HIV-2 and SIVmac Accessory Virulence Factor Vpx Down-regulates SAMHD1 Enzyme Catalysis Prior to Proteasome-dependent Degradation*

Received for publication, March 13, 2013, and in revised form, May 14, 2013 Published, JBC Papers in Press, May 15, 2013, DOI 10.1074/jbc.M113.469007

Maria DeLucia*,†, Jennifer Mehrens*,†, Ying Wu‡, and Jinwoo Ahn§

From the *Department of Structural Biology and †Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15260

Background: SAMHD1 is counteracted by the HIV-2/SIV virulence factor Vpx, which directs it for degradation.

Results: Vpx in complex with DDB1-DCAF1 binds to the C terminus of SAMHD1, inhibits its catalytic activity, and dissociates SAMHD1 tetramers.

Conclusion: The viral countermeasures by Vpx are manifested at multiple levels.

Significance: Identifying molecular mechanisms of viral countermeasures against HIV restriction factors is important for designing antiviral therapeutics.

SAMHD1, a dGTP-regulated deoxyribonucleoside triphosphate (dNTP) triphosphohydrolase, down-regulates dNTP pools in terminally differentiated and quiescent cells, thereby inhibiting HIV-1 infection at the reverse transcription step. HIV-2 and simian immunodeficiency virus (SIV) counteract this restriction via a virion-associated virulence accessory factor, Vpx (Vpr in some SIVs), which loads SAMHD1 onto CRL4-DCAF1 E3 ubiquitin ligase for polyubiquitination, programming it for proteasome-dependent degradation. However, the detailed molecular mechanisms of SAMHD1 recruitment to the E3 ligase have not been defined. Further, whether divergent, orthologous Vpx proteins, encoded by distinct HIV/SIV strains, bind SAMHD1 in a similar manner, at a molecular level, is not known. We applied surface plasmon resonance analysis to assess the requirements for and kinetics of binding between various primate SAMHD1 proteins and Vpx proteins from SIV or HIV-2 strains. Our data indicate that Vpx proteins, bound to DCAF1, interface with the C terminus of primate SAMHD1 proteins with nanomolar affinity, manifested by rapid association and slow dissociation. Further, we provide evidence that Vpx binding to SAMHD1 inhibits its catalytic activity and induces disassembly of a dGTP-dependent oligomer. Our studies reveal a previously unrecognized biochemical mechanism of Vpx-mediated SAMHD1 inhibition: direct down-modulation of its catalytic activity, mediated by the binding event that leads to SAMHD1 recruitment to the E3 ubiquitin ligase for proteasome-dependent degradation.

SAMHD1 (sterile α motif and HD domain-containing protein 1) is an antiviral factor, inhibiting HIV/SIV infection of myeloid cells and quiescent CD4+ lymphocytes at the post-entry stage (1–6). It also prevents direct transmission of HIV-1 from infected T lymphocytes to monocyte-derived dendritic cells (7). SAMHD1 is a deoxyribonucleoside triphosphate (dNTP) triphosphohydrolase, the catalytic activity of which is regulated by dGTP binding at an allosteric site (8, 9). Current models suggest that SAMHD1 maintains the cellular dNTP pools at low levels in those cells, thereby blocking viral reverse transcriptase activity (10, 11). SAMHD1 appears to have broad antiviral activity against diverse retroviruses (12, 13).

SAMHD1 comprises two structural domains: a sterile α motif (SAM) domain and a dNTPase domain, which encompasses a metal-dependent phosphohydrolase homologous region with a conserved histidine and aspartate (HD) motif. These two domains are connected by a short linker and flanked by unstructured regions (Fig. 1A). The N terminus, preceding the SAM domain, contains a nuclear localization signal (14–16). The crystal structure of the dNTPase domain has been determined and suggests, along with biochemical studies, that dGTP binding at an allosteric site, formed by two monomers, regulates its dNTPase activity (8). The same domain also contains exonuclease activity with RNA binding affinity (12, 17, 18). We recently provided biochemical and virological evidence that the biologically active form of human SAMHD1 is a tetramer and that its C terminus is required for efficient depletion of dNTP pools and inhibition of HIV-1 infection in monocytes (19).

Similar to other restriction factors, such as APOBEC3G and tetherin, SAMHD1 restriction is counteracted by an HIV/SIV accessory virulence factor, specifically Vpx, which is encoded by HIV-2 and SIV. Vpx binds DCAF1 (DDB1- and CUL4-associated factor 1), a substrate receptor for the CRL4 (Cullin4 [Cullin-4-associated factor 1]), full-length human SAMHD1 expressed as a GST fusion protein; SAM, sterile α motif; RU, response units; SPR, surface plasmon resonance; CTD, C-terminal domain.

* This work was supported, in whole or in part, by National Institutes of Health Grant P50GM082251. This work was also supported by funds from the University of Pittsburgh School of Medicine.

† This article contains supplemental Figs. 1 and 2.

‡ To whom correspondence should be addressed: Dept. of Structural Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260. Tel.: 412-383-6933; Fax: 412-648-9008; E-mail: jahn@structbio.pitt.edu.

§ The abbreviations used are: SIV, simian immunodeficiency virus;
RING ubiquitin ligase) E3 ubiquitin ligase, and recruits SAMHD1 to the E3 ligase for proteasome-dependent degradation (1–3, 20). The discovery of an interplay between the virulence factor and these host cell factors resolved a longstanding unanswered observation; Vpx facilitates transduction of dendritic cells and macrophages and relieves the inhibition of HIV-1 infection in restricting cells (21–24).

Phylogenetic tree and functional analyses of Vpx and its homolog, Vpr, combined with similar analyses of SAMHD1, indicate that these virulence accessory factors and the host restriction factor have undergone an evolutionary arms race (25–27). Interestingly, two distinct regions of SAMHD1, the SAM domain, at the N terminus, and the C terminus, distal to the dNTPase domain, display strong positive selection during primate evolution. However, the detailed molecular mechanisms by which Vpx recruits SAMHD1 to CRL4-DCAF1 are yet to be specified.

Here, we show that HIV-2 and SIVmac (isolated from Macaque monkey) Vpx recruit human and several simian SAMHD1 proteins to the CRL4-DCAF1 E3 ubiquitin ligase by interacting with the highly conserved C terminus of SAMHD1. Further, we show that a highly conserved, specific sequence motif at the Vpx N terminus is essential for efficient recruitment of SAMHD1. Real-time binding assays, using surface plasmon resonance (SPR) analysis, suggest that Vpx significantly increases the association rate and decreases the dissociation rate of SAMHD1 binding to the substrate adaptor-receptor complex of the E3 ubiquitin ligase. Surprisingly, Vpx-mediated recruitment of SAMHD1 to the substrate adaptor-receptor complex deactivates its dNTPase catalysis and subsequently disassembles GTP-dependent tetramers to dimer and monomers. These results suggest that Vpx employs an additional viral countermeasure, prior to and independently of proteasome-dependent down-regulation of SAMHD1.

