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To cite this version:
Nicolas Roy, Grégory Pacini, Clarisse Berlioz-Torrent, Katy Janvier. Mechanisms underlying HIV-1 Vpu-mediated viral egress. Frontiers in microbiology, Frontiers Research Foundation, 2014, 5, pp.177. 10.3389/fmicb.2014.00177. inserm-01068976

HAL Id: inserm-01068976
https://www.hal.inserm.fr/inserm-01068976
Submitted on 26 Sep 2014

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Mechanisms underlying HIV-1 Vpu-mediated viral egress

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INTRODUCTION
Viral egress and replication rely on a complex interplay between viral and cellular proteins. During their replication cycle, viruses, notably lentiviruses, have to face several levels of the host immune defense mechanisms and must counteract these barriers to persist and disseminate in the host. Understanding the mechanisms underlying lentiviruses evasion from host antiviral activities has been the focus of many studies of the past decades. Not only did they contribute to major advances in the characterization of host defense mechanisms and must counteract these barriers to interfere with the host machinery regulating protein turnover and vesicular trafficking. This review will focus on our current understanding of the mechanisms whereby Vpu down-regulates CD4 and BST2 expression levels to favor viral egress.

Keywords: Vpu, CD4, BST2/Tetherin, HIV-1, ESCRT, degradation, cell surface down-regulation, NF-κB

CHARACTERISTICS AND FUNCTIONS OF THE ACCESSORY PROTEIN VPU

The accessory protein Vpu is an 81-amino acid type I integral membrane phosphoprotein, expressed by the genome of HIV-1, the related SIVgsn (Strebel et al., 1988) and the SIVmac lineages including the greater spot-nosed monkey (SIVgsn), the mona monkey (SIVmon), the mustached monkey (SIVmus) and Dent’s mona monkey (SIVden) isolates (Gao et al., 1999; Courgnaud et al., 2002, 2003; Bailey et al., 2003). Vpu contains a short luminal N-terminal domain, a 23-amino acid transmembrane domain and a large cytoplasmic C-terminal domain (Strebel et al., 1988; Maldarelli et al., 1993). Vpu localizes mainly in the endoplasmic reticulum (ER), the trans-Golgi network (TGN) and endosomal compartments. Two major functions have been attributed to Vpu during HIV-1 replication cycle. Firstly, Vpu targets the newly synthesized CD4 receptor for proteasomal degradation (Willey et al., 1992a). Secondly, it favors the release of viral particles from most human cell types through counteracting the inhibitory effect of BST2 (Neil et al., 2008; Van Damme et al., 2008). More recently, Vpu was shown to down-regulate cell surface expression of two additional mediators of the immune response: the lipid-antigen presenting protein CD1d expressed by antigen-presenting cells (Moll et al., 2010) and the natural killer cells ligand NTB-A (Shah et al., 2010). Vpu appears therefore as a key factor for HIV evasion from the host immune system.

VPU-INDUCED DOWN-REGULATION OF CD4

CD4 constitutes the major component of the receptor complex used by primate lentiviruses to infect the cells. It is a 54 kDa
type I integral glycoprotein expressed at the surface of helper T-lymphocytes, cells of the monocyte/macrophage lineage and hematopoietic progenitor cells.

Infection of CD4+ cells by primate lentiviruses results in a rapid and constant down-modulation of cell surface CD4 expression level (Ray and Doms, 2006). CD4 down-regulation was proposed to prevent lethal superinfection of cells by additional virions (Wildum et al., 2006), contribute to the escape of infected cells from the immune system and favor viral fitness (Willey et al., 1992b). CD4 depletion in infected cells is achieved by the concerted, though mechanically distinct, action of three viral proteins: Nef, Vpu and, to a lower extent, Env (Wildum et al., 2006). Nef, produced shortly after infection, enhances internalization of pre-existing CD4 from the cell surface and targets the receptor for lysosomal degradation (Chaudhuri et al., 2007). Env precursor gp160 binds CD4 in the ER and blocks its transport to the cell surface (Grise et al., 1990; Jabbar and Nayak, 1990).

Vpu targets CD4 molecules present in the ER for proteasomal degradation (Willey et al., 1992a).

Vpu induces degradation of newly synthesized CD4 by a multistep process involving binding of Vpu with CD4 via their transmembrane domains (TMD), retention and poly-ubiquitination of CD4 in the ER, followed by its delivery to the ER-associated degradation pathway (ERAD) for further proteasomal degradation (Magadan et al., 2010; Magadan and Bonifacino, 2012; Figure 1). Vpu-induced degradation of CD4 requires the integrity of two phosphoserines S52/S56 present in a canonical DSGXXS motif within the cytoplasmic tail of Vpu and involved in an interaction with the β-transducin repeat-containing protein 1 or 2 (β-TrCP1; β-TrCP2), two adaptors for the SKP1-cullin1-F-box (SCF) E3 ubiquitin ligase complex (Margottin et al., 1998). Recruitment of the SCFβ-TrCP complex by Vpu results in poly-ubiquitination of the cytoplasmic tail of CD4 on lysine, serine and threonine residues (Magadan et al., 2010). Interestingly, Vpu-induced SCF-mediated poly-ubiquitination of CD4 contributes to retain the receptor in the ER and enables the recruitment of the ERAD VCP-UFD1L-NPL4 dislocase complex, leading to the extraction of CD4 from the ER membrane and its subsequent degradation by the proteasome (Magadan et al., 2010; Figure 1).

