siRNA, cells were transfected with a pool of siRNAs 24 hours before being either transfected with pNL4-3 or infected with virus. Virus-containing supernatants were harvested and filtered to remove debris, and viral p24 antigens were measured with an ELISA kit (Zepto Metrix). The cell lysates were prepared with HBST buffer [10 mM Hepes (pH 7.4), 150 mM NaCl, 0.5% Triton X-100] containing protease inhibitor cocktail (Roche). Western blotting analysis and the antibodies used have been described previously (*77*). In some experiments, OA (Calbiochem) was added 18 hours before harvesting. In experiments with IFN-β, virus-producing cells were washed twice with phosphate-buffered saline (PBS) and treated with IFN-β for 8 hours before being harvested. The culture supernatants and cell lysates were subjected to p24 ELISA measurement or Western blotting analysis, as described earlier.

Flow cytometry

Cells in 12-well plates were transfected with vectors encoding Vpu (100 ng), green fluorescent protein (GFP) (100 ng), and either HA-SCYL2 or empty plasmid (0, 1, and 2 μg). Alternatively, vector encoding KSHV-K5 was used instead of vector encoding Vpu. Eighteen hours later, cells were harvested in PBS containing 5 mM EDTA and washed with PBS containing 1% bovine serum albumin (BSA). The cells were blocked with 10% normal goat serum and then stained with an anti-BST2 antibody (a gift from Chugai Pharmaceutical Co.) and a phycoerythrin (PE)–conjugated secondary antibody (Beckman Coulter) or a PE/Cy7-conjugated anti-CD4 antibody (BioLegend). All samples were fixed with 4% paraformaldehyde and analyzed with a FACSCanto II instrument (BD Biosciences) and FlowJo software (Treestar) gated for the GFP-positive fraction.

Detection of pVpu

For phosphate-affinity (Phos-tag) gel analysis, cells in 12-well plates were transfected with vectors encoding either wild-type Vpu or the Vpu(S52,56A) mutant (100 ng) together with plasmid encoding SCYL2 (at 0, 500, or 1000 ng). Two days later, cell lysates were loaded onto an 8% polyacrylamide gel containing 50 μM $MnCl_2$ and 25 μM Phos-tag acrylamide (Wako). After electrophoresis, gels were soaked in a general transfer buffer with 1 mM EDTA for 10 min to eliminate Mn^{2+} ions and then subjected to Western blotting analysis with anti-Vpu antibody (a gift from K. Strebel). A phospho-specific polyclonal antibody against Vpu phosphorylated at Ser^{52} and Ser^{56} was produced and purified by Scrum Inc.

Microscopy

One day before transfection, cells were seeded onto glass-bottomed dishes (Matsunami). At 48 hours after transfection, the cells were fixed and stained as described previously (*77*). Microscopic imaging was performed with an FV1000-D confocal laser scanning microscope (Olympus) equipped with a 60× oil-immersion objective.

In vitro and in vivo binding assays

For in vitro GST pull-down assays, biotinylated SCYL2 was incubated with either GST-Vpu or GST–dihydrofolate reductase (DHFR) at 26°C for 2 hours before being co-incubated with glutathione-Sepharose beads (GE Healthcare) at 4°C for 3 hours. The beads were then washed three times, and bound proteins were analyzed by Western blotting with streptavidin–horseradish peroxidase (HRP) conjugate (GE Healthcare). For immunoprecipitation analysis, cells expressing epitope-tagged proteins were lysed with HBST buffer containing protease inhibitor cocktail (Roche). Cell lysates were immunoprecipitated with EZview Red Affinity Gel (Sigma), and bound proteins were analyzed by Western blotting.

Quantitative phosphatase assays

Phosphorylated or unphosphorylated Vpu peptides including Ser^{52} and Ser^{56} ($AEDS_{52}GNES_{56}EGE$) were chemically synthesized by Scrum Inc. Cells in six-well plates were transfected with pCMV-HA-SCYL2. Two days later, cell lysates were prepared with HBST buffer containing protease inhibitor cocktail alone or with 10 nM OA. Cell lysates were immunoprecipitated with an anti-HA antibody and incubated with 10 μl of 1 mM of either phosphorylated or unphosphorylated Vpu peptide in 50 μl of reaction buffer [50 mM imidazole (pH 7.2), 200 μM EGTA, 0.25% β-mercaptoethanol, BSA (0.1 mg/ml)] at 37°C for 8 hours. To stop the reactions, 50 μl of 20 mM ammonium molybdate was added to the mixture. The amounts of free phosphate ion were measured by absorbance at 630 nm. Standard curves were obtained with a Ser/Thr phosphatase assay kit (Promega) according to the manufacturer's instructions.

Luciferase reporter assays

Cells in 24-well plates were transfected with the pGL4-based luciferase reporter plasmid and with pGL4-TK-Rluc (Promega) as a transfection control. Two days later, cells were treated with IFN-β (1000 U/ml) for 8 hours before being harvested. Luciferase activity was determined with a Dual-Luciferase Reporter assay system (Promega) and normalized to *Renilla* luciferase activity.

Statistical analysis

All graphs present the means and SDs. Statistical significance of differences between two groups was tested by two-tailed unpaired *t* test with Prism 6 software (GraphPad). In cases where multiple comparisons within an experiment were made, one-way analysis of variance (ANOVA) was used. A P value of <0.05 was considered statistically significant.

SUPPLEMENTARY MATERIALS

www.sciencesignaling.org/cgi/content/full/5/245/ra73/DC1

Fig. S1. Schematic representation of the initial screening method.

Fig. S2. The CKII inhibitor DRB phenocopies the effect of increased SCYL2 abundance.

Fig. S3. SCYL2 fails to inhibit SIV_{GSN} Vpu–induced counteraction of BST2.

Fig. S4. SCYL2 inhibits Vpu function through a phosphorylation-dependent mechanism.

REFERENCES AND NOTES

1. M. H. Malim, M. Emerman, HIV-1 accessory proteins—Ensuring viral survival in a hostile environment. *Cell Host Microbe* **3**, 388–398 (2008).
2. P. D. Bieniasz, The cell biology of HIV-1 virion genesis. *Cell Host Microbe* **5**, 550–558 (2009).
3. N. J. Arhel, F. Kirchhoff, Implications of Nef: Host cell interactions in viral persistence and progression to AIDS. *Curr. Top. Microbiol. Immunol.* **339**, 147–175 (2009).
4. F. Kirchhoff, Immune evasion and counteraction of restriction factors by HIV-1 and other primate lentiviruses. *Cell Host Microbe* **8**, 55–67 (2010).

5. J. Guatelli, How innate immunity can inhibit the release of HIV-1 from infected cells. *N. Engl. J. Med.* **362**, 553–554 (2010).
6. K. Strebel, T. Klimkait, M. A. Martin, A novel gene of HIV-1, vpu, and its 16-kilodalton product. *Science* **241**, 1221–1223 (1988).
7. E. A. Cohen, E. F. Terwilliger, J. G. Sodroski, W. A. Haseltine, Identification of a protein encoded by the *vpu* gene of HIV-1. *Nature* **334**, 532–534 (1988).
8. T. Huet, R. Cheynier, A. Meyerhans, G. Roelants, S. Wain-Hobson, Genetic organization of a chimpanzee lentivirus related to HIV-1. *Nature* **345**, 356–359 (1990).
9. R. L. Willey, F. Maldarelli, M. A. Martin, K. Strebel, Human immunodeficiency virus type 1 Vpu protein induces rapid degradation of CD4. *J. Virol.* **66**, 7193–7200 (1992).
10. S. J. Neil, T. Zang, P. D. Bieniasz, Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. *Nature* **451**, 425–430 (2008).
11. N. Van Damme, D. Goff, C. Katsura, R. L. Jorgenson, R. Mitchell, M. C. Johnson, E. B. Stephens, J. Guatelli, The interferon-induced protein BST-2 restricts HIV-1 release and is downregulated from the cell surface by the viral Vpu protein. *Cell Host Microbe* **3**, 245–252 (2008).
12. S. Bour, C. Perrin, K. Strebel, Cell surface CD4 inhibits HIV-1 particle release by interfering with Vpu activity. *J. Biol. Chem.* **274**, 33800–33806 (1999).
13. J. Lama, A. Mangasarian, D. Trono, Cell-surface expression of CD4 reduces HIV-1 infectivity by blocking Env incorporation in a Nef- and Vpu-inhibitable manner. *Curr. Biol.* **9**, 622–631 (1999).
14. R. L. Willey, F. Maldarelli, M. A. Martin, K. Strebel, Human immunodeficiency virus type 1 Vpu protein regulates the formation of intracellular gp160-CD4 complexes. *J. Virol.* **66**, 226–234 (1992).
15. J. L. Douglas, K. Viswanathan, M. N. McCarroll, J. K. Gustin, K. Früh, A. V. Moses, Vpu directs the degradation of the human immunodeficiency virus restriction factor BST-2/Tetherin via a βTrCP-dependent mechanism. *J. Virol.* **83**, 7931–7947 (2009).
16. E. Miyagi, A. J. Andrew, S. Kao, K. Strebel, Vpu enhances HIV-1 virus release in the absence of Bst-2 cell surface down-modulation and intracellular depletion. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 2868–2873 (2009).
17. C. Goffinet, I. Allespach, S. Homann, H. M. Tervo, A. Habermann, D. Rupp, L. Oberbremer, C. Kern, N. Tibroni, S. Welsch, J. Krijnse-Locker, G. Banting, H. G. Kräusslich, O. T. Fackler, O. T. Keppler, HIV-1 antagonism of CD317 is species specific and involves Vpu-mediated proteasomal degradation of the restriction factor. *Cell Host Microbe* **5**, 285–297 (2009).
18. K. Sato, S. P. Yamamoto, N. Misawa, T. Yoshida, T. Miyazawa, Y. Koyanagi, Comparative study on the effect of human BST-2/Tetherin on HIV-1 release in cells of various species. *Retrovirology* **6**, 53 (2009).
19. Y. Iwabu, H. Fujita, M. Kinomoto, K. Kaneko, Y. Ishizaka, Y. Tanaka, T. Sata, K. Tokunaga, HIV-1 accessory protein Vpu internalizes cell-surface BST-2/tetherin through transmembrane interactions leading to lysosomes. *J. Biol. Chem.* **284**, 35060–35072 (2009).
20. H. Hauser, L. A. Lopez, S. J. Yang, J. E. Oldenburg, C. M. Exline, J. C. Guatelli, P. M. Cannon, HIV-1 Vpu and HIV-2 Env counteract BST-2/tetherin by sequestration in a perinuclear compartment. *Retrovirology* **7**, 51 (2010).
21. M. Skasko, Y. Wang, Y. Tian, A. Tokarev, J. Munguia, A. Ruiz, E. B. Stephens, S. J. Opella, J. Guatelli, HIV-1 Vpu protein antagonizes innate restriction factor BST-2 via lipid-embedded helix-helix interactions. *J. Biol. Chem.* **287**, 58–67 (2012).
22. E. Bartee, A. McCormack, K. Früh, Quantitative membrane proteomics reveals new cellular targets of viral immune modulators. *PLoS Pathog.* **2**, e107 (2006).
23. B. Mangeat, G. Gers-Huber, M. Lehmann, M. Zufferey, J. Luban, V. Piguet, HIV-1 Vpu neutralizes the antiviral factor Tetherin/BST-2 by binding it and directing its β-TrCP2-dependent degradation. *PLoS Pathog.* **5**, e1000574 (2009).
24. R. S. Mitchell, C. Katsura, M. A. Skasko, K. Fitzpatrick, D. Lau, A. Ruiz, E. B. Stephens, F. Margottin-Goguet, R. Benarous, J. C. Guatelli, Vpu antagonizes BST-2–mediated restriction of HIV-1 release via β-TrCP and endo-lysosomal trafficking. *PLoS Pathog.* **5**, e1000450 (2009).
25. R. K. Gupta, S. Hué, T. Schaller, E. Verschoor, D. Pillay, G. J. Towers, Mutation of a single residue renders human tetherin resistant to HIV-1 Vpu-mediated depletion. *PLoS Pathog.* **5**, e1000443 (2009).
26. D. Perez-Caballero, T. Zang, A. Ebrahimi, M. W. McNatt, D. A. Gregory, M. C. Johnson, P. D. Bieniasz, Tetherin inhibits HIV-1 release by directly tethering virions to cells. *Cell* **139**, 499–511 (2009).
27. K. Fitzpatrick, M. Skasko, T. J. Deerinck, J. Crum, M. H. Ellisman, J. Guatelli, Direct restriction of virus release and incorporation of the interferon-induced protein BST-2 into HIV-1 particles. *PLoS Pathog.* **6**, e1000701 (2010).
28. J. Hammonds, J. J. Wang, H. Yi, P. Spearman, Immunoelectron microscopic evidence for Tetherin/BST2 as the physical bridge between HIV-1 virions and the plasma membrane. *PLoS Pathog.* **6**, e1000749 (2010).
29. U. Schubert, T. Schneider, P. Henklein, K. Hoffmann, E. Berthold, H. Hauser, G. Pauli, T. Porstmann, Human-immunodeficiency-virus-type-1-encoded Vpu protein is phosphorylated by casein kinase II. *Eur. J. Biochem.* **204**, 875–883 (1992).
30. U. Schubert, K. Strebel, Differential activities of the human immunodeficiency virus type 1-encoded Vpu protein are regulated by phosphorylation and occur in different cellular compartments. *J. Virol.* **68**, 2260–2271 (1994).
31. F. Margottin, S. P. Bour, H. Durand, L. Selig, S. Benichou, V. Richard, D. Thomas, K. Strebel, R. Benarous, A novel human WD protein, h-βTrCp, that interacts with HIV-1 Vpu connects CD4 to the ER degradation pathway through an F-box motif. *Mol. Cell* **1**, 565–574 (1998).
32. C. Butticaz, O. Michielin, J. Wyniger, A. Telenti, S. Rothenberger, Silencing of both β-TrCP1 and HOS (β-TrCP2) is required to suppress human immunodeficiency virus type 1 Vpu-mediated CD4 down-modulation. *J. Virol.* **81**, 1502–1505 (2007).
33. A. A. Tokarev, J. Munguia, J. C. Guatelli, Serine-threonine ubiquitination mediates downregulation of BST-2/tetherin and relief of restricted virion release by HIV-1 Vpu. *J. Virol.* **85**, 51–63 (2011).
34. R. Vigan, S. J. Neil, Determinants of tetherin antagonism in the transmembrane domain of the human immunodeficiency virus type 1 Vpu protein. *J. Virol.* **84**, 12958–12970 (2010).
35. C. F. Basler, A. Garcia-Sastre, Viruses and the type I interferon antiviral system: Induction and evasion. *Int. Rev. Immunol.* **21**, 305–337 (2002).
36. C. E. Samuel, Antiviral actions of interferons. *Clin. Microbiol. Rev.* **14**, 778–809 (2001).
37. M. J. de Veer, M. Holko, M. Frevel, E. Walker, S. Der, J. M. Paranjape, R. H. Silverman, B. R. Williams, Functional classification of interferon-stimulated genes identified using microarrays. *J. Leukoc. Biol.* **69**, 912–920 (2001).
38. A. L. Blasius, E. Giurisato, M. Cella, R. D. Schreiber, A. S. Shaw, M. Colonna, Bone marrow stromal cell antigen 2 is a specific marker of type I IFN-producing cells in the naive mouse, but a promiscuous cell surface antigen following IFN stimulation. *J. Immunol.* **177**, 3260–3265 (2006).
39. G. Poli, J. M. Orenstein, A. Kinter, T. M. Folks, A. S. Fauci, Interferon-alpha but not AZT suppresses HIV expression in chronically infected cell lines. *Science* **244**, 575–577 (1989).
40. A. Okumura, G. Lu, I. Pitha-Rowe, P. M. Pitha, Innate antiviral response targets HIV-1 release by the induction of ubiquitin-like protein ISG15. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 1440–1445 (2006).
41. S. D. Barr, J. R. Smiley, F. D. Bushman, The interferon response inhibits HIV particle production by induction of TRIM22. *PLoS Pathog.* **4**, e1000007 (2008).
42. T. Sawasaki, T. Ogasawara, R. Morishita, Y. Endo, A cell-free protein synthesis system for high-throughput proteomics. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 14652–14657 (2002).
43. E. F. Ullman, H. Kirakossian, S. Singh, Z. P. Wu, B. R. Irvin, J. S. Pease, A. C. Switchenko, J. D. Irvine, A. Dafforn, C. N. Skold, D. B. Wagner, Luminescent oxygen channeling immunoassay: Measurement of particle binding kinetics by chemiluminescence. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 5426–5430 (1994).
44. A. Von Leoprechting, R. Kumpf, S. Menzel, D. Reulle, R. Griebel, M. J. Valler, F. H. Büttner, Miniaturization and validation of a high-throughput serine kinase assay using the AlphaScreen platform. *J. Biomol. Screen.* **9**, 719–725 (2004).
45. M. Mansouri, K. Viswanathan, J. L. Douglas, J. Hines, J. Gustin, A. V. Moses, K. Früh, Molecular mechanism of BST2/tetherin downregulation by K5/MIR2 of Kaposi's sarcoma-associated herpesvirus. *J. Virol.* **83**, 9672–9681 (2009).
46. C. Pardieu, R. Vigan, S. J. Wilson, A. Calvi, T. Zang, P. Bieniasz, P. Kellam, G. J. Towers, S. J. Neil, The RING-CH ligase K5 antagonizes restriction of KSHV and HIV-1 particle release by mediating ubiquitin-dependent endosomal degradation of tetherin. *PLoS Pathog.* **6**, e1000843 (2010).
47. E. Kinoshita, M. Takahashi, H. Takeda, M. Shiro, T. Koike, Recognition of phosphate monoester dianion by an alkoxide-bridged dinuclear zinc(II) complex. *Dalton Trans.* **8**, 1189–1193 (2004).
48. E. Kinoshita, E. Kinoshita-Kikuta, K. Takiyama, T. Koike, Phosphate-binding tag, a new tool to visualize phosphorylated proteins. *Mol. Cell. Proteomics* **5**, 749–757 (2006).
49. E. Kinoshita, E. Kinoshita-Kikuta, T. Koike, Separation and detection of large phosphoproteins using Phos-tag SDS-PAGE. *Nat. Protoc.* **4**, 1513–1521 (2009).
50. S. D. Conner, S. L. Schmid, CVAK104 is a novel poly-L-lysine-stimulated kinase that targets the β2-subunit of AP2. *J. Biol. Chem.* **280**, 21539–21544 (2005).
51. M. Düwel, E. J. Ungewickell, Clathrin-dependent association of CVAK104 with endosomes and the *trans*-Golgi network. *Mol. Biol. Cell* **17**, 4513–4525 (2006).
52. G. H. Borner, A. A. Rana, R. Forster, M. Harbour, J. C. Smith, M. S. Robinson, CVAK104 is a novel regulator of clathrin-mediated SNARE sorting. *Traffic* **8**, 893–903 (2007).
53. V. Janssens, J. Goris, C. Van Hoof, PP2A: The expected tumor suppressor. *Curr. Opin. Genet. Dev.* **15**, 34–41 (2005).
54. M. Dubé, B. B. Roy, P. Guiot-Guillain, J. Binette, J. Mercier, A. Chiasson, E. A. Cohen, Antagonism of tetherin restriction of HIV-1 release by Vpu involves binding and sequestration of the restriction factor in a perinuclear compartment. *PLoS. Pathog.* **6**, e1000856 (2010).
55. H. M. Tervo, S. Homann, I. Ambiel, J. V. Fritz, O. T. Fackler, O. T. Keppler, β-TrCP is dispensable for Vpu's ability to overcome the CD317/Tetherin-imposed restriction to HIV-1 release. *Retrovirology* **8**, 9 (2011).
56. T. K. Kerppola, Design and implementation of bimolecular fluorescence complementation (BiFC) assays for the visualization of protein interactions in living cells. *Nat. Protoc.* **1**, 1278–1286 (2006).
57. T. Kobayashi, H. Ode, T. Yoshida, K. Sato, P. Gee, S. P. Yamamoto, H. Ebina, K. Strebel, H. Sato, Y. Koyanagi, Identification of amino acids in the human tetherin transmembrane