**EXPERIMENTAL PROCEDURES**

**Cloning and Plasmid Construction**—The cDNAs encoding full-length SAMHD1 (SAMHD1-FL) or residues 113–626 (ΔN-SAMHD1) or residues 1–595 (SAMHD1-ΔC) of human, rhesus, or De Brazza’s SAMHD1 were cloned into pCDNA3.1 (Invitrogen) with an HA tag at its N terminus or into pET28 (EMD Biosciences) with His6 and T7 tags at its N terminus. The cDNAs encoding SAMHD1 from other primates, including African green monkey, mandrill, red capped monkey, and sooty mangabey were also cloned into the pET28 vector. SAMHD1 cDNAs, as indicated, using LipofectAMINE 2000 (Invitrogen), were provided by M. Emerman (Fred Hutchinson Cancer Center, Seattle, WA). Site-specific mutants of Vpx and SAMHD1 were prepared using QuikChange mutagenesis kits (Agilent).

**Protein Expression and Purification**—The various SAMHD1 and NusA-Vpx fusion proteins were expressed in Escherichia coli Rosetta 2 (DE3) cultured in Luria-Bertani medium with 0.4 mM isopropyl 1-thio-β-d-galactopyranoside at 18 °C for 16 h. Proteins were first purified using a 5-ml nickel-nitrilotriacetic acid column (GE Healthcare), and then the aggregates were removed by gel filtration column chromatography (Hi-Load Superdex200 16/60, GE Healthcare) equilibrated with a buffer containing 25 mM sodium phosphate, pH 7.5, 150 mM NaCl, 2 mM DTT, 10% glycerol, and 0.02% sodium azide. The DDB1-DCAF1CB (DCAF1 residues 1045–1396) complex was expressed and purified from SF21 cells co-infected with recombinant baculoviruses at a multiplicity of infection of 2 for 40 h, as described previously (20). For preparation of multiprotein complexes, DDB1-DCAF1CB and NusA-Vpx proteins were mixed at a molar ratio of 1:3, digested with tobacco etch virus protease, and purified over an 8-ml MONO Q column (GE Healthcare) at pH 7.5, using a 0–1 M NaCl gradient. All other proteins were prepared as described previously (20, 28).

**Mammalian Cell Lines, Transfection, and Immunoblotting**—Human embryonic kidney cell lines (HEK293 from ATCC) were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum with antibiotics. Cells were plated on 6-cm plates, 24 h prior to transient transfection, and grown to 90–95% confluence. HEK293 cells were transfected with 7 μg of a mixture of pCDNA3.1 plasmids encoding specific cDNAs, as indicated, using LipofectAMINE 2000 (Invitrogen), according to the manufacturer’s protocol. When indicated, the transfected cells were treated with 25 μM MG132 (Boston Biochem) for 6 h, 42 h after transfection. Transfected cells were harvested and treated with 300 μl of lysis buffer containing 25 mM sodium phosphate, pH 7.5, 300 mM NaCl, 1 mM EDTA, 0.3% Nonidet P-40, 1% Tween, 5% glycerol, and 1 mM phenylmethylsulfonyl fluoride. Proteins in the lysate were separated by 4–20% gradient SDS-PAGE, transferred to PVDF membrane, and subsequently identified by immunoblotting. For detection of proteins, anti-HA (Covance), anti-V5 (Sigma), and anti-actin (Sigma) antibodies were used.

**Analytical Size Exclusion Column Chromatography**—SAMHD1 proteins (0.5 μM) or their mixtures with dGTP (200 μM) in 25 mM sodium phosphate, pH 7.5, 150 mM NaCl, 5% glycerol, 1 mM dTT, and 0.02% sodium azide were injected into a 24-ml analytical Superdex200 column (10 × 300 mm, GE Healthcare) at a flow rate of 0.8 ml/min, equilibrated with the same buffer lacking the reducing agent. The elution profiles were recorded by monitoring fluorescence, with excitation at 282 nm and emission at 313 nm.

**SPR**—SPR experiments were performed using a BLAcore 2000 instrument (GE Healthcare) at 12 °C. CM5 sensor chips were coated with anti-GST antibodies according to the manufacturer’s protocol (GST capture kit, GE Healthcare). GST-SAMHD1 variants, at a concentration of 10 nM in running buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.005% P20, 0.02% sodium azide, 1 mM tris(2-carboxyethyl)phosphine),
Inhibition of SAMHD1 Catalysis

FIGURE 1. Schematic representation of human SAMHD1 and alignment of Vpx protein sequences. A, SAMHD1 comprises two folded domains: a SAM (residues 42–110) and a dNTPase domain (residues 128–576). The dNTPase domain encompasses a metal-dependent phosphohydrolase homologous region with a conserved HD motif (residues 160–325). Several residues that have been characterized for their roles in SAMHD1 function are indicated. The nuclear localization signal is at residues 11–14 (14, 15). Residues Asp-137, Gln-142, Arg-145, and Arg-451 are located at the dGTP-binding allosteric site, and residues Arg-164, His-206, Asp-207, and Asp-311 are at the catalytic site (8). The C-terminal residues Arg-617, Leu-620, Phe-621, and Met-626 are recognized by Vpx for proteasome-dependent down-regulation (20, 27). B, alignment of Vpx amino acid sequences encoded by SIVmac, HIV-2 Rod9, and HIV-2 7312a. The residues critical for SAMHD1 recruitment and down-regulation (20) are indicated in the box.