**VPU-MEDIATED ANTAGONISM OF THE RESTRICTION FACTOR BST2**

A major breakthrough in understanding how Vpu promotes the release of HIV-1 particles was made by the identification of BST2 as a restriction factor for HIV-1 release (Neil et al., 2008; Van Damme et al., 2008).

**CHARACTERISTICS OF BST2**

BST2 is a 30–36 kDa highly glycosylated type II integral membrane protein, constitutively expressed in several cell types and can be up-regulated by type-I interferon and pro-inflammatory stimuli (Neil, 2013). BST2 is composed of a short N-terminal cytoplasmic tail, linked to a transmembrane domain and an extracellular domain anchored to the membrane through a C-terminal glycosyl-phosphatidylinositol (GPI) moiety (Kupzig et al., 2003). Recently, a short isoform of BST2 produced by an alternative translation initiation from the methionine residue at position 13 has been identified (Cocka and Bates, 2012). BST2 is localized at the plasma membrane (PM) in cholesterol–rich microdomains (rafts) and in intracellular compartments such as the TGN as well as early and recycling endosomes (Kupzig et al., 2003; Masuyama et al., 2009). BST2 was proposed to assemble as a “picket fence” around the lipid rafts, playing a role in organizing membrane microdomains (Billcliff et al., 2013). BST2 was shown to physically trap the de novo formed mature viral particles at the surface of infected cells, thereby considerably reducing virus release (Neil et al., 2008; Van Damme et al., 2008; Perez-Caballero et al., 2009; Hammond et al., 2010). This activity relies on BST2 ability to form parallel disulfide–bond homo-dimers and to bridge virions and cellular membranes via its N– and C–terminal membrane anchoring domains (Iwabu et al., 2009; Perez-Caballero et al., 2009; Schubert et al., 2010), with a preference for an “axial” configuration in which the GPI anchors are inserted into viros, and the N-termini transmembrane anchors remain in the infected cells membrane (Venkatesh and Bieniasz, 2013).

Although initially identified as the factor responsible for defective release of HIV-1 mutants lacking the accessory gene vpu (Neil et al., 2008; Van Damme et al., 2008), it is now well established that BST2 restricts the release of nearly all enveloped viruses (retroviruses, herpes viruses, filoviruses, rhabdoviruses, paramyxoviruses, and arenaviruses) (Neil, 2013). BST2 therefore appears as a major mediator of the innate immune defense against viral dissemination. Primates lentiviruses deploy three proteins to antagonize BST2 antiviral activity: Vpu in HIV-1 (Neil et al., 2008; Van Damme et al., 2008); Env in HIV-2 ROD10, HIV-2 RODA, SIVagm, Tan and SIVmac239Δ nef isolates (Gupta et al., 2009b; Le Tortorec and Neil, 2009; Hauser et al., 2010; Serra-Moreno et al., 2011) and Nef in most isolates of SIV (Jia et al., 2009; Sauter et al., 2009; Zhang et al., 2009). HIV-1 Vpu, HIV-2 Env, and SIV Nef were shown to down-regulate the cell surface expression level of BST2 to favor its removal from viral budding sites and further viral release (Neil, 2013). To date, the precise mechanism involved in this process has not been fully characterized.

**BINDING OF VPU WITH BST2**

Binding of Vpu with BST2 through their respective TMD was shown to be essential to counteract BST2 antiviral activity. Mutagenesis analyzes have unraveled the critical role of residues I34, I37, L41 of BST2 and A14, W22 and to some extent A18 of Vpu in this interaction (Vigan and Neil, 2010; Kobayashi et al., 2011). These residues were proposed to form an anti-parallel helix-helix interface (Skasko et al., 2012). Interestingly, recent studies have identified additional residues, located at the periphery of the TMD of BST2 and Vpu respectively, required for the interaction between both proteins and antagonism of BST2 (McNatt et al., 2013; Pickering et al., 2014).