domain responsible for HIV-1 Vpu interaction and susceptibility. *J. Virol.* **85**, 932–945 (2011).
58. R. A. Liberatore, P. D. Bieniasz, Tetherin is a key effector of the antiretroviral activity of type I interferon in vitro and in vivo. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 18097–18101 (2011).
59. T. Terabayashi, Y. Funato, M. Fukuda, H. Miki, A coated vesicle-associated kinase of 104 kDa (CVAK104) induces lysosomal degradation of frizzled 5 (Fzd5). *J. Biol. Chem.* **284**, 26716–26724 (2009).
60. D. Schwartz, G. M. Church, Collection and motif-based prediction of phosphorylation sites in human viruses. *Sci. Signal.* **3**, rs2 (2010).
61. A. G. Bukrinskaya, A. Ghorpade, N. K. Heinzinger, T. E. Smithgall, R. E. Lewis, M. Stevenson, Phosphorylation-dependent human immunodeficiency virus type 1 infection and nuclear targeting of viral DNA. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 367–371 (1996).
62. L. A. Pinna, Protein kinase CK2: A challenge to canons. *J. Cell Sci.* **115**, 3873–3878 (2002).
63. M. C. Mumby, G. Walter, Protein serine/threonine phosphatases: Structure, regulation, and functions in cell growth. *Physiol. Rev.* **73**, 673–699 (1993).
64. V. Janssens, J. Goris, Protein phosphatase 2A: A highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling. *Biochem. J.* **353**, 417–439 (2001).
65. K. Okamoto, H. Li, M. R. Jensen, T. Zhang, Y. Taya, S. S. Thorgeirsson, C. Prives, Cyclin G recruits PP2A to dephosphorylate Mdm2. *Mol. Cell* **9**, 761–771 (2002).
66. D. Lau, W. Kwan, J. Guatelli, Role of the endocytic pathway in the counteraction of BST-2 by human lentiviral pathogens. *J. Virol.* **85**, 9834–9846 (2011).
67. T. Kueck, S. J. Neil, A cytoplasmic tail determinant in HIV-1 Vpu mediates targeting of tetherin for endosomal degradation and counteracts interferon-induced restriction. *PLoS Pathog.* **8**, e1002609 (2012).
68. N. Belaïdouni, C. Marchal, R. Benarous, C. Besnard-Guérin, Involvement of the βTrCP in the ubiquitination and stability of the HIV-1 Vpu protein. *Biochem. Biophys. Res. Commun.* **357**, 688–693 (2007).
69. D. Tadokoro, S. Takahama, K. Shimizu, S. Hayashi, Y. Endo, T. Sawasaki, Characterization of a caspase-3-substrate kinome using an N- and C-terminally tagged protein kinase library produced by a cell-free system. *Cell Death Dis.* **1**, e89 (2010).
70. T. Sawasaki, R. Morishita, M. D. Gouda, Y. Endo, Methods for high-throughput materialization of genetic information based on wheat germ cell-free expression system. *Methods Mol. Biol.* **375**, 95–106 (2007).
71. K. Takai, T. Sawasaki, Y. Endo, Practical cell-free protein synthesis system using purified wheat embryos. *Nat. Protoc.* **5**, 227–238 (2010).
72. A. Adachi, H. E. Gendelman, S. Koenig, T. Folks, R. Willey, A. Rabson, M. A. Martin, Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. *J. Virol.* **59**, 284–291 (1986).
73. K. L. Nguyen, M. Ilano, H. Akari, E. Miyagi, E. M. Poeschla, K. Strebel, S. Bour, Codon optimization of the HIV-1 *vpu* and *vif* genes stabilizes their mRNA and allows for highly efficient Rev-independent expression. *Virology* **319**, 163–175 (2004).
74. T. Klimkait, K. Strebel, M. D. Hoggan, M. A. Martin, J. M. Orenstein, The human immunodeficiency virus type 1-specific protein vpu is required for efficient virus maturation and release. *J. Virol.* **64**, 621–629 (1990).
75. R. E. Means, S. M. Lang, J. U. Jung, The Kaposi's sarcoma-associated herpesvirus K5 E3 ubiquitin ligase modulates targets by multiple molecular mechanisms. *J. Virol.* **81**, 6573–6583 (2007).
76. T. Ohnishi, A. Yamashita, I. Kashima, T. Schell, K. R. Anders, A. Grimson, T. Hachiya, M. W. Hentze, P. Anderson, S. Ohno, Phosphorylation of hUPF1 induces formation of mRNA surveillance complexes containing hSMG-5 and hSMG-7. *Mol. Cell* **12**, 1187–1200 (2003).
77. K. Miyakawa, A. Ryo, T. Murakami, K. Ohba, S. Yamaoka, M. Fukuda, J. Guatelli, N. Yamamoto, BCA2/Rabring7 promotes tetherin-dependent HIV-1 restriction. *PLoS Pathog.* **5**, e1000700 (2009).

Acknowledgments: We thank K. Strebel (NIH), P. Cannon (University of Southern California), and Chugai Pharmaceutical Co. for various reagents. We also thank all members of the Ryo laboratory for technical support and useful suggestions. **Funding:** This work was in part supported by a research grant from the Ministry of Health, Labour, and Welfare, Japan; a grant from Special Coordination Funds for Promoting Science and Technology; grants from Takeda Science Foundation and Uehara Memorial Foundation to A.R.; National University of Singapore School of Medicine Start-up Grant (R-182-000-160-733, R-182-000-160-133); a grant from the National Medical Research Council, Singapore (NMRC/R-182-000-182-213) to N.Y.; and a grant from the NIH (AI081668) to J.G. **Author contributions:** K.M. designed and performed the research, analyzed the data, and wrote the manuscript; T.S. developed the screening system; S.M. and A.T. performed the research; G.Q., H.K., and N.Y. analyzed the data and edited the manuscript; M.N. and A.A. contributed reagents and analyzed the data; J.G. analyzed the data and edited the manuscript; and A.R. directed the research, analyzed the data, and wrote the manuscript. **Competing interests:** The authors declare that they have no competing interests.

Submitted 11 May 2012
Accepted 20 September 2012
Final Publication 9 October 2012
10.1126/scisignal.2003212
Citation: K. Miyakawa, T. Sawasaki, S. Matsunaga, A. Tokarev, G. Quinn, H. Kimura, M. Nomaguchi, A. Adachi, N. Yamamoto, J. Guatelli, A. Ryo, Interferon-induced SCYL2 limits release of HIV-1 by triggering PP2A-mediated dephosphorylation of the viral protein Vpu. *Sci. Signal.* **5**, ra73 (2012).

frontiers in
MICROBIOLOGY

REVIEW ARTICLE
published: 10 August 2012
doi: 10.3389/fmicb.2012.00297

SAMHD1-dependent and -independent functions of HIV-2/SIV Vpx protein

Mikako Fujita[1]*, Masako Nomaguchi[2], Akio Adachi[2] and Masami Otsuka[3]

[1] Research Institute for Drug Discovery, School of Pharmacy, Kumamoto University, Kumamoto, Japan
[2] Department of Microbiology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan
[3] Department of Bioorganic Medicinal Chemistry, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan

Edited by:
Atsushi Koito, Kumamoto University, Japan

Reviewed by:
Yuntao Wu, George Mason University, USA
Yoshio Koyanagi, Kyoto University, Japan

***Correspondence:**
Mikako Fujita, Research Institute for Drug Discovery, School of Pharmacy, Kumamoto University, 5-1 Oe-honmachi, Chuo-ku, Kumamoto 862-0973, Japan.
e-mail: mfujita@kumamoto-u.ac.jp

Both human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) encode a unique set of accessory proteins that enhance viral replication in the host. Two similar accessory proteins, Vpx and Vpr, are encoded by HIV-2. In contrast, HIV-1 encodes Vpr but not Vpx. Recent studies have indicated that Vpx counteracts a particular host restriction factor, thereby facilitating reverse transcription in myeloid cells such as monocyte-derived macrophages and monocyte-derived dendritic cells. This mechanism of counteraction is similar to that of the accessory proteins Vif and Vpu which antagonize other host factors. In 2011, the protein SAMHD1 was identified as the restriction factor counteracted by Vpx. Studies have since revealed that SAMHD1 degrades deoxynucleoside triphosphates (dNTPs), which are components of viral genomic cDNA, in order to deprive viruses of dNTPs. Although interactions between SAMHD1 and Vpx continue to be a major research focus, Vpx has also been shown to have an apparent ability to enhance nuclear import of the viral genome in T lymphocytes. This review summarizes the current knowledge regarding SAMHD1-dependent and -independent functions of Vpx, and discusses possible reasons why HIV-2 encodes both Vpx and Vpr, unlike HIV-1.

