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HIV-1 Vpu targets cell surface markers CD4 and BST-2 through distinct mechanisms

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Abstract

Vpu is a small integral membrane protein encoded by HIV-1 and some SIV isolates. The protein is known to induce degradation of the viral receptor molecule CD4 and to enhance the release of newly formed virions from the cell surface. Vpu accomplishes these two functions through two distinct mechanisms. In the case of CD4, Vpu acts as a molecular adaptor to connect CD4 to an E3 ubiquitin ligase complex resulting in CD4 degradation by cellular proteasomes. This requires signals located in Vpu's cytoplasmic domain. Enhancement of virus release on the other hand involves the neutralization of a cellular host factor, BST-2 (also known as CD317, HM1.24, or tetherin) and requires Vpu's TM domain. The current review discusses recent advances on the role of Vpu in controlling degradation of CD4 and in regulating virus release.

1. Introduction

Viruses are obligate intracellular parasites whose primary goal is to infect host cells in order to replicate their genomes and to produce progeny virions for infection of new target cells. Some viruses cause long-lasting chronic infections while others replicate in fast, lytic cycles. Most virus infections can be controlled by the host immune system through adaptive, innate, or intrinsic immune mechanisms leading to the elimination of the virus from the infected host. Other viruses, including HIV-1, establish chronic life-long infections and are difficult if not impossible to eradicate. There are currently an estimated 33 million people living with HIV worldwide. Despite this staggering number, the initial infection by HIV-1 appears to be quite inefficient. Indeed, HIV faces an uphill battle when spreading to a new host since it has to overcome several levels of host defense mechanisms.

The overall genome organization of primate lentiviruses is quite similar. However, there are a few notable differences. For instance viruses of the HIV-1/SIVcpz lineage, as well as SIVgsn, SIVmon, SIVmus, and SIVden encode a *vpu* gene while viruses of the HIV-2/SIVsm lineage as well as SIVrcm, SIVmnd-2, and SIVdrl encode a *vpx* gene (Dazza et al., 2005). Other SIV strains encode neither *vpu* nor *vpx*. Additional genes including *vif*, *vpr*, and *nef* are encoded by most lentiviruses. Because inactivation of these genes does not consistently affect virus replication in tissue culture they were termed *accessory genes* for lack of a more functionally descriptive term. However, the past years have brought exciting new insights into the role of these proteins and it became increasingly clear that many if not all of the HIV accessory proteins

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have important functions in counteracting different levels of antiviral host cell restriction. Much progress has been made in the identification and characterization of host restriction factors affecting HIV replication. In particular, the identification of Trim-5 α , APOBEC3G, and BST-2/tetherin has significantly advanced our understanding of innate and intrinsic immune mechanisms involved in the defense against HIV (for a recent update see (Strebel et al., 2009)). This review will focus on recent advances on Vpu and its actions against CD4 and BST-2/tetherin.

2. Structure of Vpu

Vpu is an 81 amino acid type 1 integral membrane protein. Residues 1–27 constitute the N-terminal hydrophobic membrane anchor followed by 54 residues that extend into the cytoplasm. A highly conserved region spanning residues 47–58 contains a pair of serine residues that are constitutively phosphorylated by casein kinase II (Friborg et al., 1995a; Schubert et al., 1994). Structural analysis of synthetic peptides representing the cytoplasmic domain of Vpu detected two discrete α -helical structures encompassing amino acids 35–50 and 58–70, respectively, separated by a flexible segment containing the two conserved phosphorylated serine residues (Federau et al., 1996; Henklein et al., 1993; Kochendoerfer et al., 2004; Wittlich et al., 2009; Wray et al., 1995; Zheng et al., 2003). The Vpu helix 1 is amphipathic with hydrophobic, basic, and acidic residues clustered along the axis of the helix. The same is true for helix 2 albeit to a less striking degree. Using solid-state NMR it was determined that the Vpu transmembrane domain forms a stable helical structure with a tilt angle of $\sim 6^\circ$ to 15° (Kukol and Arkin, 1999; Park et al., 2006; Park et al., 2003; Park and Opella, 2005; Sharpe et al., 2006). These data along with the extensive structure information available for the Vpu cytoplasmic tail in solution have led to a model of Vpu topology in a membrane environment as depicted in Figure 1 (I). In this model, the membrane-spanning N-terminal domain forms a stable α -helix that is connected to the soluble cytoplasmic tail by a short unstructured fragment. A string of positively charged residues within that flexible arm would allow interactions with the negatively charged lipid surface. The hydrophobic side of helix 1 in the cytoplasmic domain is partially buried in the lipid bilayer, exposing the hydrophilic (or charged) side to the cytoplasm. The flexible region joining the cytoplasmic helices 1 and 2 appears to form a loop pointing away from the membrane, mostly due to the acidic nature of the two conserved phosphorylated serine residues (Coadou et al., 2002; Coadou et al., 2003; Henklein et al., 2000). The phosphorylation status of the two serine residues does not appear to have significant structural consequences (Wittlich et al., 2008). In a second model (Fig. 1, II), the two cytoplasmic helices of Vpu adopt a more compact structure where the two helices are oriented almost parallel to each other (Lemaitre et al., 2004). More recent structural analysis of micelle-associated Vpu cytoplasmic domain fragments suggested a substantial degree of structural flexibility (Wittlich et al., 2009).