**VPU-INDUCED CELL SURFACE DOWN-REGULATION OF BST2**

The mechanism whereby Vpu decreases cell surface BST2 expression appears to rely on interference with BST2 intracellular trafficking. BST2 was thought to cycle between the PM, the TGN and the endosomes, with a fraction sorted for lysosomal degradation through an Endosomal Sorting Complexes Required...
FIGURE 1 | Vpu mediates proteasomal degradation of CD4 to favor viral fitness. (A) Newly synthesized CD4 and HIV-1 envelope precursor gp160 interact in the ER through their luminal domain, preventing Env trafficking to the cell surface. (B) Vpu induces retention of CD4 in the ER through interaction via their transmembrane region and connects CD4 to SKP1-cullin1-F-Box (SCF) E3 ubiquitin ligase through binding with the SCF subunits β-TrCP1 and β-TrCP2. Interaction of Vpu with β-TrCP involves the conserved phosphorylated serines S$_{52}$ and S$_{56}$, located in the cytoplasmic tail of Vpu. Recruitment of the SCF$^\beta$-TrCP complex by Vpu induces poly-ubiquitination of CD4 on lysine, serine and threonine residues in its cytoplasmic tail. Poly-ubiquitination of CD4 partially contributes its retention in the ER, through a yet-to-be-determined mechanism. Vpu-induced SCF-mediated poly-ubiquitination of CD4 enables the recruitment of the ERAD VCP-UFD1L-NPL4 dislocase complex, leading to the extraction of CD4 from the ER membrane and its subsequent degradation by the proteasome. Degradation of CD4 by Vpu was proposed to dissociate CD4 from newly synthesized viral Env present in the ER, allowing Env maturation and trafficking to the cell surface for its subsequent incorporation in the forming virions.
FIGURE 2 | Vpu subverts the host cell trafficking and ubiquitin machineries to counteract BST2 antiviral activity. BST2 physically tethers HIV-1 particles on the surface of infected cells preventing their release and further dissemination. The accessory protein Vpu counteracts this restriction by hijacking the host machineries involved in BST2 trafficking and turnover. BST2 cycles between the plasma membrane, the TGN and the endosomes, with a fraction sorted for lysosomal degradation through an ESCRT-mediated pathway (left panel). Vpu counteracts BST2 restriction activity through (i) displacing it from viral assembly sites by a yet-to-be-determined mechanism involving a “dileucine”-like motif located in its cytoplasmic tail and (ii) down-regulating BST2 cell surface expression level and as such restores viral release. Vpu down-regulates cell surface BST2 by slowing down the PM access of internalized and newly synthesized BST2. Vpu-induced BST2 cell surface down regulation is associated with targeting of the restriction factor for lysosomal degradation through an ubiquitin-dependent mechanism that involves the recruitment of SCFβ-TrCP E3 ubiquitin ligase complex and the ESCRT machinery. Indeed, through binding to HRS, Vpu enhances the affinity of BST2 for HRS thus accelerating its sorting by the ESCRT machinery for lysosomal degradation. One might propose that through binding with the ESCRT machinery, Vpu may favor the sorting of BST2 at the level of endosomes, thereby reducing its recycling to the plasma membrane (right panel). This figure is adapted from Janvier et al. (2012).

of β-TrCP in Vpu-mediated antagonism of BST2 antiviral activity remains controversial to date (Douglas et al., 2009; Iwabu et al., 2009; Mangeat et al., 2009; Mitchell et al., 2009; Miyagi et al., 2009; Tervo et al., 2011). BST2 undergoes ubiquitination on lysine residues located in its cytoplasmic tail (Pardieu et al., 2010). Interestingly, Vpu increases BST2 ubiquitination on lysine/serine and threonine residues located in its cytoplasmic tail, as is also observed with CD4 (Tokarev et al., 2010). Mutation of these residues reduces Vpu-induced antagonism of BST2. Consistent with this observation, the short isoform of BST2 lacking these residues shows decreased sensitivity to Vpu antagonism (Tokarev et al., 2010; Cocka and Bates, 2012). One study challenged the requirement of the S3T4S5 residues in Vpu-induced ubiquitination of BST2 (Gustin et al., 2012) but no explanation has been proposed for this discrepancy.