Keywords: Vpx, HIV-2, SIV, SAMHD1, reverse transcription, dNTP, nuclear import, Vpr

INTRODUCTION

Human and simian immunodeficiency viruses (HIV/SIVs) carry a unique set of accessory proteins, Vif, Vpx, Vpr, Vpu, and Nef, which enhance viral replication in the host. Of these accessory proteins, Vpx is unique to HIV-2-type viruses, defined in this paper as the HIV/SIVs carrying both Vpr and Vpx, such as HIV-2, SIVsmm (Sooty mangabey), and SIVmac (Rhesus monkey) (Fujita et al., 2010). Vpr and Vpx are small proteins of approximately 100 amino acids and similar sequence (approximately 20–25% similarity). Both Vpr and Vpx are predicted to have a similar structure consisting of three major helices (Khamsri et al., 2006). In contrast, while HIV-1 carries Vpr, it does not carry Vpx. The answer to the question why HIV-2 viruses encode these two similar proteins while HIV-1 carries only one remains elusive, and must await the determination of their functional details.

Extensive research over the past decade has revealed that lentiviruses carry genes for accessory proteins that overcome host antiviral factors. The first such accessory protein identified was Vif, which inactivates APOBEC3 proteins, cellular cytidine deaminases that restrict the replication of retroviruses by hypermutating viral cDNA and/or inhibiting reverse transcription (Sheehy et al., 2002; Goila-Gaur and Strebel, 2008; Kitamura et al., 2011). Vif reduces the amount of APOBEC3 through proteasome-mediated degradation and other degradation-independent mechanisms. The second major finding in this area was that the viral protein Vpu counteracts host BST-2/tetherin, which normally blocks the release of virions by directly tethering viral particles to the membranes of infected cells (Neil et al., 2008; Van Damme et al., 2008; Arias et al., 2011). The mechanism through which Vpu antagonizes the function of BST-2/tetherin may be proteasome/lysosome degradation or relocalization from the cell surface.

Recently, it was reported that the viral accessory protein Vpx inhibits the host restriction factor SAMHD1 in monocyte-derived macrophages (MDMs) and monocyte-derived dendritic cells (MDDCs) (Hrecka et al., 2011; Laguette et al., 2011), stimulating interest in SAMHD1 and Vpx. In addition to inhibiting SAMHD1 in MDMs and MDDCs, Vpx is also capable of enhancing viral replication in T lymphocytes (Guyader et al., 1989; Kappes et al., 1991; Yu et al., 1991; Akari et al., 1992; Gibbs et al., 1994; Kawamura et al., 1994; Tokunaga et al., 1997; Ueno et al., 2003; Doi et al., 2011). In this review, we summarize current research into SAMHD1-dependent and -independent functions of Vpx and discuss the virological significance of this protein.

SAMHD1-DEPENDENT FUNCTIONS OF Vpx

Several studies have shown that while wild-type HIV-2-type viruses grow well in MDMs, growth of these Vpx-deletion mutants is completely suppressed, demonstrating that Vpx is essential for viral replication in MDMs (Ueno et al., 2003; Fujita et al., 2008a). It is known that Vpx is packaged in virions and functions in the target cell. Independent work in our laboratory and that of another group revealed that Vpx is critical for reverse transcription of the viral RNA genome in MDMs (Fujita et al., 2008a; Srivastava et al., 2008), correcting the long-held misconception that Vpx contributes to nuclear import of the viral genome but does not play a role in reverse transcription. Furthermore, Vpx was shown to

induce proteasome-degradation of an unknown restriction factor to facilitate reverse transcription of the viral genome. It was demonstrated that degradation of the unknown factor involves formation of a Cul4-DDB1-DCAF1 E3 ligase complex (Sharova et al., 2008; Bergamaschi et al., 2009; Kaushik et al., 2009). Considerable effort was subsequently directed toward identification of the unknown factor, and in 2011 SAMHD1 was identified as the MDM host factor from co-immunoprecipitation studies of Vpx expressed in THP-1 cells and in 293T cells (Hrecka et al., 2011; Laguette et al., 2011). SAMHD1 has a tandem sterile alpha motif (SAM) and HD domain with potential phosphohydrolase activity. The SAMHD1 protein was initially identified from MDDCs as a homolog of mouse interferon-γ-induced protein (Li et al., 2000), and is upregulated in response to viral infection (Preheud et al., 2005; Hartman et al., 2007; Zhao et al., 2008). Furthermore, SAMHD1 is believed to be involved in regulating cellular intrinsic antiviral responses (Rice et al., 2009).

The identification of SAMHD1 as a target of Vpx was not sufficient to explain all the related phenomena, suggesting the involvement of another factor (Hrecka et al., 2011; Planelles, 2011). However, based on reports indicating that SAMHD1 is a deoxynucleoside triphosphate (dNTP) triphosphohydrolase (Goldstone et al., 2011; Powell et al., 2011), it was hypothesized that SAMHD1 degrades dNTPs (which are small molecule components of viral genomic cDNA) in order to deprive viruses of dNTPs by keeping their concentration low. Lahouassa et al. (2012) recently demonstrated the validity of this hypothesis (**Figures 1** and **2**). Thus, the additional factor targeted by Vpx appears to be dNTPs. Although dNTPs are utilized for reverse transcription in the cytosol, they are small enough to freely diffuse through nuclear pores in and out of the nucleus. Since SAMHD1 is a nuclear protein (Rice et al., 2009), it is most likely that the concentration of dNTPs in the cytosol is controlled by SAMHD1 in the nucleus. In fact, it was suggested that Vpx-mediated degradation of SAMHD1 is initiated in the nucleus (Brandariz-Nuñez et al., 2012; **Figure 2**).

In addition to being components of the viral genome, dNTPs are components of the host genome; thus, proliferative $CD4^+$ T cells do not express SAMHD1, and maintain the concentration of dNTPs at an optimal level for cell proliferation (2–4 μM; Lahouassa et al., 2012). In contrast, since MDMs do not proliferate, they do not require high levels of dNTPs, and the low dNTP levels (20–40 nM) resulting from SAMHD1-mediated degradation are therefore not harmful to MDMs (Lahouassa et al., 2012). HIV-2-type viruses carry Vpx for proteasome-mediated degradation of SAMHD1 in order to facilitate replication in MDMs. In contrast, HIV-1 does not require Vpx in order to replicate in MDMs (Fujita et al., 2010) because its reverse transcriptase (RT) is capable of catalyzing viral cDNA synthesis from very low levels of dNTPs (Diamond et al., 2004; Lahouassa et al., 2012). The activity of HIV-2 RT is probably lower [Michaelis constant (K_m) of HIV-2 RT is higher] than that of HIV-1 RT, and therefore, to overcome this disadvantage, HIV-2-type viruses may have evolved to carry Vpx.

We previously mapped the functional region of Vpx involved in viral replication in MDMs (Fujita et al., 2008a,b; **Figure 3**). It is known that the region in major helix 3 containing amino acids Q^{76} and F^{80} interacts with DCAF1, a subunit of the Cullin4-based E3 ubiquitin ligase complex (Srivastava et al., 2008). This region could overlap with a region that is critical for virion incorporation (Park and Sodroski, 1995; Jin et al., 2001). Gramberg et al. (2010) suggested that another region, which includes amino acids P^9, N^{12}, E^{15}, E^{16}, and T^{17} in the N-terminal loop, binds to a restriction factor; this region was later confirmed to be a SAMHD1-binding region (Ahn et al., 2012).

We also identified several other functional regions in Vpx, including a central region located between major helix 1 and major helix 2, and a polyproline motif in a loop in the C-terminus (Fujita et al., 2008a,b). We revealed that the C-terminal polyproline motif is critical for stable expression of Vpx. Although the function of the central region remains unknown, it has been confirmed that this region is not involved in virion incorporation.

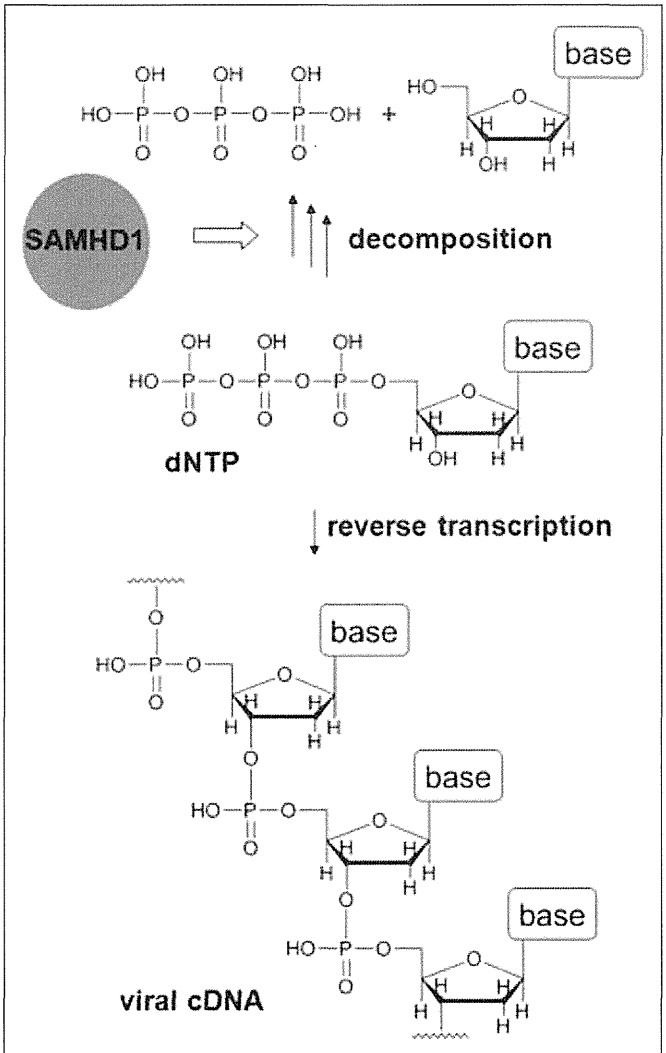

FIGURE 1 | Function of SAMHD1 in MDMs. In reverse transcription, viral cDNA is synthesized from dNTPs. The host restriction factor SAMHD1 is a dNTP triphosphohydrolase that degrades dNTPs. Thus, in the presence of SAMHD1, the concentration of dNTPs becomes low and reverse transcription is inhibited.

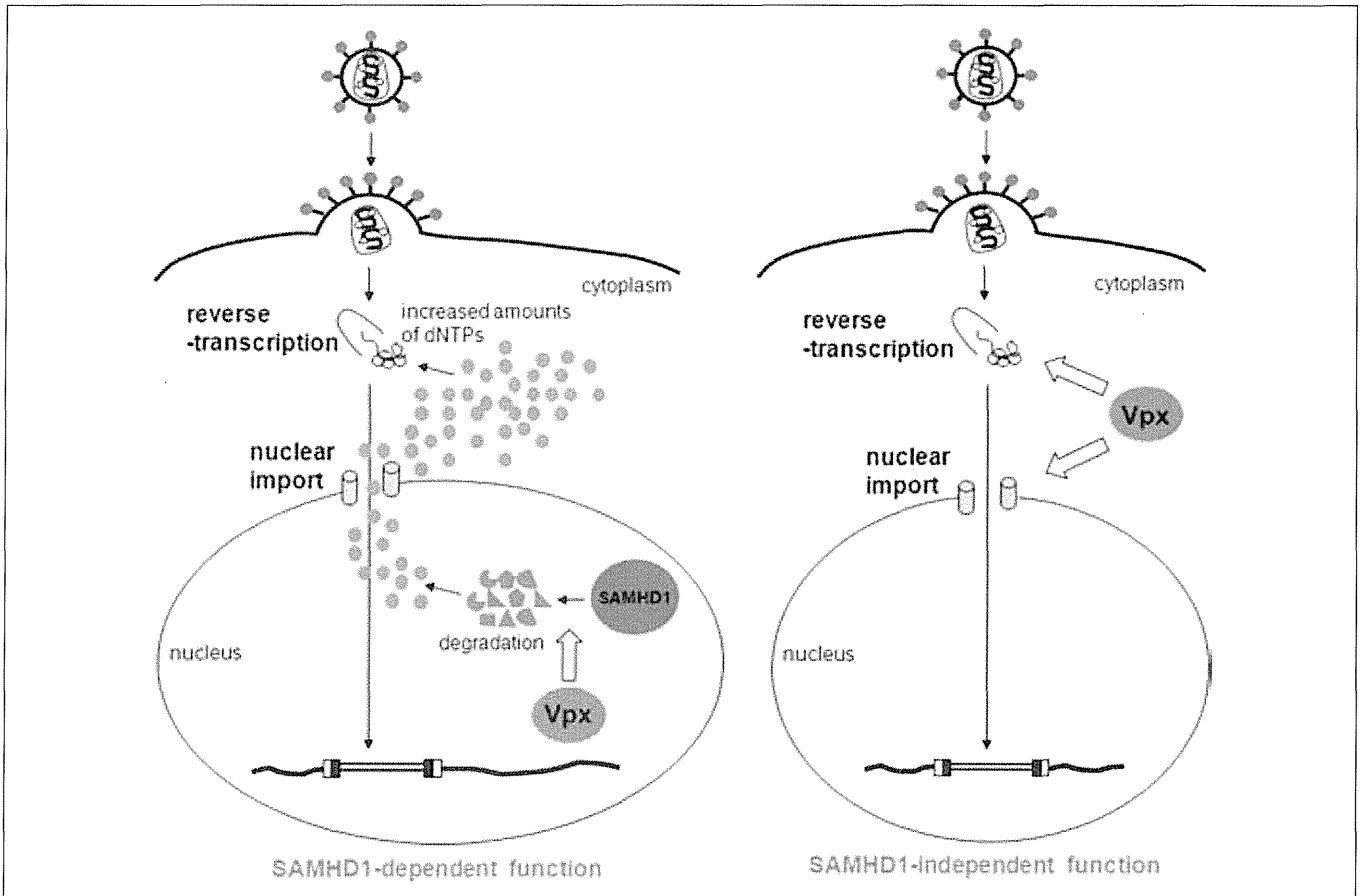

FIGURE 2 | SAMHD1-dependent and -independent functions of Vpx. In the former function, Vpx degrades SAMHD1 to increase amounts of dNTPs (orange circles), thus, reverse transcription proceeds (left). In the latter function, Vpx enhances reverse transcription and nuclear import by an unknown mechanism (right).