We also identified critical residues at the recruitment interface (Fig. 1) (27). Sequence alignment of other monkey SAMHD1 sequences shows strict conservation of this C-terminal region (supplemental Fig. 1) (27). Sequence alignment of Vpx proteins from SIVmac and HIV-2 indicates conservation of four N-terminal residues (Asn-12, Glu-15, Glu-16, and Thr-17 for VpxSIVmac and VpxRod9 and Asn-11, Glu-14, Glu-15, and Thr-16 for Vpx7312a) that were previously shown to be at the SAMDH1 recruitment interface (Fig. 1B) (20). Based on these observations, SIVmac and HIV-2 Vpx proteins probably

were injected into the flow cell at a rate of 5 μl/min until the increase in response units (RU) reached 50–60 RU. The first flow cell was used as an in-line reference with GST as ligand. The analytes, at different concentrations in the running buffer, were injected into the flow cells for 2 min at a flow rate of 30 μl/min, followed by a 3-min dissociation phase. The sensor surface was regenerated by repeated injections of a regeneration buffer containing 10 mM sodium acetate, pH 5.0. For steady-state analysis, the equilibrium response of each injection at 120 s was plotted against the concentration of injected protein, using a non-linear, one-site non-specific model (GraphPad Prism 5, GraphPad Software) to obtain the equilibrium dissociation constant (Kd) and binding response maximum (Rmax). No-equilibrium data, including the rate of association, koff, were globally fit to a predefined one-state model using BLAevaluation software (version 4.1).

In Vitro Ubiquitination Assays—Typically, E1 (UBA1, 0.2 μM), E2 (UbcH5b, 2.5 μM), and E3 complexes (mixtures of equimolar amounts of DDB1-DCAF1-CP1-VpxSIVmac, Vpx7312a, or VpxRod9 and CUL4A-RBX1 at 0.3 μM) were incubated with 0.6 μM SAMHD1 (SAMHD1-FL or SAMHD1-ΔC) and 2.5 μM ubiquitin, in a buffer containing 10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5% glycerol, 20 units/ml pyrophosphatase, 2 mM DTT, and 5 mM ATP at 37 °C for the indicated times. The extent of ubiquitination was assessed by immunoblotting with anti-T7 antibody (EMD Biosciences) after separation of reaction mixtures by 4–20% SDS-PAGE and transfer to PVDF membrane.

Chemical Cross-linking Assays—Typically, 60 μl of SAMHD1 proteins (1.6 μM) were mixed with an equal volume of dGTP (100 μM) in a buffer containing 25 mM sodium phosphate, pH 7.5, 50 mM NaCl, 5% glycerol, 2 mM MgCl₂, and 0.02% sodium azide. After 60 s, the mixtures were diluted with 3.13 mM glutaraldehyde, in the same buffer, and incubated for 3 min before cross-linking reactions (50 μl) were quenched with 290 mM Tris-HCl, pH 6.8 (20 μl). The resulting reactions were separated by 4–20% SDS-PAGE and visualized with Coomassie Brilliant Blue staining.

Deoxyribonucleoside Triphosphate Triphosphohydrolase (dNTPase) Assays—Assays of SAMHD1 dGTP-dependent enzymatic activities were carried out in a reaction buffer containing 20 mM Tris-HCl, pH 7.8, 50 mM NaCl, 2 mM MgCl₂, 5% glycerol, an appropriate concentration of dNTP (0–100 μM), and 0.1 μM recombinant SAMHD1. The reactions were stopped by mixing a 50-μl enzymatic reaction with 20 μl of 70 mM EDTA, after a specific time interval. Quenched reactions (50 μl) were injected into a Capcell Pak C18 reversed-phase column (4.6 × 250 mm; Phenomenex), pre-equilibrated with a buffer containing 10 mM ammonium phosphate, pH 7.8, and 4.8% methanol. Deoxyribonucleosides and dNTPs were eluted with a linear gradient (4.8–19.2%) of methanol over 22.5 min at a flow rate of 1.5 ml/min. The amounts of products were quantified by peak integration of the absorbance trace at 260 nm and converted to moles based on the calibration curve of deoxyribonucleosides.

Multiangle Light Scattering—100 μl of protein solution, typically at ~2 mg/ml, was injected into an analytical Superdex200 column (10 × 300 mm; GE Healthcare) with in-line multiangle light scattering (HELOS, Wyatt Technology), variable wavelength UV detector (Agilent 1100, Agilent Technologies), and refractive index detector (Optilab rEX, Wyatt Technology) at a flow rate of 0.5 ml/min in a buffer containing 25 mM sodium phosphate, pH 7.5, 50–150 mM NaCl, and 0.02% sodium azide. The molecular masses of eluted protein species were determined using the ASTRA version 5.3.4 program (Wyatt Technologies).

RESULTS

Kinetic Characterization of SAMHD1 Binding to the DDB1-DCAF1-Vpx Complex—We previously described that SIVmac Vpx facilitates loading of human SAMHD1 onto the substrate adaptor-receptor complex (DDB1-DCAF1) of the CRL4 E3 ubquitin ligase for proteasome-dependent degradation (20). We also identified critical residues at the recruitment interfaces. Specifically, C-terminal SAMHD1 residues Arg-617, Leu-620, and Phe-621, distal to the dNTPase domain, are essential for binding (Fig. 1A). The alignment of other monkey SAMHD1 sequences shows strict conservation of this C-terminal region (supplemental Fig. 1) (27). Sequence alignment of Vpx proteins from SIVmac and HIV-2 indicates conservation of four N-terminal residues (Asn-12, Glu-15, Glu-16, and Thr-17 for VpxSIVmac and VpxRod9 and Asn-11, Glu-14, Glu-15, and Thr-16 for Vpx7312a) that were previously shown to be at the SAMDH1 recruitment interface (Fig. 1B) (20). Based on these observations, SIVmac and HIV-2 Vpx proteins probably
Inhibition of SAMHD1 Catalysis

To gain mechanistic insight into how Vpx facilitates SAMHD1 binding to DDB1-DCAF1, we developed an SPR-based assay to evaluate the rates of association and dissociation between SAMHD1 and DDB1-DCAF1 (residues 1045–1396) in complex with Vpx. Specifically, full-length human SAMHD1 was expressed as a GST fusion protein (GST-Hu SAMHD1-FL) and immobilized onto a CM5 chip coated with anti-GST antibody. Then increasing concentrations of DDB1-DCAF1-Vpx(C)SIVmac were applied, and sensorgrams were recorded. Surprisingly, DDB1-DCAF1-Vpx(C)SIVmac dissociation from human SAMHD1 was negligible for 180 s after the injection was complete (Fig. 2A). Similar observations were made with immobilized rhesus (Fig. 2B) and De Brazza’s SAMHD1 (data not shown). In fact, the rate of association ($k_{on}$) and the dissociation constant ($K_d$) were essentially the same for all three SAMHD1 proteins, ranging from $18 \times 10^3$ M$^{-1}$ s$^{-1}$ and from 213 to 521 nM, respectively (Table 1 and Fig. 2C). Similar SPR experiments showed that SAMHD1 did not bind to the DDB1-DCAF1 complex in the absence of viral protein (supplemental Fig. 2A), confirming previous observations that Vpx mediates recruitment of SAMHD1 to the DDB1-DCAF1 substrate adaptor-receptor complex (20). Because human, rhesus, and De Brazza’s SAMHD1 showed binding kinetics similar to those of DDB1-DCAF1-Vpx complexes and have strict sequence conservation at the C terminus (supplemental Fig. 1), we speculated that the C terminus of SAMHD1 is sufficient for efficient interaction with DDB1-DCAF1-Vpx. To test this possibility, the C terminus (residues 595–626, SAMHD1-CTD) of human SAMHD1 was immobilized onto a CM5 chip, as a GST fusion protein, and sensorgrams were recorded for increasing concentrations of DDB1-DCAF1-Vpx(C)SIVmac (Fig. 2D). The binding kinetics for the SAMHD1 C-terminal fragment...
were essentially the same as those of the full-length protein, with a similar binding affinity (Table 1). Similar results were obtained when DDB1-DCAF1 in complex with Vpx7312a was subjected to binding to the C terminus of human SAMHD1 (supplemental Fig. 2, B and C, and Table 1).