Despite the similarity in the molecular mechanisms underlying Vpu-induced ubiquitination and degradation of CD4 and BST2, the fate of both proteins differs. Indeed, it is now well established that Vpu does not target BST2 for proteasomal degradation but induces β-TrCP-dependent lysosomal sorting of BST2 (Douglas et al., 2009; Iwabu et al., 2009; Mitchell et al., 2009). In agreement with this notion, we highlighted a major role of Rab7, a regulator of the endo/lysosomal trafficking, in this process (Caillet et al., 2011), and revealed that Vpu enhances ESCRT-mediated sorting of BST2 for degradation (Janvier et al., 2011). The ESCRT machinery is a set of four hetero-oligomeric protein complexes involved in the sorting of ubiquitinated membrane proteins into vesicles budding into endosomes for their subsequent degradation in the lysosomes. The ESCRT-0 protein HRS (hepatocyte growth-factor-regulated tyrosine kinase substrate) coordinates this process by linking ubiquitinated cargoes and the ESCRT-I component TSG101 (Raiborg and Stenmark, 2009). We showed that Vpu-mediated down-regulation of BST2 and viral release require HRS, and unveiled an increased affinity of BST2 for HRS upon Vpu.
expression (Janvier et al., 2011). One might propose that through binding with HRS and BST2, Vpu accelerates ESCRT-mediated sorting of BST2 to the lysosomes, thereby reducing its recycling to the PM (Janvier et al., 2012; Figure 2). Further evidence of the requirement of the ESCRT machinery in Vpu-mediated BST2 degradation was obtained by the characterization of a new component of the ESCRT-I machinery: the protein UBAP1 (Agromayor et al., 2012). Depletion of UBAP1 abolishes Vpu-induced degradation of BST2, but has no impact on Vpu antagonism of BST2 antiviral activity (Agromayor et al., 2012), consistent with the notion that degradation of BST2 is not strictly required for Vpu-mediated antagonism of BST2 (Miyagi et al., 2009; Goffinet et al., 2010).

**VPU AND BST2 VIRAL SENSING ACTIVITY**

In addition to its activity as a restriction factor of viral release, BST2 has been recently characterized as an innate immune sensor for HIV (Cocka and Bates, 2012; Galao et al., 2012; Tokarev et al., 2013). In 2003, BST2 was first reported to stimulate the activity of the NF-κB family of transcription factors, using a whole-genome cDNA screen (Matsuda et al., 2003). Recent studies further revealed that tethered HIV particles increase BST2 signaling activity, resulting in enhanced production of pro-inflammatory stimuli, consistent with a role of BST2 as a sensor for assembled viruses (Cocka and Bates, 2012; Galao et al., 2012; Tokarev et al., 2013). This function seems separable from its activity as an inhibitor of viral release and relies on the integrity of the non-canonical dual tyrosine residues (Y6XY8) regulating its trafficking (Galao et al., 2012; Tokarev et al., 2013). Whether BST2 trafficking is relevant for its signaling activity is unclear. BST2 was proposed to activate the "canonical" NF-κB pathway through engaging mediators of this pathway via its tyrosine motif (Galao et al., 2012; Tokarev et al., 2013). Regulation of this pathway depends on the activation of the transforming growth factor-β-activated kinase-1 (TAK1)/TAK1-binding protein 1 and 2 (TAB1 and TAB2) complex, through poly-ubiquitination by E3 ligases of the TNF receptor-associated factors (TRAFs) family (Skaug et al., 2009). Interestingly, BST2 was shown to co-immunoprecipitate with TRAF2, TRAF6 as well as the TAK1/TAB1 complex (Galao et al., 2012; Tokarev et al., 2013).

Vpu was shown to counteract BST2 signaling activity through a β-TrCP-dependent mechanism (Tokarev et al., 2013). Interestingly, over a decade ago, Vpu was shown to sequester β-TrCP away from its substrates and inhibit NF-κB activation by interfering with β-TrCP-mediated degradation of the NF-κB inhibitor IkB (Bour et al., 2001; Besnard-Guerin et al., 2004). Whether this mechanism accounts solely for Vpu-induced inhibition of BST2 signaling activity requires further investigation. Furthermore, many questions remain regarding how viral expression triggers BST2 sensing activity.

**CONCLUDING REMARKS**

Over the past decade, Vpu has emerged as an important asset for viral egress and evasion from the host antiviral machineries. Although tremendous progress has been made toward understanding the mechanisms underlying Vpu’s functions, many questions remain regarding how this protein contributes to viral pathogenesis. Vpu’s contribution to viral immune evasion relies on its ability to alter the trafficking of its targets by subverting cellular machineries involved this process. However, despite some similarities in the mechanisms involved, major differences have been reported regarding the site of action of Vpu as well as the fate of its targets in cells. Further characterization of the mechanisms controlling Vpu expression and distribution in cells as well as the interplay with its targets and the host cell machineries might contribute to explain these pleiotropic effects. In keeping with this line of thought, another fascinating aspect worthy of further investigation is the role of Vpu with regards to BST2, in cell-to-cell transmission of HIV through virological synapses. So far, conflicting results have been obtained regarding the impact of both proteins on this process, but intriguingly, they have underlined a multifaceted role of BST2 in HIV pathogenesis (Schubert et al., 1995; Gummeluru et al., 2006; Casartelli et al., 2010; Jolly et al., 2010). Adding to this complexity, BST2 was recently described to act as a host sensor of assembled viruses. Therefore, a more detailed characterization of BST2’s functions in cells as well as its interplay with Vpu would contribute to better comprehend the role of both proteins in viral egress and dissemination. Addressing all these questions might provide important insights into AIDS pathogenesis and contribute to the future development of therapeutic strategies.