One of the limitations of the structures depicted in figure 1 is that they are based on highly purified Vpu protein. However, biochemical studies have revealed that in HIV-infected cells Vpu interacts with several host factors, including CD4, β -TrCP, and BST-2/tetherin. It is likely that the interaction of Vpu with such host factors affects its tertiary structure and in particular the orientation of the cytoplasmic helices relative to the membrane. Also, homo-oligomerization of Vpu, which was first described by chemical cross-linking experiments (Maldarelli et al., 1993) may significantly affect the overall conformation of Vpu. Indeed, Vpu can be modelled into a pentameric complex to form membrane pores (Grice et al., 1997; Kruger and Fischer, 2009, 2010; Park et al., 2003). The formation of membrane pores was experimentally verified *in vitro* where Vpu formed cation-selective membrane pores (Ewart et al., 1996; Schubert et al., 1996b). For a more comprehensive review of Vpu structure and its ion channel forming properties see (Opella et al., 2005).

3. Vpu-induces degradation of CD4

Three HIV-1 encoded proteins, Nef, Env, and Vpu contribute to the viral effort to downregulate CD4 from the cell surface. The question arises why HIV devotes three viral proteins to target CD4. It has been suggested that CD4 downregulation could prevent super-infection of cells but the benefit to the virus is unclear since superinfection could in fact promote viral recombination and thus provide evolutionary advantages. However, CD4 was also shown to inhibit virus release and to interfere with the packaging of Env protein into viral particles (Bour et al., 1999a; Lama et al., 1999). Whatever the ultimate benefit may be, it is clear that CD4 down-modulation is a serious concern to HIV-1. It is interesting that Nef, Env, and Vpu affect surface expression of CD4 through three entirely different mechanisms. Nef is a myristoylated cytoplasmic membrane-associated protein that binds to CD4 at the cell surface and triggers internalization of CD4 through a clathrin-dependent pathway (Aiken et al., 1994; Chaudhuri et al., 2007; Rhee and Marsh, 1994). Although there is no obvious reason why Nef should not be able to interact with CD4 intracellularly, Nef is in fact not capable of preventing trafficking of CD4 to the cell surface. This task is accomplished by Env and Vpu who, in return, cannot target CD4 at the cell-surface. Instead, the gp160 Env precursor blocks trafficking of newly synthesized CD4 from the ER through the formation of stable complexes that are retained in the ER (Bour et al., 1991; Crise et al., 1990; Jabbar and Nayak, 1990) while Vpu induces proteasomal degradation of CD4 from the ER (Willey et al., 1992a) through a mechanism that involves the ERAD pathway (Binette et al., 2007; Magadan et al., 2010) (Figure 2). The formation of CD4-gp160 complexes in the ER blocks the transport and maturation of not only CD4 but of the Env protein itself (Bour et al., 1991). In cases where equimolar amounts of CD4 and Env are synthesized, this could lead to the depletion of cell surface Env and thus the production of Env-deficient, noninfectious virions (Buonocore and Rose, 1990, 1993). An important function of Vpu therefore may be to induce the degradation of CD4 molecules trapped in intracellular complexes with Env thus allowing gp160 to resume transport toward the cell surface (Willey et al., 1992b). In Vpu-expressing cells, CD4 is rapidly degraded in the ER and its half-life drops from 6 h to approximately 15 min (Willey et al., 1992a). The importance of ER localization for CD4 susceptibility to Vpu-mediated degradation suggests that cellular factors essential for CD4 degradation are located in the ER and/or that the rate limiting step of CD4 degradation is binding by Vpu (Chen et al., 1993). In support of the latter option, coimmunoprecipitation experiments showed that CD4 and Vpu physically interact in the ER and that this interaction is essential for targeting CD4 to the degradation pathway (Bour et al., 1995). The domains in Vpu and CD4 required for interaction are less well defined, suggesting that three-dimensional rather than linear structures are involved. While two conserved serine residues at positions 52 and 56 in the cytoplasmic domain of Vpu are critically important for CD4 degradation (Paul and Jabbar, 1997; Schubert and Strebel, 1994), they are not required for CD4 binding since phosphorylation-defective mutants of Vpu retained the capacity to interact with CD4 (Bour et al., 1995). Nevertheless, the cytoplasmic domains of Vpu and CD4 are critical for CD4-Vpu interaction (Bour et al., 1995; Margottin et al., 1996). Analysis of chimeric proteins suggests that neither the CD4 transmembrane (TM) domain nor the Vpu TM domain determine target specificity (Bour et al., 1995; Schubert et al., 1996a) (Figure 2). However, relatively non-specific interactions between the hydrophobic membrane helices of CD4 and Vpu may nonetheless stabilize Vpu-CD4 interactions and contribute to Vpu-induced CD4 degradation (Magadan et al., 2010).