VpxSIVmac cannot enhance SIV and HIV-1 infection of macrophages and differentiated THP-1 cells when specific N-terminal residues (Asn-12, Glu-15, Glu-16, or Thr-17) are mutated (29). Because these mutants retain their ability to interact with DCAF1, these residues were hypothesized to be at the binding interface for a cellular restriction factor (29).

Indeed, we showed that these Vpx mutants fail to down-regulate SAMHD1 in a proteasome-dependent manner (20). For these reasons, we monitored the binding kinetics for immobilized human SAMHD1-FL, DCAF1CA (residues 1040–1400), and VpxSIVmac, VpxRod9, or Vpx7312a WT or mutant (E15A/E16A for SIVmac and HIV-2 Rod9; E14A/E15A for HIV-2 7312a), as indicated. Protein levels were determined as described in B, E, human SAMHD1 proteins were co-expressed with DCAF1CA and Vpx7312a WT or two mutants (D13G or E14A/E15A) and analyzed as in B.

Instrumental for determining the binding strength was a biolayer interferometry (BLI) assay using Surface plasmon resonance (SPR). The assay was performed with DDB1-DCAF1CB, without Vpx, and all other CRL4 components with or without WT SAMHD1. Human SAMHD1-CTD DDB1-DCAF1-VpxSIVmac, Human SAMHD1-CTD DDB1-DCAF1-VpxRod9, and Human SAMHD1-CTD DDB1-DCAF1-Vpx7312a WT or two mutants (D13G or E14A/E15A) and analyzed as in B.

**TABLE 1**

**Kinetic binding constants of SAMHD and DDB1-DCAF1-Vpx interaction**

| Ligand                  | Analyte                     | \(k_{on}\) \(\times 10^7\) M\(^{-1}\) s\(^{-1}\) | \(K_d\) \(\times 10^{-7}\) M |
|-------------------------|-----------------------------|---------------------------------------------|--------------------------------|
| Human SAMHD1-FL         | DDB1-DCAF1-VpxSIVmac        | 33.6, 22.6                                 | 213, 376                       |
| Rhesus SAMHD1-FL        | DDB1-DCAF1-VpxSIVmac        | 27.0, 18.9                                 | 263, 476                       |
| De Brazza’s SAMHD1-FL   | DDB1-DCAF1-VpxSIVmac        | 30.1, 17.9                                 | 263, 521                       |
| Human SAMHD1-CTD        | DDB1-DCAF1-VpxSIVmac        | 27.2, 19.4                                 | 358, 363                       |
| Human SAMHD1-CTD        | DDB1-DCAF1-Vpx7312a         | 41.0, 31.4                                 | 242, 307                       |

**FIGURE 3. Both the C terminus of SAMHD1 and the N-terminal region of Vpx are essential for proteasome-dependent degradation of SAMHD1.** A, in vitro ubiquitination assays of WT or C-terminally deleted (-ΔC) Human, De Brazza’s, and rhesus SAMHD1 with CRL4-DCAF1CB-Vpx7312a. Control reactions were performed with DDB1-DCAF1CB without Vpx, and all other CRL4 components with or without WT SAMHD1. B, HEK293 cells were transiently co-transfected with full-length DCAF1 (DCAF1-FL), SIVmac Vpx, and various human SAMHD1 constructs (SAMHD1-FL, WT; SAMHD1-ΔC, residues 1–595; ΔN-SAMHD1, residues 113–626) as indicated. The levels of ectopically expressed proteins were determined by immunoblotting with appropriate antibodies after separating cell lysates by SDS-PAGE, 48 h after transfection. Each transfection condition was carried out twice. C, HEK293 cells were transiently co-transfected with DCAF1CA-human (Hu) SAMHD1, and SIVmac Vpx and analyzed as in B after cells were treated with MG132 (+) or mock-treated (−). D, HEK293 cells were transiently co-transfected with human SAMHD1-FL, DCAF1CA residues 1040–1400, and VpxSIVmac, VpxRod9, or Vpx7312a WT or mutant (E15A/E16A for SIVmac and HIV-2 Rod9; E14A/E15A for HIV-2 7312a), as indicated. Protein levels were determined as described in B, E, human SAMHD1 proteins were co-expressed with DCAF1CA and Vpx7312a WT or two mutants (D13G or E14A/E15A) and analyzed as in B.
Vpx not only specifically increases the rate of association between SAMHD1 and DDB1-DCAF1-Vpx but also substantially decreases their dissociation rate, which may account for subsequent polyubiquitination of SAMHD1 by the CRL4 ubiquitin ligase.

**The C Terminus of SAMHD1 and the N Terminus of Vpx Are Required for Vpx-dependent SAMHD1 Ubiquitination and Down-regulation**—To examine the functional importance of the SAMHD1 C terminus as it relates to Vpx-mediated recruitment to the CRL4-DCAF1 ligase, in vitro ubiquitination assays were performed. In the absence of Vpx, wild-type SAMHD1 proteins were not ubiquitinated by CRL4-DCAF1 E3 ubiquitin ligase (Fig. 3A, left). However, when Vpx<sup>7312a</sup> was complexed with the E3 ubiquitin ligase, both human and monkey SAMHD1 proteins were robustly polyubiquitinated (Fig. 3A, right). Further, the C-terminal region of SAMHD1 was required for polyubiquitination, because SAMHD1 proteins lacking this region did not show evidence of polyubiquitination. Similar results were obtained when in vitro ubiquitination assays were performed with Vpx<sup>SIVmac</sup> and Vpx<sup>Rod9</sup> (data not shown).