**AUTHOR CONTRIBUTIONS**

Nicolas Roy and Grégory Pacini conceived the figures and contributed to the writing of the manuscript; Clarisse Berlioz-Torrent edited the manuscript; Katy Janvier wrote the manuscript and contributed to the drawing of the figures.

**ACKNOWLEDGMENTS**

We sincerely apologize to the authors whose works will not have been cited due to space limitations. We thank Monica Naughtin for critical reading and editing of our manuscript. Nicolas Roy holds a fellowship from ANRS; Grégory Pacini holds a fellowship from the "Ministère Français de l’Enseignement Supérieur et de la Recherche". This work is funded by SIDACTION, the ANR-12-JSV3-0005-01 program and by ANRS.

**REFERENCES**

Agromayor, M., Soler, N., Caballe, A., Kueck, T., Freund, S. M., Allen, M. D., et al. (2012). The UBAP1 subunit of ESCRT-I interacts with ubiquitin via a SOUBA domain. *Structure* 20, 414–428. doi: 10.1016/j.str.2011.12.013

Bailes, E., Gao, F., Bibollet-Ruche, F., Courgnaud, V., Peeters, M., Marx, P. A., et al. (2003). Hybrid origin of SIV in chimpanzees. *Science* 300, 1713. doi: 10.1126/science.1080657

Besnard-Guerin, C., Belaidouni, N., Lassot, I., Segeral, E., Jobart, A., Marchal, C., et al. (2004). HIV-1 Vpu sequesters beta-transducin repeat-containing protein (betaTrCP) in the cytoplasm and provokes the accumulation of beta-catenin and other SCFbetaTrCP substrates. *J. Biol. Chem.* 279, 788–795. doi: 10.1074/jbc.M308068200

Bilkicfi, P. G., Rollason, R., Prior, I., Owen, D. M., Gaus, K., and Banting, G. (2013). CD317/tetherin is an organiser of membrane microdomains. *J. Cell Sci.* 126, 1553–1564. doi: 10.1242/jcs.112983

Bour, S., Perrin, C., Akari, H., and Strebel, K. (2001). The human immunodeficiency virus type 1 Vpu protein inhibits NF-kappa B activation by interfering with beta TrCP-mediated degradation of Ikappa B. *J. Biol. Chem.* 276, 15920–15928. doi: 10.1074/jbc.M010533200
Caillet, M., Janvier, K., Pelchen-Matthews, A., Delcroix-Genete, D., Camus, G., Marsh, M., et al. (2011). Rab7A is required for efficient production of infectious HIV-1. *PLoS Pathog.* 7:e1002347. doi: 10.1371/journal.ppat.1002347

Casartelli, N., Sourisseau, M., Feldmann, J., Guivel-Behagassine, E., Mallet, A., Marcelin, A. G., et al. (2010). Tetherin restricts productive HIV-1 cell-to-cell transmission. *PLoS Pathog.* 6:e1000955. doi: 10.1371/journal.ppat.1000955

Chaudhuri, R., Lindwasser, O. W., Smith, W. J., Hurley, J. H., and Bonifacino, J. S. (2007). Downregulation of CD4 by human immunodeficiency virus type 1 Nef is dependent on clathrin and involves direct interaction of Nef with the AP2 clathrin adaptor. *J. Virol.* 81, 3877–3890. doi: 10.1128/JVI.02725-06

Chu, H., Wang, J. J., Qi, M., Yoon, J. J., Chen, X., Wen, X., et al. (2012). Tetherin/BST-2 is essential for the formation of the intracellular virus-containing compartment in HIV-infected macrophages. *Cell Host Microbe* 12, 360–372. doi: 10.1016/j.chom.2012.07.011

Cocka, L. J., and Bates, P. (2012). Identification of alternatively translated Tetherin isoforms with differing antiviral and signaling activities. *PLoS Pathog.* 8:e1002931. doi: 10.1371/journal.ppat.1002931

Courgnaud, V., Fromentty, P., Akoua-Koffi, C., Noe, R., Boesch, C., Delaporte, E., Goffinet, C., Allespach, I., Homann, S., Tervola, H. M., Habermann, A., Rupp, D., Keppler, O. T., et al. (2003). Identification of alternatively translated Tetherin isoforms with differing antiviral and signaling activities. *PLoS Pathog.* 8:e1002931. doi: 10.1371/journal.ppat.1002931

Douglas, J. L., Viswanathan, K., McCarroll, M. N., Gustin, J. K., Fruh, K., and Emerman, M. (2000). An HIV-1 Vpu-induced down-regulation of CD4 and BST2 protein by HIV-1 Vpu protein does not require lysine, serine, or threonine residues within the BST-2 cytoplasmic domain. *J. Biol. Chem.* 287, 14837–14850. doi: 10.1074/jbc.M1112.349928