The role of the Vpu phosphoserine residues in the induction of CD4 degradation was elucidated when yeast two-hybrid assays as well as coimmunoprecipitation studies revealed an interaction of Vpu with the human beta Transducin-repeat Containing Protein (β -TrCP; (Margottin et al., 1998)). Interestingly, Vpu variants mutated at Ser52 and Ser56 were unable to interact with β -TrCP, providing a mechanistic explanation for the requirement for Vpu phosphorylation and strongly suggesting that β -TrCP was directly involved in the degradation of CD4. Human β

–TrCP contains seven C-terminal WD repeats, a structure known to mediate protein-protein interactions (Neer et al., 1994). Accordingly, the WD repeats of human β -TrCP were shown to mediate interactions with Vpu in a phosphoserine-dependent fashion (Margottin et al., 1998). In addition to the WD repeats, β -TrCP contains an F-box domain that functions as a connector between target proteins and the ubiquitin-dependent proteolytic machinery (Bai et al., 1996). Although the molecular mechanisms by which Vpu targets CD4 for degradation are now reasonably well defined, it remained unclear how CD4 is extracted from the endoplasmic reticulum membrane for degradation by a cytosolic ubiquitin ligase complex. Degradation of proteins through the ERAD pathway typically does not involve the cytosolic SCF β -TrCP complex used by Vpu for CD4 degradation but instead employs membrane-bound ubiquitin ligases such as HRD1-SEL1L, TEB4/MARCH-VI, or GP78-RMA1 complexes (Fang et al., 2001; Hassink et al., 2005; Kikkert et al., 2004; Lilley and Ploegh, 2005; Morito et al., 2008). While this implies that Vpu-induced degradation of CD4 is distinct from the classical ERAD pathway, inhibition of the ERAD machinery by silencing of VCP(p97), UFD1L, or NPL4, which are key components involved in the extraction or dislocation of ERAD substrates from the ER membrane indeed prevented Vpu-induced CD4 degradation (Magadan et al., 2010). The involvement of the ERAD pathway is further supported by the observation that over-expression of a trans dominant negative mutant of VCP(p97) inhibited Vpu-mediated CD4 degradation (Binette et al., 2007).

4. Vpu regulates the detachment of virions from the cell surface

The second well described function of Vpu is the regulation of virus release. Efficient virus release from HIV-infected cells is regulated by its Vpu gene product (Strebel et al., 1988; Terwilliger et al., 1989). It is generally accepted that Vpu regulates the detachment of otherwise complete and infectious virions from the cell surface (Klimkait et al., 1990; Neil et al., 2006). Interestingly, the ability to enhance virus release is not unique to Vpu. In fact, some HIV-2 isolates have been known to encode a Vpu-like activity in their Env glycoproteins (Abada et al., 2005; Bour et al., 1996; Bour and Strebel, 1996; Ritter et al., 1996). Intriguingly, a similar Vpu-like activity was now also shown for the Nef protein of several simian immunodeficiency viruses, including SIVmac and SIVagm (Jia et al., 2009; Zhang et al., 2009). It is interesting that the same three proteins previously implicated in the surface down-modulation of CD4 have now also been associated with the efficient release of virions from the cell surface. Again, each of the three proteins employs a different strategy to accomplish this common goal (see Figure 3). Also of interest, while Vpu is unable to target CD4 that has exited the ER, it appears to efficiently mediate internalization of BST-2 from a post-ER compartment (as discussed in more detail below) suggesting the two functions of Vpu are mechanistically distinct.