These findings were supported by in vitro cellular assays; wild-type human SAMHD1 or deletion constructs were expressed along with DCAF1 and Vpx in HEK293 cells by cotransfection, and Vpx-dependent depletion of SAMHD1 was assessed (Fig. 3B). Consistent with the previous report (20), the SAMHD1 lacking C-terminal residues 596–626 was resistant to down-regulation by Vpx<sup>SIVmac</sup> (Fig. 3B) and Vpx<sup>Rod</sup> and Vpx<sup>7312a</sup> (data not shown), whereas N-terminal deletion had no effect on Vpx-dependent SAMHD1 down-regulation. Similar results were obtained with rhesus or De Brazza’s SAMHD1 in combination with various Vpx proteins (data not shown). Notably, the C-terminal region of DCAF1 (DCAF1<sub>Cterminal</sub> residues 1040–1400) was sufficient to mediate Vpx-dependent modulation of SAMHD1 level by cotransfection (Fig. 3C). This decrease in SAMHD1 level by Vpx was alleviated by treatment with MG132, indicating proteasome-dependent down-regulation of SAMHD1 (1–3, 20).

In an analogous manner, we explored whether the N-terminal region of Vpx resides at the critical recruitment interface for both human and monkey SAMHD1, as suggested by our in vitro kinetic studies. Mutation of two consecutive acidic Glu residues (15 and 16) to Ala in Vpx<sup>SIVmac</sup> resulted in significant abrogation of SAMHD1 recruitment (15 and 16) to Ala in Vpx<sup>SIVmac</sup> (Fig. 3, B and C). Mutation of two consecutive acidic Glu residues in both human and monkey SAMHD1, as suggested by our in vitro kinetic studies, partially decreases their dissociation rate, which may account for subsequent polyubiquitination of SAMHD1 by the CRL4 ubiquitin ligase.

**FIGURE 4. SAMHD1 forms catalytically active dGTP-dependent tetramers.** A, quaternary states of human and six monkey SAMHD1 proteins were characterized by chemical cross-linking assays. B, dGTP-dependent tetramer formation of rhesus and De Brazza’s SAMHD1 proteins was assessed by analytical size exclusion column chromatography. The peak elution volumes are indicated. C, the relative dNTPase activities of human and monkey SAMHD1. Error bars, S.D.

**Inhibition of SAMHD1 Catalysis**

**SAMHD1 Forms dGTP-dependent Tetramers**—Previous reports established that the dNTPase activity of human SAMHD1 is positively regulated by dGTP, the binding of which, at allosteric sites, stabilizes dimerization and enhances its catalytic activity (8). However, our biochemical and biological analyses of full-length human SAMHD1 suggested that this protein is inactive as a monomer or dimer and that binding of dGTP induces formation of a tetramer, which is the catalytically active form of the protein (19). To confirm that monkey SAMHD1 behaves in a manner similar to the human protein (i.e. transits from an inactive monomer/dimer to an active tetramer in a dGTP-dependent manner) we carried out several biochemical assays. Chemical cross-linking analyses indicated that the six examined monkey SAMHD1 proteins do, indeed, form tetramers in a dGTP-dependent manner (Fig. 4A). Further, analytical size exclusion column chromatography on mix-
Inhibition of SAMHD1 Catalysis

![Graph A](image1)

![Graph B](image2)

![Graph C](image3)

![Graph D](image4)

**FIGURE 5.** The recruitment of SAMHD1 to DDB1-DCAF1-Vpx via its C terminus inhibits the dNTPase catalytic activity. A, the dNTPase activity of human SAMHD1 was determined in the presence of increasing concentrations of DDB1-DCAF1<sub>cb</sub>-Vpx<sub>SIVmac</sub> (ΔC<sub>SIVmac</sub>), as indicated. B, the relative dNTPase activity of human SAMHD1 was measured with increasing concentrations of DDB1-DCAF1<sub>cb</sub>-Vpx<sub>SIVmac</sub> (ΔC<sub>SIVmac</sub>) at molar ratios ranging from 0.25 to 2.0. C, the catalytic activity of SAMHD1 was determined with increasing concentrations of NusA-Vpx<sub>SIVmac</sub> WT and mutant (N12A/E15A/E16A/T17A) at molar ratios of 0.25–2-fold. D, the relative dNTPase activity of SAMHD1-ΔC or SAMHD1-FL with a triple residue mutation (L620A/F621A/K622A) was determined with increasing concentrations of DDB1-DCAF1<sub>cb</sub>-Vpx<sub>SIVmac</sub> (ΔC<sub>SIVmac</sub>), as indicated. Error bars, S.D.

In vitro dNTPase activity toward dATP in each reaction was calculated and normalized over the catalytic activity of SAMHD1 alone.