Gupta, R. K., Miochova, P., Pelchen-Matthews, A., Petit, S. J., Mattiuzzo, G., Pillay, D., et al. (2009b). Simian immunodeficiency virus envelope glycoprotein counteracts tetherin/BST-2/CD317 by intracellular sequestration. *Proc. Natl. Acad. Sci. U.S.A.* 106, 20889–20894. doi: 10.1073/pnas.090775109

Gustin, J. K., Douglas, I. L., Bai, Y., and Moses, A. V. (2012). Ubiquitination of BST-2 protein by HIV-1 Vpu protein does not require lysine, serine, or threonine residues within the BST-2 cytoplasmic domain. *J. Biol. Chem.* 287, 14837–14850. doi: 10.1074/jbc.M1112.349928

Hammonds, J., Wang, J. J., Yi, H., and Spearman, P. (2010). Immunoelectron microscopic evidence for Tetherin/BST2 as the physical bridge between HIV-1 virions and the plasma membrane. *PLoS Pathog.* 6:e1000749. doi: 10.1371/journal.ppat.1000749

Hauser, H., Lopez, L. A., Yang, S. J., Oldenburg, J. E., Exline, C. M., Guatelli, J. C., et al. (2010). HIV-1 Vpu and HIV-2 Env counteract BST-2/tetherin by sequestration in a perinuclear compartment. *Retrovirology* 7, 51. doi: 10.1186/1742-4676-7-51

Hill, M. S., Ruiz, A., Schmitt, K., and Stephens, E. B. (2010). Identification of amino acids within the second alpha helical domain of the human immunodeficiency virus type 1 Vpu that are critical for preventing CD4 cell surface expression. *Virology* 397, 104–112. doi: 10.1016/j.virol.2009.10.048

Iwabu, Y., Fujita, H., Kinomoto, M., Kaneko, K., Ishiizaka, Y., Tanaka, Y., et al. (2009). HIV-1 accessory protein Vpu internalizes cell-surface BST-2/tetherin through transmembrane interactions leading to lysosomes. *J. Biol. Chem.* 284, 35060–35072. doi: 10.1074/jbc.M109.058305

Jabbah, M. A., and Nayak, D. P. (1993). Intracellular interaction of human immunodeficiency virus type 1 (ARV-2) envelope glycoprotein gp160 with CD4 blocks the movement and maturation of CD4 to the plasma membrane. *J. Virol.* 64, 6297–6304.

Janvier, K., Pelchen-Matthews, A., Renaud, J. B., Caillet, M., Marsh, M., and Berlizio-Torrent, C. (2011). The ESCRT-0 component HRS is required for HIV-1 Vpu-mediated BST-2/tetherin down-regulation. *PLoS Pathog.* 7:e1001265. doi: 10.1371/journal.ppat.1001265

Janvier, K., Roy, N., and Berlizio-Torrent, C. (2012). Role of the endosomal ESCRT machinery in HIV-1 Vpu-induced down-regulation of BST2/tetherin. *Carr. HIV Res. 10*, 315–320. doi: 10.2174/1716085420110127.7x

Jia, B., Serra-Moreno, R., Neidermyer, W., Rahmberg, J., Mackey, J., Fofana, I. B., et al. (2009). Species-specific activity of SIV Nef and HIV-1 Vpu in overcoming restriction by tetherin/BST2. *PLoS Pathog.* 5:e1000429. doi: 10.1371/journal.ppat.1000429

Jolly, C., Booth, N. J., and Neil, S. J. (2010). Cell-cell spread of human immunodeficiency virus type-1 overcome tetherin/BST-2 mediated restriction in T cells. *J. Virol.* 84, 12185–12199. doi: 10.1128/JVI.01447-10

Kobayashi, T., Ode, H., Yoshida, T., Sato, K., Gee, P., Yamamoto, S. P., et al. (2011). Identification of amino acids in the human tetherin transmembrane domain responsible for HIV-1 Vpu interaction and susceptibility. *J. Virol.* 85, 932–945. doi: 10.1128/JVI.01668-10

Kucek, T., and Neil, S. J. (2012). A cytoplasmic tail determinant in HIV-1 Vpu mediates targeting of tetherin for endosomal degradation and counteracts interferon-inhibited restriction. *PLoS Pathog.* 8:e1002609. doi: 10.1371/journal.ppat.1002609

Kuzpiz, S., Korolchuk, V., Rollason, R., Sugden, A., Wilde, A., and Banting, G. (2003). Vpu-2/HM1.24 is a raft-associated apical membrane protein with an unusual topology. *Traffic* 4, 694–709. doi: 10.1046/j.1600-0854.2003.00129.x

Laguet, N., Sobbian, B., Casartelli, N., Ringard, M., Chable-Bessia, C., Segeral, E., et al. (2011). SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. *Nature 474*, 654–657. doi: 10.1038/nature10117