A number of different models have been proposed to explain how Vpu enhances virus release. First, a Vpu-associated ion channel activity was implicated in the regulation of virus release (reviewed in (Strebel et al., 2009)). This model is based on the observation that scrambling of Vpu's TM domain inhibited ion channel activity, impaired Vpu's ability to regulate virus release, but did not affect Vpu's ability to degrade CD4 (Schubert et al., 1996a; Schubert et al., 1996b). A second alternative model suggested that Vpu might interfere with the activity of TASK-1, a cellular ion channel, through the formation of hetero-oligomeric complexes (Hsu et al., 2004). However, while the TASK-1 model implicated the involvement of a host factor as predicted from the cell type-specific function of Vpu, it remains unclear whether expression of TASK-1 is tissue specific and if so whether expression matches that of Vpu-dependent cell types. It also remains unclear exactly how ion channel activity by either Vpu or TASK-1 might regulate detachment of particles from the cell surface.

A third model invokes the inactivation of a cellular inhibitor of virus release. The dependence on Vpu for efficient virus release is cell-type dependent, which lead investigators to predict

the presence of a host restriction factor in Vpu-dependent cell types (Varthakavi et al., 2003). Indeed, in addition to TASK-1, several other host factors have been identified whose overexpression was associated with reduced virus release. These include the Vpu binding protein UBP (Callahan et al., 1998) and the recently identified host factor BST-2 (also referred to as tetherin, CD317, or HM1.24 (Neil et al., 2008; Van Damme et al., 2008)). Among those, BST-2 is of particular interest since its cell type-specific expression most closely matches that of Vpu-dependent cell types and silencing of BST-2 expression in HeLa cells by siRNA or shRNA rendered virus release from these cells Vpu-independent (Neil et al., 2008; Van Damme et al., 2008).

5. BST-2: an interferon-inducible viral restriction factor

A clue to the identity of the Vpu-sensitive restriction factor was first discovered during a quantitative membrane proteomics study where Vpu expressed from an adenovirus vector was found to reduce cellular expression of BST-2 in HeLa cells (Bartee et al., 2006). Subsequent reports identified BST-2 as the IFN-inducible, Vpu-sensitive factor responsible for the restriction of HIV-1 virus release (Neil et al., 2008; Van Damme et al., 2008). Indeed, BST-2 expression was cell type dependent; the protein was constitutively expressed in Vpu-dependent cell types such as HeLa, Jurkat, or CD4⁺ T cells but was undetectable in permissive cell types such as 293T or HT1080 cells and thus corresponded to cell types known to depend on Vpu for efficient virus release (Neil et al., 2008; Van Damme et al., 2008). Importantly, BST-2 expression was induced by interferon treatment in 293T and HT1080 cells (Neil et al., 2007; Neil et al., 2008) and ectopic expression of BST-2 in 293T or HT1080 cells rendered these cells Vpu dependent (Neil et al., 2008; Van Damme et al., 2008). Finally, siRNA silencing of BST-2 caused virus release from HeLa cells to be Vpu-independent (Neil et al., 2008; Rong et al., 2009; Van Damme et al., 2008). Taken together these data provided strong evidence that BST-2 was indeed the host factor whose inhibitory effect on virus release was counteracted by Vpu.

6. BST-2: structural considerations

BST-2 was originally identified as a membrane protein in terminally differentiated human B cells of patients with multiple myeloma (Goto et al., 1994; Ohtomo et al., 1999). BST-2 is a 30-36 kDa type II transmembrane protein, consisting of 180 amino acids (Ishikawa et al., 1995). The protein has both an N-terminal transmembrane domain and a C-terminal glycosyl-phosphatidylinositol (GPI) anchor (Kupzig et al., 2003) (Figure 3). BST-2 protein associates with lipid rafts at the cell surface and on internal membranes, presumably the Trans-Golgi Network (TGN) (Dube et al., 2009; Kupzig et al., 2003; Masuyama et al., 2009). X-ray crystallography of recombinant BST-2 demonstrated that residues 47-148 of the protein's ectodomain can fold into a 90 Å parallel coil-coil structure (Hinz et al., 2010). In addition, small-angle X-ray scattering analyses predicted that the complete extracellular region of BST-2 adopts a long bent rodlike structure that separates the TM domain and GPI anchor by approximately 170 Å (Hinz et al., 2010).