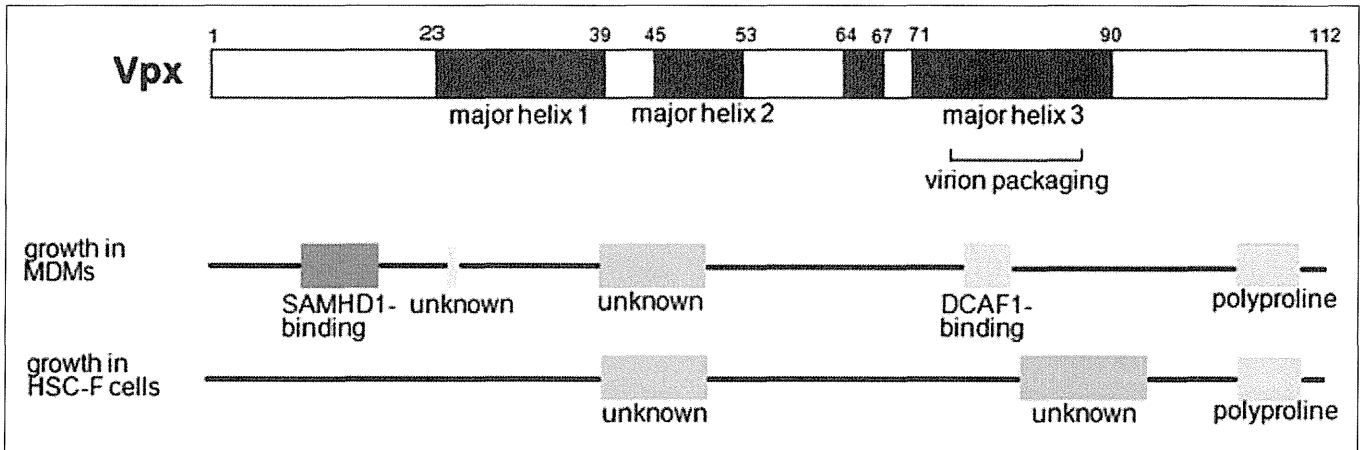

FIGURE 3 | Mapping of the functional region of Vpx. In the upper structure, the positions of predicted helices are shown in black (HIV-2 GL-AN clone; Khamsri et al., 2006). The region important for virion packaging is shown. Middle diagram shows regions critical for replication in MDMs: the SAMHD1-binding region (pink), mechanistically unknown amino acid (yellow), mechanistically unknown region (gray), DCAF1-binding region (light blue), and polyproline motif (pale orange). Lower diagram shows regions involved in replication in HSC-F cells: the mechanistically unknown region (gray), mechanistically unknown and unique region (purple), and polyproline motif (pale orange). The polyproline motif is critical for stable expression of Vpx. All functionally important regions, except those for virion packaging, SAMHD1-binding, and DCAF1-binding, are based on our report (Fujita et al., 2008a,b).

Following the identification of SAMHD1, several investigators showed that although some cells such as undifferentiated THP-1 cells express SAMHD1, both the wild-type HIV-2-type virus and its Vpx mutant infect these cells to an equivalent degree (Hrecka et al., 2011; Planelles, 2011). There are several possible explanations for the similar infectivity of wild-type and Vpx mutant viruses: (1) These cells contain large amounts of dNTPs, and thus, even in the presence of SAMHD1 there are sufficient quantities of dNTPs for viral replication, and (2) SAMHD1 does not function in these cells for some as yet unexplained reason. A plausible explanation may be posttranslational modification (phosphorylation, etc.) of the protein. Further study will be required to uncover the molecular basis for this phenomenon.

Around the time SAMHD1 was identified, it was reported that another host restriction factor, APOBEC3A, inhibits HIV-1 infection of MDMs, and that APOBEC3A is degraded by Vpx (Berger et al., 2010, 2011). In addition, APOBEC3A reportedly decreases the amount of viral cDNA synthesized during reverse transcription. Presumably, degradation of SAMHD1 alone is not sufficient to enable reverse transcription to proceed smoothly, and therefore degradation of APOBEC3A is also required for viral replication in MDMs, suggesting that Vpx functions to counteract the antiviral effects of both APOBEC3A and SAMHD1. A comparative study between APOBEC3A and SAMHD1 must be performed in order to establish each protein's contribution to restricting lentivirus infection in myeloid cells.

SAMHD1-INDEPENDENT FUNCTIONS OF Vpx

Prior to the time that Vpx was found to act on reverse transcription (Fujita et al., 2008a; Srivastava et al., 2008), it was thought that Vpx is critical for nuclear import of the viral genome (Fletcher et al., 1996; Pancio et al., 2000), based on the results of non-quantitative polymerase chain reaction (PCR) studies. This notion was supported by the tendency of Vpx to localize in the nucleus when Vpx is transduced to a cell solely (Pancio et al., 2000; Mahalingam et al., 2001). It has been well established that Vpx is critical for reverse transcription in MDMs, but this does not preclude participation of Vpx in nuclear import in these cells. We previously identified several Vpx mutants that are defective in both reverse transcription and nuclear import (Fujita et al., 2010) in MDMs, which suggests that Vpx also enhances nuclear import in these cells. We hypothesize that this function of Vpx is SAMHD1-independent, since it is plausible that there is no connection between the amount of dNTPs and nuclear import. Further investigations are underway in order to determine if this is indeed the case.

In T cells, such as peripheral blood lymphocytes (PBLs), peripheral blood mononuclear cells (PBMCs), and cultured simian cell lines immortalized by Herpesvirus saimiri such as HSC-F and M1.3S cells, HIV-2-type viruses grow well, but Vpx-deletion mutants exhibit defective replication (Guyader et al., 1989; Kappes et al., 1991; Yu et al., 1991; Akari et al., 1992; Gibbs et al., 1994; Kawamura et al., 1994; Tokunaga et al., 1997; Ueno et al., 2003; Doi et al., 2011). These results indicate that Vpx is also important for viral replication in T cells. Dispensability of Vpx for the infection of T cells has been believed by some researchers (Bergamaschi et al., 2009; Belshan et al., 2012), but this belief was probably based on the results of infectious experiments using high-titer virus. Our research showed that Vpx enhances nuclear import of the viral genome in HSC-F cells, and that the smaller effect of Vpx on reverse transcription was also observed (Ueno et al., 2003; Fujita et al., 2008a; **Figure 2**). We mapped the region of Vpx involved in viral replication in HSC-F cells (Fujita et al., 2008b; **Figure 3**) and found that as is the case in MDMs, the central region and the C-terminal polyproline motif are critical for replication. There is also a unique functional region spanning from major helix 3 to the C-terminal loop, but how this region influences infectivity is unclear. The apparent SAMHD1- and DCAF-1-binding regions are not necessary for viral replication in HSC-F cells, in contrast to MDMs. Furthermore, in HSC-F and M1.3S cells, expression of SAMHD1 was below the detectable level (Nomaguchi, M. and Adachi, A., in preparation). Thus, in these cells, Vpx enhances reverse transcription and nuclear import of the viral genome through an unknown SAMHD1-independent mechanism. Not only cultured cell lines, but also primary T cells are considered to have SAMHD1-independent functions, since SAMHD1- and DCAF-1-binding regions are dispensable for viral replication in PBLs (Fujita, M. and Adachi, A., unpublished data).

It has been reported that Vpx is important for SIV infection in monkeys, and the predominantly infected cells are the intraepithelial T lymphocytes rather than myeloid cells such as macrophages (Hirsch et al., 1998; Belshan et al., 2012). The Vpx in T cells is considered to play a significant role in infection by HIV-2-group viruses *in vivo*. Thus, we strongly suggest that SAMHD1-independent functions of Vpx are also important, although almost all the recent Vpx research has focused on SAMHD1-dependent functions.

WHY DO HIV-2 VIRUSES HAVE TWO SIMILAR PROTEINS?

Lim et al. (2012) recently revealed that Vpr, a Vpx-related protein found in HIV-1 and HIV-2-type viruses, is not involved in degradation of SAMHD1. Instead, the Vpr carried by HIV-1 and HIV-2 arrests cells in the G_2 phase of the cell cycle, a function not associated with Vpx (Fletcher et al., 1996; Stivahtis et al., 1997; Fujita et al., 2010; **Table 1**). This G_2 arrest is known to be induced via formation of a Cul4-DDB1-DCAF1 E3 ligase complex that includes Vpr as an adaptor. Formation of the complex is followed by proteasomal degradation of an unknown cellular target. This pathway is similar to that involving Vpx, which also functions as an adaptor for the Cul4-DDB1-DCAF1 E3 ligase complex to facilitate proteasomal degradation of SAMHD1 (Ahn et al., 2012). Although the virological significance of the Vpr-mediated G_2 arrest has not been determined, this function is likely to be important since it

Table 1 | The roles of Vpx and Vpr in HIV-1 and HIV-2-type viruses.

	HIV-1	HIV-2 type viruses
Reverse transcription at low dNTP concentrations (in MDMs)	Reverse transcriptase (high activity)	Vpx
Induction of G_2 arrest	Vpr	Vpr
Enhancement of nuclear import	Vpr (?)[a]	Vpx

[a] *Further study is required (see text).*

is broadly conserved among HIV/SIV. Since the activity of HIV-2 RT is lower than that of the enzyme found in HIV-1-type viruses, HIV-2 may require SAMHD1 degradation in order to increase the concentration of dNTPs, in addition to induction of G_2 arrest.

Both of these functions, SAMHD1 degradation and G_2 arrest, are mediated via the Cul4-DDB1-DCAF1 E3 ligase complex. SIVagm (African Green Monkey) is known to have only one Vpr, which induces both the degradation of SAMHD1 (Lim et al., 2012) and G_2 arrest (Planelles et al., 1996; Stivahtis et al., 1997; Zhu et al., 2001). Lim et al. proposed that in the evolution of HIV/SIVs, neofunctionalization of Vpr to degrade SAMHD1 resulted in the rapid evolution of the SAMHD1 protein, which induced the birth of a similar protein, Vpx (subfunctionalization), to maximize its SAMHD1-targeting capability. Here, we propose another reason why HIV-2 viruses have similar proteins, Vpr and Vpx. It is known that the region of HIV-1 Vpr spanning from major helix 3 to the C-terminal loop (which includes a cluster of basic amino acids) is critical for induction of G_2 arrest (Di Marzio et al., 1995; Selig et al., 1997; Jacquot et al., 2007). This region corresponds to the mechanistically unknown and unique region of Vpx required for replication in HSC-F cells (**Figure 3**; Khamsri et al., 2006; Fujita et al., 2008b), but the corresponding region in Vpx does not contain a cluster of basic amino acids in the C-terminal loop. Induction of G_2 arrest and enhancement of replication in T cells may be incompatible functions for one protein. The presence of both Vpr and Vpx may facilitate G_2 arrest and enhancement of HIV-2 replication in T cells, but a full explanation as to why HIV-2 has two proteins that are so similar will require further study.

CONCLUSION

Recent findings indicating that Vpx mediates the degradation of SAMHD1 are intriguing, and reveal yet another example of a virus with a means to counteract host defense mechanisms. **Table 1** summarizes the roles played by Vpx and Vpr in HIV-1 and HIV-2. HIV-2/SIV Vpx negates the effect of the unique host restriction factor SAMHD1 by inducing its degradation, thereby enabling reverse transcription to occur under conditions of low dNTP concentrations. In addition, Vpx enhances reverse transcription and nuclear import of the viral genome in an SAMHD1-independent manner. We are trying to isolate host factor(s) which concern with SAMHD1-independent function. Different regions of the Vpx protein are involved in mediating SAMHD1-dependent and -independent functions. A number of HIV/SIV accessory proteins have multiple functions, including HIV-1 Vpr (Fujita et al., 2010; Sharifi et al., 2012), Vpu (Nomaguchi et al., 2008; Andrew and Strebel, 2010), and Nef (Foster and Garcia, 2008; Laguette et al., 2010). Importance of those two functions of Vpx in the infected individuals should be revealed in the future.

HIV-1 Vpr has a modest effect on replication in MDMs (Fujita et al., 2010), and there have been reports that Vpr enhances nuclear import of the viral genome in these cells (Tsurutani et al., 2000; Agostini et al., 2002). It is possible that HIV-1 Vpr and HIV-2 Vpx function similarly with respect to nuclear import. However, further study is required to elucidate how these proteins impact nuclear import, since the role of HIV-1 Vpr was demonstrated using non-quantitative PCR, and we could not reproduce this result (Fujita et al., 2010).

Expression of Vpx in MDMs results in the degradation of SAMHD1 and a subsequent increase in the concentration of dNTPs, resulting in an increase in the infectivity of HIV-1. To date, studies of Vpx/SAMHD1 have been mainly restricted to HIV-1, even though Vpx is carried by HIV-2-type viruses. Through future studies involving HIV-2-type viruses, we hope to provide a more complete picture of the roles played by Vpx and Vpr.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research (C) (21590511; to Mikako Fujita) from the Japan Society for the Promotion of Science.

REFERENCES

Agostini, I., Popov, S., Hao, T., Li, J. H., Dubrovsky, L., Chaika, O., Chaika, N., Lewis, R., and Bukrinsky, M. (2002). Phosphorylation of Vpr regulates HIV type 1 nuclear import and macrophage infection. *AIDS Res. Hum. Retroviruses* 18, 283–288.

Ahn, J., Hao, C., Yan, J., DeLucia, M., Mehrens, J., Wang, C., Gronenborn, A. M., and Skowronski, J. (2012). HIV/simian immunodeficiency virus (SIV) accessory virulence factor Vpx loads the host cell restriction factor SAMHD1 onto the E3 ubiquitin ligase complex CRL4DCAF1. *J. Biol. Chem.* 287, 12550–12558.

Akari, H., Sakuragi, J., Takebe, Y., Tomonaga, K., Kawamura, M., Fukasawa, M., Miura, T., Shinjo, T., and Hayami, M. (1992). Biological characterization of human immunodeficiency virus type 1 and type 2 mutants in human peripheral blood mononuclear cells. *Arch. Virol.* 123, 157–167.