Lau, D., Kwan, W., and Guatelli, J. (2011). Role of the endocytic pathway in the reattachment of the counterfactor in HIV-2 by human lentiviral pathogens. *J. Virol.* 85, 9834–9846. doi: 10.1128/JVI.02633-10

Le Tortorec, A., and Neil, S. J. (2009). Antagonism to and intracellular sequestration of human tetherin by the human immunodeficiency virus type 2 envelope glycoprotein. *J. Virol.* 83, 11966–11978. doi: 10.1128/JVI.01497-11

Magadan, I. G., and Bonifacino, J. S. (2012). Transmembrane domain determinants of CD4 Downregulation by HIV-1 Vpu. *J. Virol.* 86, 757–772. doi: 10.1128/JVI.05933-11
Matsuda, A., Suzuki, Y., Honda, G., Muramatsu, S., Matsuzaki, O., Nagano, Y., McNatt, M. W., Zang, T., and Bieniasz, P. D. (2013). Vpu binds directly to Mitchell, R. S., Katsura, C., Skasko, M. A., Fitzpatrick, K., Lau, D., Ruiz, A., Margottin, F., Bour, S. P., Durand, H., Selig, L., Benichou, S., Richard, V., et al. Moll, M., Andersson, S. K., Smed-Sorensen, A., and Sandberg, J. K. (2010). Inhibition of tetherin and displaces it from nascent virions. Cell Host Microbe 3, 388–398. doi: 10.1016/j.chom.2008.04.008

Mangeat, B., Gers-Huber, G., Lehmann, M., Zufferey, M., Luban, J., and Piguet, V. (2009). HIV-1 Vpu neutralizes the antiviral factor Tetherin/BST-2 by binding it and directing its beta-TrCP2-dependent degradation. PLoS Pathog. 5:e1000574. doi: 10.1371/journal.ppat.1000574

Margottin, F., Bour, S. P., Durand, H., Selig, L., Benichou, S., Richard, V., et al. (1998). A novel human WD protein, h-beta TrCP, that interacts with HIV-1 Vpu connects CD4 to the ER degradation pathway through an F-box motif. Mol. Cell 1, 365–374. doi: 10.1016/S1097-2765(00)80056-8

Matsuda, A., Suzuki, Y., Honda, G., Muramatsu, S., Matsuzaki, O., Nagano, Y., et al. (2003). Large-scale identification and characterization of human genes that activate NF-kappaB and MAPK signaling pathways. Oncogene 22, 3307–3318. doi: 10.1038/sj/onc.1206406

McNatt, M. W., Zang, T., and Bieniasz, P. D. (2013). Vpu binds directly to tetherin and displaces it from nascent virions. PLoS Pathog. 9:e1003299. doi: 10.1371/journal.ppat.1003299

Mitchell, R. S., Katsura, C., Skasko, M. A., Fitzpatrick, K., Lau, D., Ruiz, A., et al. (2009). Vpu antagonizes BST-2-mediated restriction of HIV-1 release via beta-TrCP and endo-lysosomal trafficking. PLoS Pathog. 5:e1000450. doi: 10.1371/journal.ppat.1000450

Miyagi, E., Andrew, A. J., Kao, S., and Strebel, K. (2009). Vpu enhances HIV-1 virus release in the absence of BST-2 cell surface down-modulation and intracellular degradaution. Proc. Natl. Acad. Sci. U.S.A. 106, 2868–2873. doi: 10.1073/pnas.0813223106

Moll, M., Anderson, S. K., Smed-Sorensen, A., and Sandberg, J. K. (2010). Inhibition of lipid antigen presentation in dendritic cells by HIV-1 Vpu interferes with CD1d recycling from endosomal compartments. Blood 116, 1876–1884. doi: 10.1182/blood-2009-09-243667

Neil, S. J. (2013). The antiviral activities of tetherin. Curr. Top. Microbiol. Immunol. 371, 67–104. doi: 10.1007/978-3-642-77653-5_3

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. doi: 10.1038/nature06553

Pardieu, C., Vigan, R., Wilson, S. J., Calvi, A., Zang, T., Bieniasz, P., et al. (2010). 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. doi: 10.1371/journal.ppat.1000843

Perez-Caballero, D., Zang, T., Ebrahimia, A., McNatt, M. W., Gregory, D. A., Johnson, M. C., et al. (2009). Tetherin inhibits HIV-1 release by directly tethering virions to cells. Cell 139, 499–511. doi: 10.1016/j.cell.2009.08.039

Pickering, S., Hue, S., Kim, E. Y., Reddy, S., Wolinsky, S. M., and Neil, S. J. (2014). Preservation of tetherin and CD4 counter-activities in circulating Vpu alleles despite extensive sequence variation within HIV-1 infected individuals. PLoS Pathog. 10:e1003895. doi: 10.1371/journal.ppat.1003895