The recruitment of SAMHD1 to DDB1-DCAF1-Vpx via its C terminus inhibits the dNTPase catalytic activity.
Recruitment of SAMHD1 to DDB1-DCAF1 via Vpx Induces Disassembly of dGTP-dependent SAMHD1 Tetramers into Dimer and Monomers—To understand the molecular mechanisms by which DDB1-DCAF1-Vpx inhibits SAMHD1 catalysis, light scattering experiments were performed. First, SAMHD1 and DDB1-DCAF1CB-Vpx(C)SIVmac were individually subjected to analytical size exclusion column chromatography, and the molecular mass of proteins in each eluting peak was determined by multicycle light scattering. SAMHD1 alone, without dGTP, eluted between 12.5 and 14.5 ml, and the molecular mass of the protein was determined to be 125 kDa, representing a mixture of dimer and monomer populations (Fig. 6A, green trace; the theoretical molecular mass of SAMHD1 is 75 kDa). The molecular mass of DDB1-DCAF1CB-Vpx(C)SIVmac alone was estimated to be 189 kDa (data not shown). A mixture of DDB1-DCAF1CB-Vpx(C)SIVmac and SAMHD1 at a molar ratio of 1.5:1, in the absence of dGTP, yielded two major peaks, at 10.8 and 12 ml, with the molar mass of protein in each peak estimated to be 256 and 185 kDa, respectively (Fig. 6A, red trace). The mass of the first peak corresponds to a molecular complex of SAMHD1 and DDB1-DCAF1CB-Vpx(C)SIVmac, whereas the second peak may correspond to unbound DDB1-DCAF1CB-Vpx(C)SIVmac, present in slight excess of SAMHD1 in the initial mixture. Preincubation of SAMHD1 with dGTP resulted in a new peak, at ~11.0 ml, which corresponds to SAMHD1 tetramer (306 kDa) (green trace in Fig. 6B), which is consistent with our previous results (19). The elution volume of DDB1-DCAF1CB-Vpx(C)SIVmac, when preincubated with dGTP, was essentially the same (data not shown). The addition of DDB1-DCAF1CB-Vpx(C)SIVmac to the SAMHD1-dGTP mixture (at an ~1.25:1 stoichiometric ratio; Fig. 6B, red trace) produced a new peak at ~8.8 ml. The average molecular mass of proteins in this peak was estimated to be ~830–940 kDa, close to the mass of a supramolecular protein complex comprising four SAMHD1 (75 kDa each) and four DDB1-DCAF1CB-Vpx(C)SIVmac (181 kDa each). Interestingly, upon increasing the concentration of DDB1-DCAF1CB-Vpx(C)SIVmac (to an ~2.5:1 stoichiometric ratio; Fig. 6B, blue trace), the UV 280 nm absorbance height of the 8.8 ml peak was reduced, and a second peak at ~9.6 ml emerged. The average molecular mass of protein in the second peak was estimated to be 470–570 kDa, which is close to the mass of a protein complex comprising two SAMHD1 and two DDB1-DCAF1CB-Vpx(C)SIVmac proteins. A peak eluting at ~10.8 ml that had a molar mass of 260–310 kDa and probably represents one SAMHD1 bound to DDB1-DCAF1CB-Vpx(C)SIVmac showed a concomitant increase in its UV 280 nm absorbance height, upon increasing the amount of DDB1-DCAF1CB-Vpx(C)SIVmac in the mixture. The three protein peaks, eluting at three distinctive elution volumes (8.8, 9.6, and 10.8 ml), were composed of nearly stoichiometric amounts of SAMHD1, DDB1, DCAF1CB, and Vpx(C)SIVmac, as judged by SDS-PAGE analysis (Fig. 6B, bottom, compare lanes 3, 7, and 11). Taken together, these data suggest that DDB1-DCAF1CB-Vpx(C)SIVmac binding to dGTP-induced SAMHD1 tetramer results in disassembly of the supramolecular complex into dimeric and monomeric SAMHD1-DDB1-DCAF1CB-Vpx(C)SIVmac protein complexes. At the same time, the recruitment of active forms of SAMHD1 to the virus-host protein complex also results in inhibition of its catalysis (Fig. 5). Because we did not observe further reduction of SAMHD1 dNTPase activity upon increasing the molar ratio, from 1 to 2, of DDB1-DCAF1CB-Vpx(C)SIVmac over SAMHD1 (Fig. 5A), inhibition of SAMHD1 catalysis may precede the disassembly of dGTP-SAMHD1 tetramer.
DISCUSSION

The discovery of SAMHD1 as an antiviral restriction factor shed light on the molecular mechanisms underlying the inability of HIV-1 to infect monocytes and dendritic cells (30–34). SAMHD1 exerts its antiviral function by maintaining cellular dNTP pools at a level insufficient for HIV-1 reverse transcription (6, 10, 11). HIV-2, on the other hand, encodes a virulence accessory factor, Vpx, which overcomes the SAMHD1-mediated antiviral response (1–3). Vpx has been demonstrated to reprogram the CRL4 E3 ubiquitin ligase by binding DCAF1, a substrate receptor of the ligase (35–37), and recruiting SAMHD1 for proteasome-dependent degradation (2, 3, 20). However, the precise molecular mechanism of SAMHD1 activation and the interplay between Vpx and host cellular factors, with respect to SAMHD1 down-regulation, are yet to be defined. In this report, we provide extensive biochemical analyses showing 1) a common mode of human and monkey SAMHD1 recruitment by SIVmac and HIV-2 Vpx, in complex with the substrate adaptor-receptor component of CRL4 E3 ubiquitin ligase, 2) real-time binding kinetics of SAMHD1 recruitment in vitro, 3) dGTP-induced tetramerization and allosteric activation of human and monkey SAMHD1 proteins, and 4) regulation of SAMHD1 catalysis by Vpx during the course of recruitment to the substrate adaptor-receptor complex.

HIV-2 Vpx and SIVmac Vpx show strong sequence conservation, implying a common binding mode to DDB1-DCAF1.

FIGURE 7. Model of SAMHD1 activation by dGTP and recruitment to CRL4-DCAF1 reprogrammed by Vpx. A, dGTP-induced SAMHD1 tetramerization and activation. Binding of dGTP (black in the tetramer) to each allosteric site of four SAMHD1 monomers induces tetramerization via a transient head-to-tail dimer. Residues near the allosteric sites are shown in different colors for each monomer to help with orientation: Asp-137 (head) and Arg-451 (tail). The catalytic site is indicated with His-206 (H206) and Asp-207 (D207). The structural model of the HD domain was generated based on the crystal structures of EF1143 (Protein Data Bank code 3IRH) (44) and the HD domain of SAMHD1 (3UN1) (8) and was rendered faintly to emphasize residues and dGTP molecules. B, SAMHD1 monomer, dimer, and tetramer are recruited to CRL4-DCAF1 by Vpx. Vpx, while binding to DCAF1 via its middle region (16), interacts with the C terminus of SAMHD1 via its N terminus. DDB1-DCAF1-Vpx binding to the SAMHD1 tetramer deactivates its dNTPase activity and subsequently disassembles it to dimers and monomers and induces proteasome-dependent degradation.
Inhibition of SAMHD1 Catalysis

and SAMHD1. Especially, the N terminus of these Vpx proteins contain Asn-12, Glu-15, Glu-16, and Thr-17 residues (Asn-11, Glu-14, Glu-15, and Thr-16 for HIV-2 7312a), which are critical for binding to human and monkey SAMHD1 proteins by their conserved C terminus. Each of these residues at the N terminus appears to contribute equally to SAMHD1 binding (Fig. 2F), and mutation of a single residue in the region is sufficient to abrogate Vpx-dependent SAMHD1 down-regulation (1, 20, 38). Interestingly, HIV-2 Vpx<sup>3124</sup> shows a sequence divergence in this region, at position 13 (Fig. 1B); however, this residue is not important for SAMHD1 down-regulation (Fig. 3E). Notably, the N-terminal region of Vpx is not involved in the direct binary protein interaction with DCAF1 (29). However, recruitment of SAMHD1 requires both Vpx and DCAF1 in complex with DDB1 (20). These results suggest that the N-terminal region of these Vpx proteins creates a new interface in complex with DCAF1. This mode of interaction is distinct from the Vpr-mediated UNG2 recruitment to CRL4-DCAF1. Vpr directly interacts with UNG2 and forms a stable binary complex (28, 39). Interestingly, other SIV Vpr and Vpx proteins interact with the SAM domain of their cognate SAMHD1 proteins (26), and the interfaces mediating these interactions are yet to be identified. All of these Vpr and Vpx proteins contain a common sequence motif that interacts with DCAF1 (16). Taken together, these observations suggest that HIV Vpr and Vpx, although bound to DCAF1 using a common sequence motif, create unique interfaces at separate regions for two distinct host cellular factors (UNG2 and SAMHD1).