Andrew, A., and Strebel, K. (2010). HIV-1 Vpu targets cell surface markers CD4 and BST-2 through distinct mechanisms. *Mol. Aspects Med.* 31, 407–417.

Arias, J. F., Iwabu, Y., and Tokunaga, K. (2011). Structural basis for the antiviral activity of BST-2/tetherin and its viral antagonism. *Front. Microbiol.* 2:250. doi:10.3389/fmicb.2011.00250

Belshan, M., Kimata, J. T., Brown, C., Cheng, X., McCulley, A., Larsen, A., Thippeshappa, R., Hodara, V., Giavedoni, L., Hirsch, V., and Ratner, L. (2012). Vpx is critical for SIVmne infection of pigtail macaques. *Retrovirology* 9, 32.

Bergamaschi, A., Ayinde, D., David, A., Le Rouzic, E., Morel, M., Collin, G., Descamps, D., Damond, F., Brun-Vezinet, F., Nisole, S., Margottin-Goguet, F., Pancino, G., and Transy, C. (2009). The human immunodeficiency virus type 2 Vpx protein usurps the CUL4A-DDB1 DCAF1 ubiquitin ligase to overcome a postentry block in macrophage infection. *J. Virol.* 83, 4854–4860.

Berger, A., Münk, C., Schweizer, M., Cichutek, K., Schüle, S., and Flory, E. (2010). Interaction of Vpx and apolipoprotein B mRNA-editing catalytic polypeptide 3 family member A (APOBEC3A) correlates with efficient lentivirus infection of monocytes. *J. Biol. Chem.* 285, 12248–12254.

Berger, G., Durand, S., Fargier, G., Nguyen, X.-N., Cordeil, S., Bouaziz, S., Muriaux, D., Darlix, J.-L., and Cimarelli, A. (2011). APOBEC3A is a specific inhibitor of the early phases of HIV-1 infection in myeloid cells. *PLoS Pathog.* 7, e1002221. doi:10.1371/journal.ppat.1002221

Brandariz-Nuñez, A., Valle-Casuso, J. C., White, T. E., Laguette, N., Benkirane, M., Brojatsch, J., and Diaz-Griffero, F. (2012). Roles of SAMHD1 nuclear localization in restriction of HIV-1 and SIVmac. *Retrovirology* 9, 49.

Di Marzio, P., Choe, S., Ebright, M., Knoblauch, R., and Landau, N. R. (1995). Mutational analysis of cell cycle arrest, nuclear localization, and virion packaging of human immunodeficiency virus type 1 Vpr. *J. Virol.* 69, 7909–7916.

Diamond, T. L., Roshal, M., Jamburuthugoda, V. K., Reynolds, H. M., Merriam, A. R., Lee, K. Y., Balakrishnan, M., Bambara, R. A., Planelles, V., Dewhurst, S., and Kim, B. (2004). Macrophage tropism of HIV-1 depends on efficient cellular dNTP utilization by reverse transcriptase. *J. Biol. Chem.* 279, 51545–51553.

Doi, N., Fujiwara, S., Adachi, A., and Nomaguchi, M. (2011). Rhesus M1.3S cells suitable

for biological evaluation of macaque-tropic HIV/SIV clones. *Front. Microbiol.* 2:115. doi:10.3389/fmicb.2011.00115

Fletcher, T. M. III, Brichacek, B., Sharova, N., Newman, M. A., Stivahtis, G., Sharp, P. M., Emerman, M., Hahn, B. H., and Stevenson, M. (1996). Nuclear import and cell cycle arrest functions of the HIV-1 Vpr protein are encoded by two separate genes in HIV-2/SIV(SM). *EMBO J.* 15, 6155–6165.

Foster, J. L., and Garcia, J. V. (2008). HIV-1 Nef: at the crossroads. *Retrovirology* 22, 84.

Fujita, M., Otsuka, M., Miyoshi, M., Khamsri, B., Nomaguchi, M., and Adachi, A. (2008a). Vpx is critical for reverse transcription of the human immunodeficiency virus type 2 genome in macrophages. *J. Virol.* 82, 7752–7756.

Fujita, M., Otsuka, M., Nomaguchi, M., and Adachi, A. (2008b). Functional region mapping of HIV-2 Vpx protein. *Microbes Infect.* 10, 1387–1392.

Fujita, M., Otsuka, M., Nomaguchi, M., and Adachi, A. (2010). Multifaceted activity of HIV Vpr/Vpx proteins: the current view of their virological functions. *Rev. Med. Virol.* 20, 68–76.

Gibbs, J. S., Regier, D. A., and Desrosiers, R. C. (1994). Construction and in vitro properties of SIVmac mutants with deletions in "nonessential" genes. *AIDS Res. Hum. Retroviruses* 10, 607–616.

Goila-Gaur, R., and Strebel, K. (2008). HIV-1 Vif, APOBEC, and intrinsic immunity. *Retrovirology* 5, 51.

Goldstone, D. C., Ennis-Adeniran, V., Hedden, J. J., Groom, H. C., Rice, G. I., Christodoulou, E., Walker, P. A., Kelly, G., Haire, L. F., Yap, M. W., de Carvalho, L. P., Stoye, J. P., Crow, Y. J., Taylor, I. A., and Webb, M. (2011). HIV-1 restriction factor SAMHD1 is a deoxynucleoside triphosphate triphosphohydrolase. *Nature* 480, 379–382.

Gramberg, T., Sunseri, N., and Landau, N. R. (2010). Evidence for an activation domain at the amino terminus of simian immunodeficiency virus Vpx. *J. Virol.* 84, 1387–1396.

Guyader, M., Emerman, M., Montagnier, L., and Peden, K. (1989). VPX mutants of HIV-2 are infectious in established cell lines but display a severe defect in peripheral blood lymphocytes. *EMBO J.* 8, 1169–1175.

Hartman, Z. C., Kiang, A., Everett, R. S., Serra, D., Yang, X. Y., Clay, T. M., and Amalfitano, A. (2007). Adenovirus infection triggers rapid, MyD88-regulated transcriptome response critical to acute-phase and adaptive immune responses in vivo. *J. Virol.* 81, 1796–1812.

Hirsch, V. M., Sharkey, M. E., Brown, C. R., Brichacek, B., Goldstein, S., Wakefield, J., Byrum, R., Elkins, W. R., Hahn, B. H., Lifson, J. D., and Stevenson, M. (1998). Vpx is required for dissemination and pathogenesis of SIVSMPBj: evidence of macrophage-dependent viral amplification. *Nat. Med.* 4, 1401–1408.

Hrecka, K., Hao, C., Gierszewska, M., Swanson, S. K., Kesik-Brodacka, M., Srivastava, S., Florens, L., Washburn, M. P., and Skowronski, J. (2011). Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. *Nature* 474, 658–661.

Jacquot, G., Le Rouzic, E., David, A., Mazzolini, J., Bouchet, J., Bouaziz, S., Niedergang, F., Pancino, G., and Benichou, S. (2007). Localization of HIV-1 Vpr to the nuclear envelope: impact on Vpr functions and virus replication in macrophages. *Retrovirology* 4, 84.

Jin, L., Zhou, Y., and Ratner, L. (2001). HIV type 2 Vpx interaction with Gag and incorporation into virus-like particles. *AIDS Res. Hum. Retroviruses* 17, 105–111.

Kappes, J. C., Conway, J. A., Lee, S.-W., Shaw, G. M., and Hahn, B. H. (1991). Human immunodeficiency virus type 2 vpx protein augments viral infectivity. *Virology* 184, 197–209.

Kaushik, R., Zhu, X., Stranska, R., Wu, Y., and Stevenson, M. (2009). A cellular restriction dictates the permissivity of nondividing monocytes/macrophages to lentivirus and gammaretrovirus infection. *Cell Host Microbe* 6, 68–80.

Kawamura, M., Sakai, H., and Adachi, A. (1994). Human immunodeficiency virus Vpx is required for the early phase of replication in peripheral blood mononuclear cells. *Microbiol. Immunol.* 38, 871–878.

Khamsri, B., Murao, F., Yoshida, A., Sakurai, A., Uchiyama, T., Shirai, H., Matsuo, Y., Fujita, M., and Adachi, A. (2006). Comparative study on the structure and cytopathogenic activity of HIV Vpr/Vpx proteins. *Microbes Infect.* 8, 10–15.

Kitamura, S., Ode, H., and Iwatani, Y. (2011). Structural features of antiviral APOBEC3 proteins are linked to their functional activities. *Front. Microbiol.* 2:258. doi:10.3389/fmicb.2011.00258

Laguette, N., Brégnard, C., Benichou, S., and Basmaciogullari, S. (2010). Human immunodeficiency virus (HIV) type-1, HIV-2 and simian immunodeficiency virus Nef proteins. *Mol. Aspects Med.* 31, 418–433.

Laguette, N., Sobhian, B., Casartelli, N., Ringeard, M., Chable-Bessia, C., Segeral, E., Yatim, A., Emiliani, S., Schwartz, O., and Benkirane, M. (2011). SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. *Nature* 474, 654–657.

Lahouassa, H., Daddacha, W., Hofmann, H., Ayinde, D., Logue, E. C., Dragin, L., Bloch, N., Maudet, C., Bertrand, M., Gramberg, T., Pancino, G., Priet, S., Canard, B., Laguette, N., Benkirane, M., Transy, C., Landau, N. R., Kim, B., and Margottin-Goguet, F. (2012). SAMHD1 restricts the replication of human immunodeficiency virus type 1 by depleting the intracellular pool of deoxynucleoside triphosphates. *Nat. Immunol.* 13, 223–228.

Li, N., Zhang, W., and Cao, X. (2000). Identification of human homologue of mouse IFN-γ induced protein from human dendritic cells. *Immunol. Lett.* 74, 221–224.

Lim, E. S., Fregoso, O. I., McCoy, C. O., Matsen, F. A., Malik, H. S., and Emerman, M. (2012). The ability of primate lentiviruses to degrade the monocyte restriction factor SAMHD1 preceded the birth of the viral accessory protein Vpx. *Cell Host Microbe* 11, 194–204.

Mahalingam, S., Van Tine, B., Santiago, M. L., Gao, F., Shaw, G. M., and Hahn, B. H. (2001). Functional analysis of the simian immunodeficiency virus Vpx protein: identification of packaging determinants and a novel nuclear targeting domain. *J. Virol.* 75, 362–374.

Neil, S. J., Zang, T., and Bieniasz, P. D. (2008). Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. *Nature* 451, 425–430.

Nomaguchi, M., Fujita, M., and Adachi, A. (2008). Role of HIV-1 Vpu protein for virus spread and pathogenesis. *Microbes Infect.* 10, 960–967.

Pancio, H. A., Vander Heyden, N., and Ratner, L. (2000). The C-terminal proline-rich tail of human immunodeficiency virus type 2 Vpx is necessary for nuclear localization of the viral preintegration complex in nondividing cells. *J. Virol.* 74, 6162–6167.

Park, I.-W., and Sodroski, J. (1995). Amino acid sequence requirements for the incorporation of the Vpx protein of simian immunodeficiency virus into virion particle. *J. Acquir. Immune. Defic. Syndr. Hum. Retrovirol.* 10, 506–510.

Planelles, V. (2011). Restricted access to myeloid cells explained. *Viruses* 3, 1624–1633.

Planelles, V., Jowett, J. B., Li, Q. X., Xie, Y., Hahn, B., and Chen, I. S. (1996). Vpr-induced cell cycle arrest is conserved among primate lentiviruses. *J. Virol.* 70, 2516–2524.

Powell, R. D., Holland, P. J., Hollis, T., and Perrino, F. W. (2011). Aicardi-Goutieres syndrome gene and HIV-1 restriction factor SAMHD1 is a dGTP-regulated deoxynucleotide triphosphohydrolase. *J. Biol. Chem.* 286, 43596–43600.

Prehaud, C., Megret, F., Lafage, M., and Lafon, M. (2005). Virus infection switches TLR-3-positive human neurons to become strong producers of beta interferon. *J. Virol.* 79, 12893–12904.

Rice, G. I., Bond, J., Asipu, A., Brunette, R. L., Manfield, I. W., Carr, I. M., Fuller, J. C., Jackson, R. M., Lamb, T., Briggs, T. A., Ali, M., Gornall, H., Couthard, L. R., Aeby, A., Attard-Montalto, S. P., Bertini, E., Bodemer, C., Brockmann, K., Brueton, L. A., Corry, P. C., Desguerre, I., Fazzi, E., Cazorla, A. G., Gener, B., Hamel, B. C., Heiberg, A., Hunter, M., van der Knaap, M. S., Kumar, R., Lagae, L., Landrieu, P. G., Lourenco, C. M., Marom, D., McDermott, M. F., van der Merwe, W., Orcesi, S., Prendiville, J. S., Rasmussen, M., Shalev, S. A., Soler, D. M., Shinawi, M., Spiegel, R., Tan, T. Y., Vanderver, A., Wakeling, E. L., Wassmer, E., Whittaker, E., Lebon, P., Stetson, D. B., Bonthron, D. T., and Crow, Y. J. (2009). Mutations involved in Aicardi-Goutières syndrome implicate SAMHD1 as regulator of the innate immune response. *Nat. Genet.* 41, 829–832.

Selig, L., Benichou, S., Rogel, M. E., Wu, L. I., Vodicka, M. A., Sire, J., Benarous, R., and Emerman, M. (1997). Uracil DNA glycosylase specifically interacts with Vpr of both human immunodeficiency virus type 1 and simian immunodeficiency virus of sooty mangabeys, but binding does not correlate with cell cycle arrest. *J. Virol.* 71, 4842–4846.

Sharifi, H. J., Furuya, A. M., and de Noronha, C. M. (2012). The role of HIV-1 Vpr in promoting the infection of nondividing cells and in cell

cycle arrest. *Curr. Opin. HIV AIDS* 7, 187–194.

Sharova, N., Wu, Y., Zhu, X., Stanska, R., Kaushik, R., Sharkey, M., and Stevenson, M. (2008). Primate lentiviral Vpx commandeers DDB1 to counteract a macrophage restriction. *PLoS Pathog.* 4, e1000057. doi:10.1371/journal.ppat.1000057

Sheehy, A. M., Gaddis, N. C., Choi, J. D., and Malim, M. H. (2002). Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. *Nature* 418, 646–650.