Raiborg, C., and Stenmark, H. (2009). The ESCRT machinery in endosomal sorting of ubiquitylated membrane proteins. Nature 458, 445–452. doi: 10.1038/nature07961

Ray, N., and Doms, R. W. (2006). HIV-1 coreceptors and their inhibitors. Curr. Top. Microbiol. Immunol. 303, 97–120. doi: 10.1007/978-3-540-33397-5_5

Rollason, R., Korolchuk, V., Hamilton, C., Schu, P., and Banting, G. (2007). Clathrin-mediated endocytosis of a lipid-raft-associated protein is mediated through a dual tyrosine motif. J. Cell Sci. 120, 3850–3858. doi: 10.1242/jcs.003343

Sauter, D., Schindler, M., Specht, A., Landford, W. N., Munch, J., Kim, K. A., et al. (2009). Tetherin-driven adaptation of Vpu and Nef function and the evolution of pandemic and non-pandemic HIV-1 strains. Cell Host Microbe 6, 409–421. doi: 10.1016/j.chom.2009.10.004

Schubert, H. L., Zhai, Q., Sandrin, V., Eckert, D. M., Garcia-Maya, M., Saul, L., et al. (2010). Structural and functional studies on the extra- cellular domain of BST2/tetherin in reduced and oxidized conformations. Proc. Natl. Acad. Sci. U.S.A. 107, 17951–17956. doi: 10.1073/pnas.1008206107

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. doi: 10.1038/nature00939

Skaug, B., Jiang, X., and Chen, Z. J. (2009). The role of ubiquitin in NK-kappaB regulatory pathways. Annu. Rev. Biochem. 78, 769–796. doi: 10.1146/annurev.biochem.78.070907.102730

Strebel, K., Klimkait, T., and Martin, M. A. (1988). A novel gene of HIV-1, vpu, and its 16-kilodalton product. Science 241, 1221–1223. doi: 10.1126/science.318888

Stremlau, M., Owens, C. M., Perron, M. J., Kiessling, M., Autissier, P., and Sodroski, J. (2004). The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in old world monkeys. Nature 427, 848–853. doi: 10.1038/nature02343

Tervo, H. M., Homann, S., Ambiel, I., Fritz, J. V., Fackler, O. T., and Keppler, O. T. (2011). Beta-TrCP is dispensable for Vpu’s ability to overcome the CD317/Tetherin-imposed restriction to HIV-1 release. Retrovirology 8, 9. doi: 10.1186/1742-4690-8-9

Tokarev, A., Suarez, M., Kwan, W., Fitzpatrick, K., Singh, R., and Guatelli, J. (2013). Stimulation of NF-kappaB activity by the HIV restriction factor BST2. J. Virol. 87, 2046–2057. doi: 10.1128/JVI.02272-12

Tokarev, A. A., Munguia, J., and Guatelli, J. C. (2010). Serine-threonine ubiquitination mediates downregulation of BST-2/tetherin and relief of restricted virion release by HIV-1 Vpu. J. Virol. 85, 51–63. doi: 10.1128/JVI.01795-10

Van Damme, N., Goff, D., Katsura, C., Jorgenson, R. L., Mitchell, R., Johnson, M. C., et al. (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. doi: 10.1016/j.chom.2008.03.001

Venkatesh, S., and Bieniasz, P. D. (2013). Mechanism of HIV-1 virion entrapment by tetherin. PLoS Pathog. 9:e1003483. doi: 10.1371/journal.ppat.1003483
Vigan, R., and Neil, S. J. (2010). Determinants of tetherin antagonism in the transmembrane domain of the human immunodeficiency virus type-1 (HIV-1) Vpu protein. J. Virol. 84, 12958–12970. doi: 10.1128/JVI.01699-10

Wildum, S., Schindler, M., Munch, J., and Kirchhoff, F. (2006). Contribution of Vpu, Env, and Nef to CD4 down-modulation and resistance of human immunodeficiency virus type 1-infected T cells to superinfection. J. Virol. 80, 8047–8059. doi: 10.1128/JVI.00252-06

Willey, R. L., Maldarelli, F., Martin, M. A., and Strebel, K. (1992a). Human immunodeficiency virus type 1 Vpu protein induces rapid degradation of CD4. J. Virol. 66, 7193–7200.

Willey, R. L., Maldarelli, F., Martin, M. A., and Strebel, K. (1992b). Human immunodeficiency virus type 1 Vpu protein regulates the formation of intracellular gp160-CD4 complexes. J. Virol. 66, 226–234.

Zhang, F., Wilson, S. J., Landford, W. C., Virgen, B., Gregory, D., Johnson, M. C., et al. (2009). Nef proteins from simian immunodeficiency viruses are tetherin antagonists. Cell Host Microbe 6, 54–67. doi: 10.1016/j.chom.2009.05.008

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: 09 February 2014; accepted: 31 March 2014; published online: 01 May 2014.

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