We confirmed and extended our previous observation that the C terminus of SAMHD1 is required for efficient recruitment to the CRL4-DCAF1 E3 ubiquitin ligase in complex with Vpx, interacting with DCAF1 for transfer of multiple ubiquitins to SAMHD1 (Figs. 2 and 3). Kinetic studies of substrate polyubiquitination, with SCF E3 ligase, indicate that a relatively slow monoubiquitination (at a rate of 0.03 s<sup>-1</sup>) is followed by fast polyubiquitination (at a rate of ∼3–5 s<sup>-1</sup>) (40). Our SPR data indicate that the rate of SAMHD1 dissociation from DDB1-DCAF1-Vpx is extremely slow (Fig. 2). This would allow sufficient time for initial monoubiquitination and subsequent multiple rounds of ubiquitin transfer from E2 to SAMHD1 bound to the Vpx-modified substrate receptor of the E3 ubiquitin ligase.

Our current findings along with our previous report (19) suggest that human and monkey SAMHD1 undergo dGTP-induced tetramerization and activation of catalysis. We propose that primate SAMHD1, in the absence of dGTP, interconverts between monomer and dimer, which are catalytically inactive forms. Binding of dGTP at the allosteric site induces formation of tetramer, which is a catalytically active dNTPase (Fig. 7A).

Our biochemical characterization of SAMHD1 catalysis upon binding to the DDB1-DCAF1-Vpx complex suggests that Vpx counteracts SAMHD1 restriction at an enzymatic level in addition to proteasome-dependent degradation (1–3, 20). Vpx, bound to DDB1-DCAF1, inhibits its dNTPase activity and induces disassembly of the dGTP-induced tetramer by interacting with the C terminus, distal to the oligomeric HD domain (Fig. 7B). Light-scattering analyses indicate that DDB1-DCAF1-Vpx binding to the dGTP-induced tetramer results in several distinctive quaternary states of protein complexes (Fig. 6B). Although a small region of Vpx is required for SAMHD1 binding, a large molecular interface would probably be created by both DCAF1 and Vpx, and this extended interface may negatively influence the conformation of the catalytically active form. This notion is supported by our finding that SAMHD1-Vpx interaction is not sufficiently strong to inhibit dNTPase catalysis (Fig. 5C). Structural studies of SAMHD1 in complex with Vpx as well as components of the CRL4-DCAF1 E3 ubiquitin ligase would shed light on these important interfaces.

The direct inhibition of SAMHD1 catalysis by Vpx bound to DDB1-DCAF1 is analogous to the direct inhibition of APOBEC3G catalysis by Vif. In particular, the cytidine deaminase activity of APOBEC3G was inhibited by Vif binding, in the absence of degradation, when investigated in bacteria and in a cell-free system (41–43). Given that our observations were made using a cell-free system, our model of Vpx-mediated inhibition of SAMHD1 catalysis needs to be validated in cells.

Acknowledgments—We thank Dr. Teresa Brosenitsch for careful reading of the manuscript and editorial help. We also thank Drs. Jacek Skowronski and Michael Emerman for helpful discussion and for sharing reagents.

REFERENCES

1. Berger, A., Sommer, A. F., Zwarg, J., Hamdorf, M., Welzel, K., Esly, N., Panitz, S., Reuter, A., Ramos, I., Jatiani, A., Mulder, L. C., Fernandez-Sesma, A., Rutsch, F., Simon, V., König, R., and Flory, E. (2011) SAMHD1-deficient CD4<sup>+</sup> cells from individuals with Aicardi-Goutieres syndrome are highly susceptible to HIV-1 infection. *PloS Pathog.* 7, e1002425

2. Hrebec, 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

3. Lagutte, N., Sohbián, B., Casartelli, N., Ringeard, M., Chable-Bessia, C., Ségéral, 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

4. Descours, B., Cribier, A., Chable-Bessia, C., Ayinde, D., Rice, G., Crow, Y., Yatim, A., Schwartz, O., Lagutte, N., and Benkirane, M. (2012) SAMHD1 restricts HIV-1 reverse transcription in quiescent CD4<sup>+</sup> T-cells. *Retrovirology* 9, 87

5. Baldauf, H. M., Pan, X., Erikson, E., Schmidt, S., Daddacha, W., Burgraf, M., Schenkova, K., Ambiel, I., Wabnitz, G., Gramberg, T., Panitz, S., Flory, E., Landau, N. R., Sertel, S., Rutsch, F., Lasitschka, F., Kim, B., König, R., Fackler, O. T., and Keppler, O. T. (2012) SAMHD1 restricts HIV-1 infection in resting CD4<sup>+</sup> T cells. *Nat. Med.* 18, 1682–1687

6. St Gelais, C., de Silva, S., Amie, S. M., Coleman, C. M., Hoy, H., Hollenbaugh, J. A., Kim, B., and Wu, L. (2012) SAMHD1 restricts HIV-1 infection in dendritic cells (DCs) by dNTP depletion, but its expression in DCs and primary CD4<sup>+</sup> T-lymphocytes cannot be upregulated by interferons. *Retrovirology* 9, 105

7. Puigdomènech, I., Casartelli, N., Porrot, F., and Schwartz, O. (2013) SAMHD1 restricts HIV-1 cell-to-cell transmission and limits immune detection in monocyte-derived dendritic cells. *J. Virol.* 87, 2846–2856

8. 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

9. 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.*
Inhibition of SAMHD1 Catalysis

286, 43596 – 43600

10. Kim, B., Nguyen, L. A., Daddacha, W., and Hollenbaugh, J. A. (2012) Tight interplay among SAMHD1 protein level, cellular dNTP levels, and HIV-1 proviral DNA synthesis kinetics in human primary monocyte-derived macrophages. J. Biol. Chem. 287, 21570–21574

11. 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-Gougé, F. (2012) SAMHD1 restricts the replication of human immunodeficiency virus type 1 by depleting the intracellular pool of deoxyribonucleoside triphosphates. Nat. Immunol. 13, 223–228