Srivastava, S., Swanson, S. K., Manel, N., Florens, L., Washburn, M. P., and Skowronski, J. (2008). Lentiviral Vpx accessory factor targets VprBP/DCAF1 substrate adaptor for cullin 4 E3 ubiquitin ligase to enable macrophage infection. *PLoS Pathog.* 4, e1000059. doi:10.1371/journal.ppat.1000059

Stivahtis, G. L., Soares, M. A., Vodicka, M. A., Hahn, B. H., and Emerman, M. (1997). Conservation and host specificity of Vpr-mediated cell cycle arrest suggest a fundamental role in primate lentivirus evolution and biology. *J. Virol.* 71, 4331–4338.

Tokunaga, K., Ishimoto, A., Ikuta, K., and Adachi, A. (1997). Growth ability of auxiliary gene mutants of human immunodeficiency virus types 1 and 2 in unstimulated peripheral blood mononuclear cells. *Arch. Virol.* 142, 177–181.

Tsurutani, N., Kubo, M., Maeda, Y., Ohashi, T., Yamamoto, N., Kannagi, M., and Masuda, T. (2000). Identification of critical amino acid residues in human immunodeficiency virus type 1 IN required for efficient proviral DNA formation at steps prior to integration in dividing and nondividing cells. *J. Virol.* 74, 4795–4806.

Ueno, F., Shiota, H., Miyaura, M., Yoshida, A., Sakurai, A., Tatsuki, J., Koyama, A. H., Akari, H., Adachi, A., and Fujita, M. (2003). Vpx and Vpr proteins of HIV-2 up-regulate the viral infectivity by a distinct mechanism in lymphocytic cells. *Microbes Infect.* 5, 387–395.

Van Damme, N., Goff, D., Katsura, C., Jorgenson, R. L., Mitchell, R., Johnson, M. C., Stephens, E. B., and Guatelli, J. (2008). The interferon-induced protein BST-2 restricts HIV-1 release and is downregulated from the cell surface by the viral Vpu protein. *Cell Host Microbe* 3, 245–252.

Yu, X.-F., Yu, Q.-C., Essex, M., and Lee, T.-H. (1991). The vpx gene of simian immunodeficiency virus facilitates efficient viral replication in fresh lymphocytes and macrophages. *J. Virol.* 65, 5088–5091.

Zhao, D., Peng, D., Li, L., Zhang, Q., and Zhang, C. (2008). Inhibition of GIP3 expression found in the different display study on respiratory syncytial virus infection. *Virol. J.* 5, 114.

Zhu, Y., Gelbard, H. A., Roshal, M., Pursell, S., Jamieson, B. D., and Planelles, V. (2001). Comparison of cell cycle arrest, transactivation, and apoptosis induced by the simian immunodeficiency virus SIVagm and human immunodeficiency virus type 1 vpr genes. *J. Virol.* 75, 3791–3801.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 25 April 2012; paper pending published: 16 May 2012; accepted: 25 July 2012; published online: 10 August 2012.

Citation: Fujita M, Nomaguchi M, Adachi A and Otsuka M (2012) SAMHD1-dependent and -independent functions of HIV-2/SIV Vpx protein. Front. Microbio. 3:297. doi: 10.3389/fmicb.2012.00297

This article was submitted to Frontiers in Virology, a specialty of Frontiers in Microbiology.

frontiers in MICROBIOLOGY

REVIEW ARTICLE
published: 26 July 2012
doi: 10.3389/fmicb.2012.00267

Species tropism of HIV-1 modulated by viral accessory proteins

Masako Nomaguchi, Naoya Doi, Yui Matsumoto, Yosuke Sakai, Sachi Fujiwara and Akio Adachi*

Department of Microbiology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan

Edited by:
Mikako Fujita, Kumamoto University, Japan

Reviewed by:
Mikako Fujita, Kumamoto University, Japan
Yasuyuki Miyazaki, The University of Tokushima Graduate School, Japan

****Correspondence:***
Akio Adachi, Department of Microbiology, Institute of Health Biosciences, The University of Tokushima Graduate School, 3-18-15 Kuramoto-cho, Tokushima-shi, Tokushima 770-8503, Japan. e-mail: adachi@basic.med.tokushima-u.ac.jp

Human immunodeficiency virus type 1 (HIV-1) is tropic and pathogenic only for humans, and does not replicate in macaque monkeys routinely used for experimental infections. This specially narrow host range (species tropism) has impeded much the progress of HIV-1/acquired immunodeficiency syndrome (AIDS) basic research. Extensive studies on the underlying mechanism have revealed that Vif, one of viral accessory proteins, is critical for the HIV-1 species tropism in addition to Gag-capsid protein. Another auxiliary protein Vpu also has been demonstrated to affect this HIV-1 property. In this review, we focus on functional interactions of these HIV-1 proteins and species specific-restriction factors. In addition, we describe an evolutional viewpoint that is relevant to the species tropism of HIV-1 controlled by the accessory proteins.

Keywords: HIV-1, species tropism, accessory protein, Vif, Vpu

INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) is strictly adapted to humans, and cause disease-inducing persistent infection only in humans (Nomaguchi et al., 2008). This property is unique among primate immunodeficiency viruses, and represent one of the most evident and important viral characteristics to understand the biology/molecular biology of HIV-1. Of numerous primate immunodeficiency viruses so far identified (Kirchhoff, 2009; Sharp and Hahn, 2011), HIV-1 with an extremely limited host range exhibits exceptionally high replication ability, transmissibility, and pathogenicity in sensitive host humans. For basic HIV-1 researchers, it would be final goal to realize the basis/mechanism underlying these properties by experimental approaches.

Primate immunodeficiency viruses can be divided into three groups based on their genome structure in the central regions (Kirchhoff, 2009; Fujita et al., 2010; Sharp and Hahn, 2011). While viruses of HIV-1 type contain *vpr* and *vpu* genes, viruses of HIV-2 type carry *vpx* and *vpr* genes in tandem (**Figure 1**). The other simian immunodeficiency viruses (SIVs), the prototype virus, have only the *vpr* gene in the corresponding genomic region. HIV-1 is believed to emerge from the prototype virus via SIVmon/mus/gsn (isolated from the mona, mustached, and greater spot-nosed monkeys), SIVcpz (isolated from the chimpanzees), and SIVgor (isolated from the gorilla) through mutational and recombinational events. SIVmon/mus/gsn is known to recombine with SIVrcm (isolated from the red-capped mangabey monkey) to generate SIVcpz (for genome structures, see, **Figure 1**). SIVcpz served as parental virus for HIV-1 (M and N) and SIVgor (and finally for HIV-1 P).

The biological and molecular biological bases for species tropism of HIV-1 should reside in the above outlined evolutional processes. In particular, the so-called accessory proteins encoded by extra genes are important. Each virus group has a unique set of the accessory proteins in terms of their combinations and of their activities. Therefore, studies on viral accessory proteins are also meaningful for understanding viral evolution by cross-species transmission.

VIRAL AND CELLULAR DETERMINANTS FOR HIV-1 SPECIES TROPISM

Our early studies have already suggested the possible viral determinants and viral replication stage involved in the HIV-1 species tropism described above (Shibata et al., 1991, 1995; Shibata and Adachi, 1992). By the use of numerous chimeric molecular clones between HIV-1 and dual-tropic (tropic for human and monkey cells) SIVmac (isolated from the macaque monkey), we have claimed in essence, together with a work on the cyclophilin A (CypA; Dorfman and Gottlinger, 1996), that Gag-capsid (CA) and a viral protein(s) encoded by the central genomic region of HIV-1 are the determinants. We also have showed that HIV-1 is replication-incompetent in monkey cells because a certain replication step(s) before/during reverse transcription, other than the viral entry into cells, does not proceed normally. Subsequent extensive studies by us and others have clearly indicated that the interactions of Gag-CA/CypA, Gag-CA/tripartite motif (TRIM) proteins, and Vif/apolipoprotein B mRNA-editing enzyme-catalytic (APOBEC) proteins are major determinants for the HIV-1 species tropism (Nomaguchi et al., 2008, 2011; Nakayama and Shioda, 2012; Sakuma and Takeuchi, 2012) as summarized in **Table 1**. Gag-CA, CypA, and TRIM proteins have been described in detail in two articles in the Research Topic of this journal (Nakayama and Shioda, 2012; Sakuma and Takeuchi, 2012).

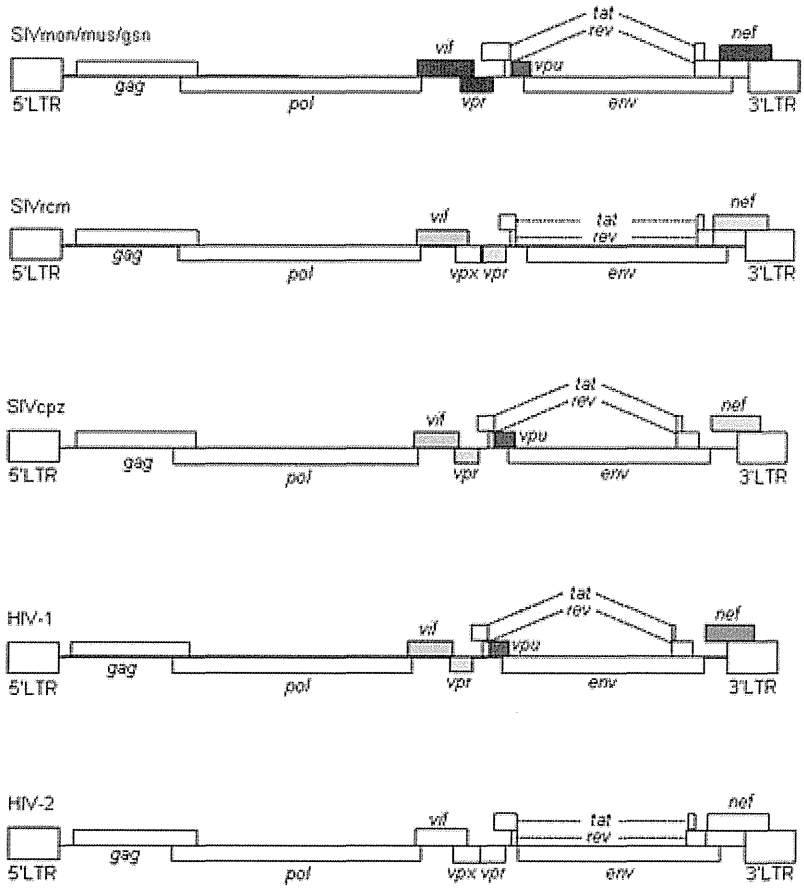

FIGURE 1 | Genome organization of primate immunodeficiency viruses. Various proviral genomes are schematically shown. As indicated by colored boxes, the *vpr* and *vpu* genes of SIVcpz/HIV-1 came from those of SIVrcm and SIVmon/mus/gsn, respectively. Also, the *vif* genes of SIVcpz/HIV-1 originated from that of SIVrcm. In addition, as shown by colored boxes, HIV-1 *nef* gene is similar to but distinct from SIVcpz *nef* gene. HIV-1 *nef* gene is different from those of SIVmon/mus/gsn, SIVrcm, and HIV-2 as indicated. For virus designations, see text.

Table 1 | Major viral and cellular determinants for HIV-1 species tropism.

Virus	Cell	Viral replication step affected
Gag-CA	CypA	
Gag-CA	TRIM5α	Uncoating (early phase)
Gag-CA	TRIMCyp	Uncoating (early phase)
Vif	APOBEC3G	Reverse transcription (early phase)
	APOBEC3F	Reverse transcription (early phase)
Vpu	Tetherin/BST-2	Virion release (late phase)

For details, see references (Nakayama and Shioda, 2012; Sakuma and Takeuchi, 2012) for Gag-CA, and Figures 3 and 4 for Vif/Vpu.

ACCESSORY PROTEINS OF PRIMATE IMMUNODEFICIENCY VIRUSES

All primate immunodeficiency viruses encode a number of extra proteins (Vif, Vpx, Vpr, Vpu, and Nef) in addition to regulatory (Tat and Rev) and structural (Gag, Pol, and Env) proteins (**Figure 1**). Structural proteins are common to all retroviruses, but the regulatory and accessory proteins are unique to the complex primate lentiviruses and not found in the other simple mammalian retroviruses. Regulatory Tat and Rev proteins are trans-activators for transcription and for the expression of late viral proteins, respectively. While the regulatory and structural proteins are essential for viral replication, the extra proteins, unfairly generically called "accessory," are dispensable under certain circumstances. However, in some cells, some of them are essential and the others are quite critical/important for optimal viral replication as illustrated for ΔVif and ΔVpu viruses (viruses that lack Vif or Vpu) in **Figure 2**. Another point to be mentioned here is relating to Vpr/Vpx proteins. Although Vpr and Vpx are genetically very similar (Khamsri et al., 2006), some primate immunodeficiency viruses bear two of them as described above (Fujita et al., 2010). Furthermore, the other viruses have Vpr only. What about the functional relationship of the two proteins? At present, the function of Vpr/Vpx is least well understood relative to that of the other accessory proteins (Malim and Emerman, 2008; Fujita et al., 2010). **Table 2** summarizes the important information regarding these accessory proteins so

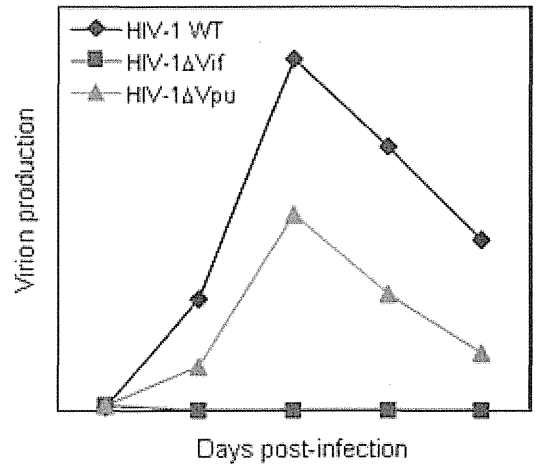

FIGURE 2 | A schema of replication kinetics by HIV-1 wild-type and mutant viruses. Viral growth properties in cells are illustrated based on numerous infection experiments in our laboratory. WT, wild-type.

Table 2 | Accessory proteins of primate immunodeficiency viruses.