12. Wei, W., Guo, H., Han, X., Liu, X., Zhou, X., Zhang, W., and Yu, X. F. (2012) Role of SAMHD1 nuclear localization in restriction of HIV-1 and SIVmac. Retrovirology 9, 49

13. Hofmann, H., Logue, E. C., Bloch, N., Daddacha, W., Polsky, S. B., Schultz, M. L., Kim, B., and Landau, N. R. (2012) The Vpx lentiviral accessory protein targets SAMHD1 for degradation in the nucleus. J. Virol. 86, 12552–12560

14. Wei, W., Guo, H., Han, X., Liu, X., Zhou, X., Zhang, W., and Yu, X. F. (2012) A novel DCAF1-binding motif required for Vpx-mediated degradation of nuclear SAMHD1 and Vpr-induced G0 arrest. Cell. Microbiol. 14, 1745–1756

15. Beloglozova, N., Flick, R., Tchigvintsev, A., Brown, G., Popovic, A., Noceti, B., and Yakunin, A. F. (2013) Nuclease activity of the human SAMHD1 protein implicated in the Aicardi-Goutieres syndrome and HIV-1 restriction. J. Biol. Chem. 288, 8101–8110

16. Goncalves, A., Karayel, E., Rice, G. J., Bennett, K. L., Crow, Y. J., Superti-Furga, G., and Bückerstümmer, T. (2012) SAMHD1 is a nucleic-acid binding protein that is mislocalized due to aicardi-goutieres syndrome-associated mutations. Hum. Mutat. 33, 1116–1122

17. Yu, J., Kaur S, Delucia M, Rao C, Mehrens J, Wang G, Golczak M, Paclezewski K, Gronenborn AM, Ahn J, Skowronski J. (2013) Tetramerization of SAMHD1 is required for biological activity and inhibition of HIV infection. J. Biol. Chem. 288, 10406–10417

18. 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

19. 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 macrophage. J. Virol. 65, 5088–5091

20. 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

21. 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 SIV(SM) PBj. Evidence of macrophage-dependent viral amplification. J. Virol. 72, 3358–3368

22. Lima, 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

23. Laugette, N., Rahm, N., Sobhian, B., Chable-Bessa, C., Münch, J., Snoeck, J., Sauter, D., Switzer, W. M., Heneine, W., Kirchhoff, F., Deluc, F., Teltenti, A., and Benkirane, M. (2012) Evolutionary and functional analyses of the interaction between the myeloid restriction factor SAMHD1 and the lentiviral Vpx protein. Cell Host Microbe 11, 205–217

24. Ahn, J., Vu, T., Novince, Z., Guerrero-Santoro, J., Rapic-Otrin, V., and Gronenborn, A. M. (2010) HIV-1 Vpr loads uracil DNA glycosylase-2 onto DCAF1, a substrate recognition subunit of a cullin 4A-ring E3 ubiquitin ligase for proteasome-dependent degradation. J. Biol. Chem. 285, 37333–37341

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

26. Yan, N., and Lieberman, J. (2012) SAMHD1 does it again, now in resting T cells. Nat. Med. 18, 1611–1612

27. Arons, N., Wu, L. (2012) SAMHD1: a new contributor to HIV-1 restriction in resting CD4+ T-cells. Retrovirology 9, 88

28. Schaller, T., Goujon, C., and Malim, M. H. (2012) AIDS/HIV. HIV interaction with SAMHD1. Science 335, 1313–1314

29. Pananlaves, V. (2012) SAMHD1 joins the red queen’s court. Cell Host Microbe 11, 103–105

30. Lanub, J. (2012) Innate immune sensing of HIV-1 by dendritic cells. Cell Host Microbe 12, 408–418

31. Sharova, N., Wu, Y., Zhu, X., Stranska, R., Kaushik, R., Sharkey, M., and Stevenson, M. (2008) Primate lentiviral Vpx commandeer DDB1 to counteract a macrophage restriction. PLoS Pathog. 4, e1000057

32. Srivastava, S., Swanson, S. K., Manel, F., Florens, L., Washburn, M. P., and Skowronski, J. (2008) Lentiviral Vpx accessory factor targets VprBP/ DCAF1 substrate adaptor for cullin 4D ubiquitin ligase to enable macrophage infection. PLoS Pathog 4, e1000059

33. Bergamaschi, A., Ayinde, D., David, A., Le Rouzic, E., Morel, M., Collin, G., Descamps, D., Damond, F., Brun-Verinet, F., Nisole, S., Martignetti, 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

34. Yu, H., Usmani, S. M., Borch, A., Krämer, J., Stürzel, C. M., Khalid, M., Li, X., Krenave, D., van der Ende, M. E., Osterhaus, A. D., Gruters, R. A., and Kirchhoff, F. (2013) The efficiency of Vpx-mediated SAMHD1 antagonism does not correlate with the potency of viral control in HIV-2-infected individuals. Retrovirology 10, 27

35. Chen, R., Le Rouzic, E., Kearney, J. A., Monsky, L. M., and Benichou, S. (2004) Vpr-mediated incorporation of UNG2 into HIV-1 particles is required to modulate the virus mutation rate and for replication in macrophages. J. Biol. Chem. 279, 28419–28425

36. Pierce, N. W., Kleger, G., Shan, S. O., and Deshaies, R. J. (2009) Detection of sequential polyubiquitylation on a millisecond timescale. Nature 462, 615–619

37. Santa-Marta, M., da Silva, F. A., Fonseca, A. M., and Goncalves, J. (2005) HIV-1 Vif can directly inhibit apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3G-mediated cytidine deamination by using a single amino acid interaction and without protein degradation. J. Biol. Chem. 280, 8765–8775

38. Santa-Marta, M., Aires da Silva, F., Fonseca, A. M., Rato, S., and Goncalves, J. (2007) HIV-1 Vif protein blocks the cytidine deaminase activity of B-cell specific AID in E. coli by a similar mechanism of action. Mol. Immunol. 44, 583–590

39. Birin-Rosich, E., Nowarski, R., and Kotler, M. (2011) Multicapped counter-APOBEC3G mechanisms employed by HIV-1 Vif. J. Mol. Biol. 410, 1065–1076

40. Vorontsov, I. I., Minasov, G., Kiryukhina, O., Brunzelle, J. S., Shuvalova, L., and Anderson, W. F. (2011) Characterization of the deoxyribonucleotide triphosphate phosphohydrolase (dNTPase) activity of the E1143 protein from Enterococcus faecalis and crystal structure of the activator-substrate complex. J. Biol. Chem. 286, 33158–33166