Viral Proteins	Major functions for viral replication reported so far
Vif	Neutralize APOBEC3G/F. Essential for viral replication in natural target cells.
Vpx	Degrade SAMHD1/APOBEC3A. Critical for viral replication in natural target cells.
Vpr	Important for viral replication in macrophages (HIV-1).
Vpu	Down-regulate Tetherin/BST-2. Important for viral replication in CD4-positive cells.
Nef	Down-regulate cell surface molecules (CD4, MHC-I etc.).

far reported. In total, it is fairly reasonable to believe that the accessory proteins are regulators to optimize viral replication and persistence *in vivo* thereby enhancing viral transmission between individuals.

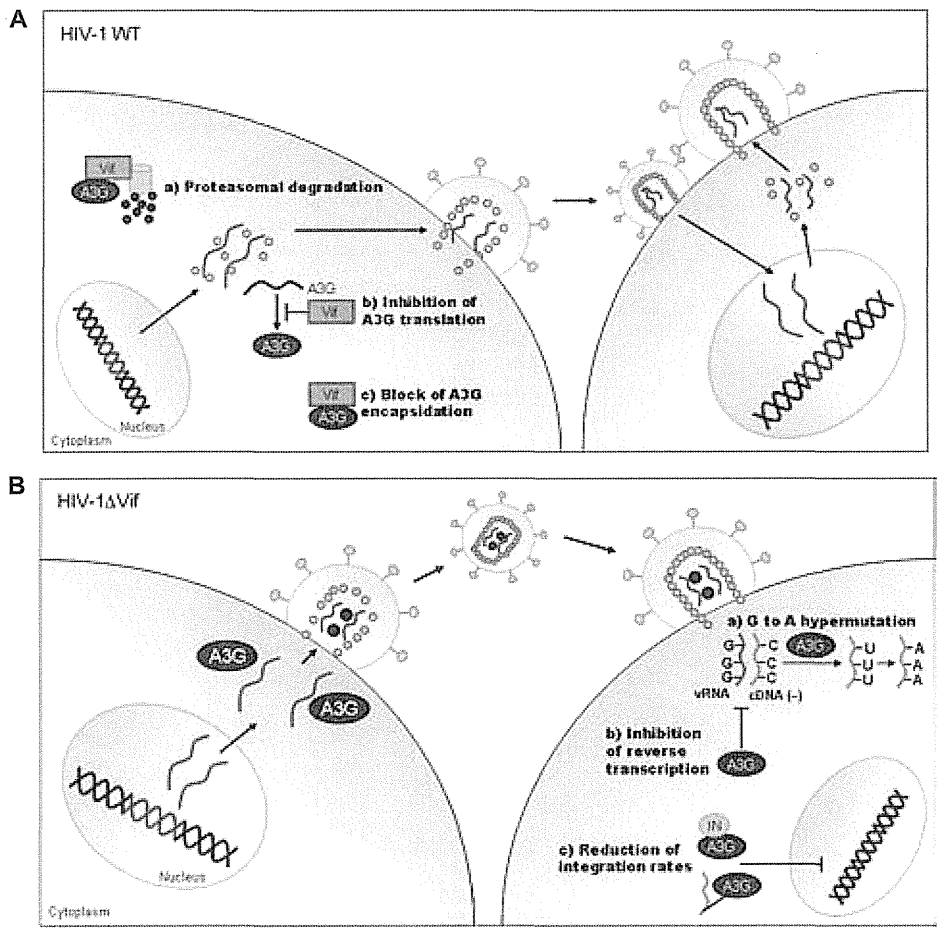

FIGURE 3 | HIV-1 replication and APOBEC3G. On the basis of results reported so far, the action mechanism of Vif is depicted. Replication process for wild-type (WT) and ΔVif mutant viruses are schematically shown on the basis of previously reported review articles (Holmes et al., 2007; Huthoff and Towers, 2008; Strebel et al., 2009). A3G, APOBEC3G; IN, viral integrase protein.

Vif AND Vpu PROTEINS

Vif protein is essential for viral replication in natural target cells such as CD4-positve lymphocytes and macrophages. Recent identification of its cellular object for attack (Sheehy et al., 2002) has clearly revealed the biological and biochemical bases for the growth property of ΔVif virus in natural target cells. This finding (identification of a family of APOBEC3 proteins, cellular cytidine deaminases, as potent inhibitors of HIV-1 replication in primary cells) has also contributed much to establish the concept of "the restriction factor" to well understand virus–cell interaction (Malim and Emerman, 2008; Sato et al., 2012). Of the APOBEC3 family proteins, APOBEC3G and APOBEC3F (Kitamura et al., 2011) strongly inhibit viral replication in the absence of Vif (**Figure 3**). Although HIV-1 Vif can abrogate the activities of human APOBEC3, it cannot do so against monkey APOBEC3. In contrast, SIVmac Vif can neutralize the antiviral activity of APOBEC3 of both origins. Finally, it has been demonstrated that Vif and APOBEC3 are the major determinants for the HIV-1 species tropism by constructing macaque-tropic HIV-1 (HIV-1mt) and monitoring the HIV-1mt growth property in various genetic contexts of macaques (Hatziioannou et al., 2006, 2009; Kamada et al., 2006; Igarashi et al., 2007; Thippeshappa et al., 2011).

Vpu protein, unique to viruses of the HIV-1 group (**Figure 1**), modulates viral replication in human CD4-positive cell lines and primary cells. Mutant HIV-1 without Vpu (ΔVpu virus) grows poorly relative to wild-type virus. Recently, a cellular protein named Tetherin (also called BST-2) has been identified as a restriction factor against HIV-1 and is antagonized by Vpu (Neil et al., 2008; Van Damme et al., 2008). Vpu down-regulates the Tetherin from cell surface, and thereby promotes extracellular production of progeny virions (Malim and Emerman, 2008; Arias et al., 2011; Sato et al., 2012). The baseline mechanism for this action of Vpu is well studied as shown in **Figure 4**. Here, it must be attentive that the anti-Tetherin activity of Vpu is host species-specific as observed for Vif. HIV-1 Vpu acts against human but not (or poorly) macaque Tetherins (Sauter et al., 2009, 2010). Although the biological effect of Vpu is much milder than that of Vif as judged by the growth kinetics of mutant viruses (**Figure 2**), Vpu may be critical for interspecies transmission

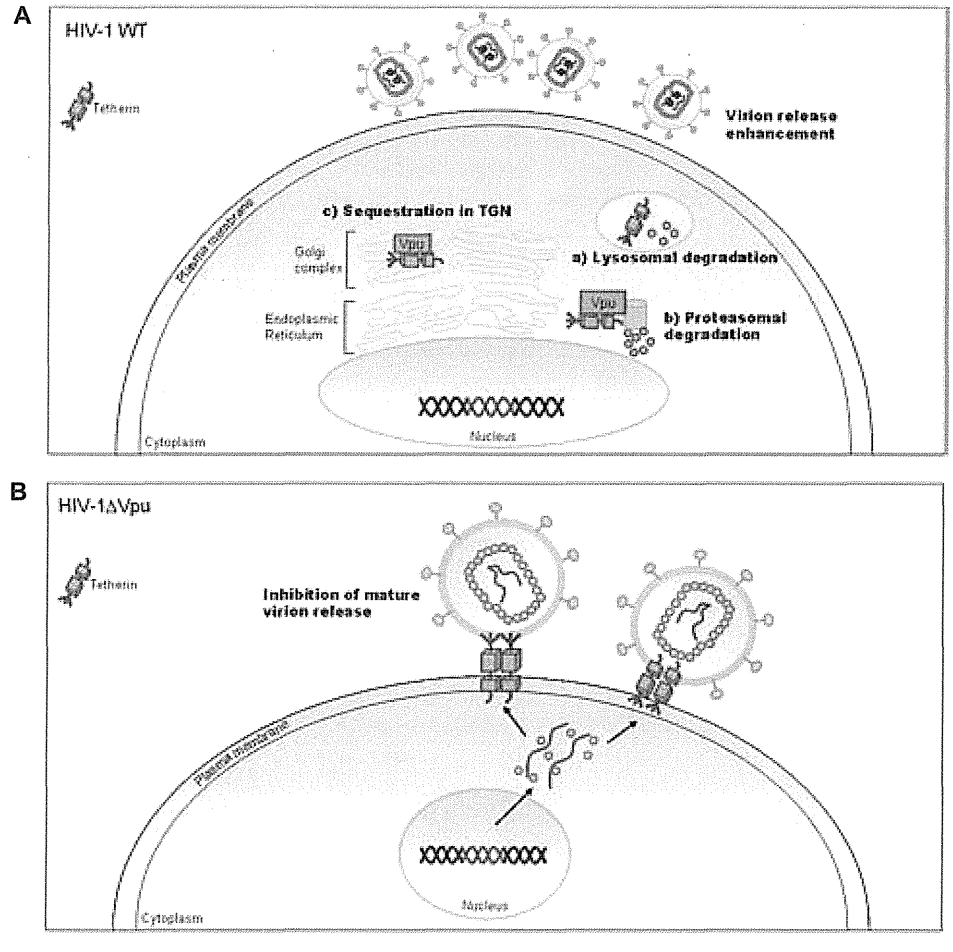

FIGURE 4 | HIV-1 replication and Tetherin. On the basis of results reported so far, the action mechanism of Vpu is depicted. Replication process for wild-type (WT) and ΔVpu mutant viruses are schematically shown on the basis of previously reported review articles (Tokarev et al., 2009; Douglas et al., 2010; Evans et al., 2010). TGN, trans-Golgi network.

through mutation/adaptation/recombinations (Kirchhoff, 2009; Sauter et al., 2009, 2010; Sharp and Hahn, 2011). Thus, Vpu and Tetherin affect the HIV-1 species tropism, but the effect may be relatively small.

In sum, Vif and Vpu counteract the major restriction factors APOBEC3 proteins and Tetherin/BST-2, respectively, and represent viral determinants for the host range of HIV-1 (**Tables 1 and 2**). It is intriguing to note that these factors would have shaped HIV-1 and made it unique among various primate immunodeficiency viruses (**Figure 1**).

Vpx AND Vpr PROTEINS

Vpx and Vpr proteins are necessary for efficient viral replication (Malim and Emerman, 2008; Fujita et al., 2010). In macrophages, ΔVpx replication is not detectable and this defect has been shown to be present at post-entry and before/during the reverse transcription process (Fujita et al., 2008, 2010; Srivastava et al., 2008). Also in some lymphocyte cell lines and in primary lymphocytes, Vpx protein is critical for viral replication (Ueno et al., 2003; Fujita et al., 2010; Doi et al., 2011). Because ΔVpr virus is somewhat replication-defective in some cells (for both HIV-1 and HIV-2), it is not unreasonable to assume that Vpr may play a role in the viral growth cycle. As such, Vpx and Vpr are important for *in vivo* viral replication and finally for viral pathogenicity (Fujita et al., 2010).

Very recently, SAMHD1 and APOBEC3A have been reported to be myeloid cell-specific restriction factors against HIV-1 counteracted by Vpx (Berger et al., 2011; Hrecka et al., 2011; Laguette et al., 2011). Whether these proteins are associated with the HIV-1 species tropism described in this review article, and whether they can explain the *in vitro* and *in vivo* situation of HIV-2/SIVmac mutant viruses mentioned above remain to be determined (Fujita et al., 2010; Nomaguchi et al., 2011).

CONCLUSION

In this review, we have described the major determinants for the species tropism of HIV-1. Structural Gag-CA and accessory Vif and Vpu proteins are clearly involved in this host range of HIV-1 as viral factors (**Table 1**). Cellular proteins that interact with these and contribute to this tropism are definitely the restriction factors (**Table 1**). In total, interplays between the viral and cellular responsible factors decide this unique and limited tropism of HIV-1. Whether there are the other factors that affect the HIV-1 species tropism is awaiting further investigations. In this regard, the biology of Vpx deserves attention. Because Vpx is present in SIVmac but not in HIV-1 (**Figure 1**), it may inactivate a cellular anti-viral protein(s) which is not recognized by HIV-1 proteins.

In both basic and applicable points of view, the narrow host range of HIV-1 is burdensome obstacle to overcome. Assuming that HIV-1mt can grow and cause disease similarly with SIVmac in macaques, we would be able to better perform model studies to precisely analyze viral replication and pathogenicity *in vivo*, and to establish the effective anti-HIV-1/AIDS strategies. To the best of our knowledge, there are no such HIV-1mt clones so far (Hatziioannou et al., 2006, 2009; Kamada et al., 2006; Igarashi et al., 2007; Kuroishi et al., 2009; Saito et al., 2011; Thippeshappa et al., 2011). We may further improve the ability of HIV-1mt by today's powerful methodology if we knew all the cellular determinants for the species tropism of HIV-1. Studies in this direction are in progress in our laboratory.

ACKNOWLEDGMENTS

We thank Ms Kazuko Yoshida for excellent editorial assistance. This work was supported by a grant from the Ministry of Health, Labour and Welfare of Japan (Research on HIV/AIDS project no. H22-003).

REFERENCES

Arias, J. F., Iwabu, Y., and Tokunaga, K. (2011). Structural basis for the antiviral activity of BST-2/tetherin and its viral antagonism. *Front. Microbiol.* 2:250. doi: 10.3389/fmicb.2011.00250

Berger, G., Durand S., Fargier, G., Nguyen, X.-N., Cordeil, S., Bouaziz, S., Muriaux, D., Darlix, J.-L., and Cimarelli, A. (2011). APOBEC3A is a specific inhibitor of the early phases of HIV-1 infection in myeloid cells. *PLoS Pathog.* 7, e1002221. doi: 10.1371/journal.ppat.1002221

Doi, N., Fujiwara, S., Adachi, A., and Nomaguchi, M. (2011). Rhesus M1.3S cells suitable for biological evaluation of macaque-tropic HIV/SIV clones. *Front. Microbiol.* 2:115. doi: 10.3389/fmicb.2011.00115

Dorfman, T., and Gottlinger, H. G. (1996). The human immunodeficiency virus type 1 capsid p2 domain confers sensitivity to the cyclophilin-binding drug SDZ NIM 811. *J. Virol.* 70, 5751–5757.

Douglas, J. L., Gustin, J. K., Viswanathan, K., Mansouri, M., Moses, A. V., and Fruh, K. (2010). The great escape: viral strategies to counter BST-2/tetherin. *PLoS Pathog.* 6, e1000913. doi: 10.1371/journal.ppat.1000913

Evans, D. T., Serra-Moreno, R., Singh, R. K., and Guatelli, J. C. (2010). BST-2/tetherin: a new component of the innate immune response to enveloped viruses. *Trends Microbiol.* 18, 388–396.

Fujita, M., Otsuka, M., Miyoshi, M., Khamsri, B., Nomaguchi, M., and Adachi, A. (2008). Vpx is critical for reverse transcription of the human immunodeficiency virus type 2 genome in macrophages. *J. Virol.* 82, 7752–7756.

Fujita, M., Otsuka, M., Nomaguchi, M., and Adachi, A. (2010). Multifaceted activity of HIV Vpr/Vpx proteins: the current view of their virological functions. *Rev. Med. Virol.* 20, 68–76.

Hatziioannou, T., Ambrose, Z., Chung, N. P. Y., Piatak, M. Jr., Yuan, F., Trubey, C. M., Coalter, V., Kiser, R., Schneider, D., Smedley, J., Pung, R., Gathuka, M., Estes, J. D., Veazey, R. S., KewalRamani, V. N., Lifson, J. D., and Bieniasz, P. D. (2009). A macaque model of HIV-1 infection. *Proc. Natl. Acad. Sci. U.S.A.* 106, 4425–4429.

Hatziioannou, T., Princiotta, M., Piatak, M. Jr., Yuan, F., Zhang, F., Lifson, J. D., and Bieniasz, P. D. (2006). Generation of simian-tropic HIV-1 by restriction factor evasion. *Science* 314, 95.

Holmes, R. K., Malim, M. H., and Bishop, K. N. (2007). APOBEC-mediated viral restriction: not simply editing? *Trends Biochem. Sci.* 32, 118–128.

Hrecka, K., Hao, C., Gierszewska, M., Swanson, S. K., Kesik-Brodacka, M., Srivastava, S., Florens, L., Washburn, M. P., and Skowronski, J. (2011). Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. *Nature* 474, 658–661.

Huthoff, H., and Towers, G. J. (2008). Restriction of retroviral replication by APOBEC3G/F and TRIM5alpha. *Trends Microbiol.* 16, 612–619.

Igarashi, T., Iyengar, R., Byrum, R. A., Buckler-White, A., Dewar, R. L., Buckler, C. E., Lane, H. C., Kamada, K., Adachi, A., and Martin, M. A. (2007). Human immunodeficiency virus type 1 derivative with 7% simian immunodeficiency virus genetic content is able to establish infections in pig-tailed macaques. *J. Virol.* 81, 11549–11552.

Kamada, K., Igarashi, T., Martin, M. A., Khamsri, B., Hatcho, K., Yamashita, T., Fujita, M., Uchiyama, T., and Adachi, A. (2006). Generation of HIV-1 derivatives that productively infect macaque monkey lymphoid cells. *Proc. Natl. Acad. Sci. U.S.A.*103, 16959–16964.

Khamsri, B., Murao, F., Yoshida, A., Sakurai, A., Uchiyama, T., Shirai, H., Matsuo, Y., Fujita, M., and Adachi, A. (2006). Comparative study on the structure and cytopathogenic activity of HIV Vpx/Vpr proteins. *Microbes Infect.* 8, 10–15.

Kirchhoff, F. (2009). Is the high virulence of HIV-1 an unfortunate coincidence of primate lentiviral evolution? *Nat. Rev. Microbiol.* 7, 467–476.

Kitamura, S., Ode, H., and Iwatani, Y. (2011). Structual features of antiviral APOBEC3 proteins are linked to their functional activities. *Front. Microbiol.* 2:258. doi: 10.3889/fmicb.2011.00258

Kuroishi, A., Saito, A., Shingai, Y., Shioda, T., Nomaguchi, M., Adachi, A., Akari, H., and Nakayama, E. E. (2009). Modification of a loop sequensce between alpha-helices 6 and 7 of virus capsid (CA) protein in a human immunodeficiency virus type 1 (HIV-1) derivative that has simian immunodeficiency virus (SIVmac239) Vif and CA alpha-helices 4 and 5 loop improves replication in cynomolgus monkey cells. *Retrovirology* 6, 70.

Laguette, N., Sobhian, B., Casartelli, N., Ringeard, M., Chable-Bessia, C., Segeral, E., Yatim, A., Emiliani, S., Schwartz, O., and Benkirane, M. (2011). SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. *Nature* 474, 654–657.

Malim, M. H., and Emerman, M. (2008). HIV-1 accessory proteins-ensuring viral survival in a hostile environment. *Cell Host Microbe* 3, 388–398.

Nakayama, E. E., and Shioda, T. (2012). TRIM5α and species tropism of HIV/SIV. *Front. Microbiol.* 3:13. doi: 10.3389/fmicb.2012.00013

Neil, S. J., Zang, T., and Bieniasz, P. D. (2008). Tether ininhibits retrovirus release and is antagonized by HIV-1 Vpu. *Nature* 451, 425–430.

Nomaguchi, M., Doi, N., Kamada, K., and Adachi, A. (2008). Species barrier of HIV-1 and its jumping by virus engineering. *Rev. Med. Virol.* 18, 261–275.

Nomaguchi, M., Fujita, M., and Adachi, A. (2011). The fourth major restriction factor against HIV/SIV. *Front. Microbiol.* 2:132. doi: 10.3389/fmicb.2011.00132

Saito, A., Nomaguchi, M., Iijima, S., Kuroishi, A., Yoshida, T., Lee, Y. J., Hayakawa, T., Kono, K., Nakayama, E. E., Shioda, T., Yasutomi, Y., Adachi, A., Matano, T., and Akari, H. (2011). Improved capacity of a monkey-tropic HIV-1 derivative to replicate in cynomolgus monkeys with minimal modifications. *Microbes Infect.* 13, 58–64.

Sakuma, R., and Takeuchi, H. (2012). SIV replication in human cells. *Front. Microbiol.* 3:162. doi: 10.3389/fmicb.2012.00162

Sato, K., Gee, P., and Koyanagi, Y. (2012). Vpu and BST-2: still not there yet? *Front. Microbiol.* 3:131. doi: 10.3389/fmicb.2012.00131

Sauter, D., Schindler, M., Specht, A., Landford, W. N., Munch, J., Kim, K.-A., Votteler, J., Schubert, U., Bibollet-Ruche, F., Keele, B. R. F., Takehisa, J., Ogando, Y., Ochsenbauer, C., Kappes, J. C., Ayouba, A., Peeters, M., Learn, G. H., Shaw, G., Sharp, P. M., Bieniasz, P., Hahn, B. H., Hatzioannou, T., and Kirchhoff, F. (2009). Tetherin-driven adaptation of Vpu and Nef function and the evolution of pandemic and nonpandemic HIV-1 strains. *Cell Host Microbe* 6, 409–421.

Sauter, D., Specht, A., and Kirchhoff, F. (2010). Tetherin: holding on and letting go. *Cell* 141, 392–398.

Sharp, P. M., and Hahn, B. H. (2011). Origins of HIV and the AIDS pandemic. *Cold Spring Harb. Perspect. Med.* 1, a006841.

Sheehy, A. M., Gaddis, N. C., Choi, J. D., and Malim, M. H. (2002). Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. *Nature* 418, 646–650.

Shibata, R., and Adachi, A. (1992). SIV/HIV recombinants and their use in studying biological properties. *AIDS Res. Hum. Retroviruses* 8, 403–409.

Shibata, R., Kawamura, M., Sakai, H., Hayami, M., Ishimoto, A., and Adachi, A. (1991). Generation of a chimeric human and simian immunodeficiency virus infectious to monkey peripheral blood mononuclear cells. *J. Virol.* 65, 3514–3520.

Shibata, R., Sakai, H., Kawamura, M., Tokunaga, K., and Adachi, A. (1995). Early replication block of human immunodeficiency type 1 in monkey cells. *J. Gen. Virol.* 76, 2723–2730.

Srivastava, S., Swanson, S. K., Manel, N., Florens, L., Washburn, M. P., and Skowronski, J. (2008). Lentiviral Vpx accessory factor targets VprBP/DCAF1 substrate adaptor for cullin 4 E3 ubiquitin ligase to enable to enhance macrophage infection. *PLoS Pathog.* 4, e1000059. doi: 10.1371/journal.ppat.1000059

Strebel, K., Luban, J., and Jeang, K. T. (2009). Human cellular restriction factors that target HIV-1 replication. *BMC Med.* 7, 48. doi: 10.1186/1741-7015-7-48

Thippeshappa, R., Polacino, P., Kimata, M. T. Y., Siwak, E. B., Anderson, D., Wang, W., Sherwood, L., Arora, R., Wen, M., Zhou, P., Hu, S.-L., and Kimata, J. T. (2011). Vif substitution enables persistent infection of pig-tailed macaques by human immunodeficiency virus type 1. *J. Virol.* 85, 3767–3779.

Tokarev, A., Skasko, M., Fitzpatrick, K., and Guatelli, J. (2009). Antiviral activity of the interferon-induced cellular protein BST-2/tetherin. *AIDS Res. Hum. Retroviruses* 25, 1197–1210.

Ueno, F., Shiota, H., Miyaura, M., Yoshida, A., Sakurai, A., Tatsuki, J., Koyama, A. H., Akari, H., Adachi, A., and Fujita, M. (2003). Vpx and Vpr proteins up-regulate the viral infectivity by a distinct mechanism in lymphocytic cells. *Microbes Infect.* 5, 387–395.

Van Damme, N., Goff, D., Katsura, C., Jorgenson, R. L., Mitchell, R., Johnson, M. C., Stephens, E. B., and Guatelli, J. (2008). The interferon-induced protein BST-2 restricts HIV-1 release and is down-regulated from the cell surface by the Vpu protein. *Cell Host Microbe* 3, 245–252.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 02 July 2012; paper pending published: 08 July 2012; accepted: 09 July 2012; published online: 26 July 2012.

Citation: Nomaguchi M, Doi N, Matsumoto Y, Sakai Y, Fujiwara S and Adachi A (2012) Species tropism of HIV-1 modulated by viral accessory proteins. Front. Microbio. 3:267. doi: 10.3389/fmicb.2012.00267

This article was submitted to Frontiers in Virology, a specialty of Frontiers in Microbiology.

SCIENTIFIC REPORTS

SUBJECT AREAS:
CANCER GENOMICS
HAEMATOLOGICAL CANCER
DNA DAMAGE AND REPAIR
ONCOGENES

Received
2 August 2012

Accepted
10 October 2012

Published
13 November 2012

Correspondence and requests for materials should be addressed to K.S. (shind009@kuhp.kyoto-u.ac.jp)

APOBEC3B can impair genomic stability by inducing base substitutions in genomic DNA in human cells

Masanobu Shinohara, Katsuhiro Io, Keisuke Shindo, Masashi Matsui, Takashi Sakamoto, Kohei Tada, Masayuki Kobayashi, Norimitsu Kadowaki & Akifumi Takaori-Kondo

Department of Hematology and Oncology, Graduate school of medicine, Kyoto University, Kyoto 606-8507, Japan.

Human APOBEC3 proteins play pivotal roles in intracellular defense against viral infection by catalyzing deamination of cytidine residues, leading to base substitutions in viral DNA. Activation-induced cytidine deaminase (AID), another member of the APOBEC family, is capable of editing immunoglobulin (Ig) and non-Ig genes, and aberrant expression of AID leads to tumorigenesis. However, it remains unclear whether APOBEC3 (A3) proteins affect stability of human genome. Here we demonstrate that both A3A and A3B can induce base substitutions into human genome as AID can. A3B is highly expressed in several lymphoma cells and somatic mutations occur in some oncogenes of the cells highly expressing A3B. Furthermore, transfection of *A3B* gene into lymphoma cells induces base substitutions in *cMYC* gene. These data suggest that aberrant expression of A3B can evoke genomic instability by inducing base substitutions into human genome, which might lead to tumorigenesis in human cells.

It is widely recognized that the accumulation of genetic changes in tumor-related genes is essential for cancer development[1]. With the innovation of high-throughput sequencing technology, genome-wide analyses on various types of cancer cells have revealed numerous somatic mutations in tumor-related genes[2]. Some of these mutations are caused by defects in DNA repair systems (e.g., DNA mismatch repair deficiencies give rise to hereditary non-polyposis colon cancer[3]), whereas mechanisms that account for the majority of genetic changes in cancer cells are poorly understood. Referring to somatic base substitution spectra in cancer cells, C/G to T/A transitions are most prevalent, especially in gastric cancer, colorectal cancer, glioma, and melanoma[2,4,5]. This strong bias in somatic mutations suggests the existence of active mechanisms that induce C/G to T/A transitions into genomic DNA. It is obviously attributable to ultraviolet irradiation and following repair process against pyrimidine dimer in case of melanoma, but not in others.

The human APOBEC family proteins can induce C to T (G to A, in complementary sequences) transitions into target DNA through cytidine deamination. The APOBEC family is comprised of a series of molecules with conserved cytidine deaminase domains (CDAs), including AID, APOBEC1, APOBEC2, APOBEC3A to H, and APOBEC4[6,7]. Among them, AID plays a crucial role in somatic hypermutation and class switch recombination of Ig genes, which enables diversification of immune system[8]. AID has been considered the only molecule that can induce C/G to T/A transitions into genomic DNA. The expression of AID is highly regulated and restricted in germinal center B-cells under physiological conditions, but with inflammatory stimulations, AID can be overexpressed in not only B-cells but also other types of cells (e.g., epithelial cells) via activation of NF-κB[9]. Aberrant expression of AID results in the accumulation of mutations in non-Ig genes[10], which leads to development of various cancers such as gastric and hepatic cancers as well as lymphomas[9,11–13].

A series of seven A3 genes are tandemly arrayed on human chromosome 22, and the main function of the resulting gene products is to protect the cells from retroviruses and endogenous mobile retroelements[14,15]. A3B, A3D, A3F, and A3G contain two CDAs, instead of one in A3A, A3C, and A3H. A3G is a powerful anti-retroviral molecule that induces cytidine deamination in viral genome and acts as a host defensive factor against viruses such as HIV-1[16]. A3A and A3B have been reported as potent inhibitors of retrotransposons[17]. Thus, A3 proteins act as sentinels in innate immunity against mobile DNA/RNA including viruses, while little is known about the effect of these proteins on nuclear DNA, in other words, host human genome. Recent studies have demonstrated that A3A impairs nuclear DNA under the condition of suppressing uracil DNA-glycosylase (UNG) which prevents base alterations by eliminating uracil from DNA and initiating the base-excision repair pathway[18,19].