Biochemical analyses demonstrated that BST-2 forms stable cysteine-linked dimers (Andrew et al., 2009; Goto et al., 1994; Ohtomo et al., 1999; Perez-Caballero et al., 2009) and is modified by N-linked glycosylation (Andrew et al., 2009; Kupzig et al., 2003; Ohtomo et al., 1999). The formation of cysteine-linked dimers can be catalyzed by any one of three cysteine residues in the BST-2 ectodomain. Interestingly, BST-2 dimerization was not essential for BST-2 cell-surface expression or sensitivity to Vpu (Andrew et al., 2009; Perez-Caballero et al., 2009) but is critical for inhibition of HIV-1 release (Andrew et al., 2009; Perez-Caballero et al., 2009). Similarly, deletion of the coil-coil domain inhibited BST-2 function without affecting dimerization or cell surface expression (Perez-Caballero et al., 2009). The functional role of

BST-2 glycosylation on the other hand is still debated. Two studies found that glycosylation of BST-2 was not important for inhibition of HIV-1, Lassa, or Marburg virus release (Andrew et al., 2009; Sakuma et al., 2009) while a third study found that lack of glycosylation almost completely inactivated BST-2 function presumably due to a trafficking defect (Perez-Caballero et al., 2009). The reason for these discrepant observations is not clear. Unlike the first two studies, which used untagged or N-terminally tagged protein, respectively, the third study employed a BST-2 variant encoding an internal HA-epitope tag. It is conceivable that the presence of an internal epitope tag in conjunction with the lack of carbohydrate modifications induced a conformational change in the protein and resulted in the loss of activity.

7. BST-2, a molecular crosslinker?

How does BST-2 inhibit virus release? Vpu-defective particles produced in BST-2 positive cells accumulate at the cell surface. This has led to a model in which BST-2, by means of its N-terminal transmembrane domain and its C-terminal GPI anchor, tethers otherwise fully detached virions to the producer cell (Neil et al., 2008). Such a model is consistent with the observation that Vpu-defective particles can be released by protease treatment (Fitzpatrick et al., 2010; Kaletsky et al., 2009; Miyakawa et al., 2009; Neil et al., 2007; Neil et al., 2008) or by physical force (Klimkait et al., 1990; Miyagi et al., 2009). While the tethering model is elegant and simple, it awaits formal experimental validation.

One approach to validate the tetherin model is to employ immune-electron microscopy to study the localization of BST-2 on virus producing cells (Fitzpatrick et al., 2010; Habermann et al., 2010; Hammonds et al., 2010; Perez-Caballero et al., 2009). In these studies, tethered virions stained positive for BST-2 in support of a tethering function of BST-2. As noted above, BST-2 is capable of bridging a gap of about 17 nm due to its rod-like structure (Hinz et al., 2010). Surprisingly, the measured distance between virus particles and the plasma membrane or among tethered virions was frequently significantly larger than 17 nm (Fitzpatrick et al., 2010; Hammonds et al., 2010; Perez-Caballero et al., 2009). Also, some of the Vpu-defective particles appeared to be connected by a stalk rather than having the appearance of being fully detached virions (Habermann et al., 2010; Hammonds et al., 2010; Klimkait et al., 1990). While these data do not rule out direct BST-2 mediated tethering of viral and/or cellular membranes located less than 17 nm apart as originally proposed by Neil et al. (Neil et al., 2008), tethering of virions across distances greater than 17 nm cannot be explained by such a mechanism. In that case it is possible that virions are not fully detached from the plasma membrane or from each other but remain connected via a membrane stalk. Such a membrane stalk could be lined with multiple BST-2 molecules that interact via coil-coil interactions thereby stabilizing the membrane stalk. In support of such a model, several EM studies noted short stalks connecting virions to the plasma membrane (Habermann et al., 2010; Klimkait et al., 1990) or observed long filamentous structures decorated with BST-2 (Hammonds et al., 2010). A stalk model would be consistent with the noted sensitivity of tethered virions to physical shearing or protease treatment since such treatments would destabilize BST-2-supported membrane stalks resulting in the detachment of the virions.

8. How does Vpu counteract the BST-2 imposed restriction of virus release?

It is generally accepted that Vpu regulates the detachment of otherwise complete and infectious virions from the cell surface (Klimkait et al., 1990; Neil et al., 2006). This effect of Vpu is not limited to HIV-1 but was shown to affect other retroviruses such as HIV-2, visna virus, Moloney murine leukemia virus, and XMRV, as well as unrelated enveloped viruses such as Ebola (Bour et al., 1996; Gottlinger et al., 1993; Groom et al., 2010; Jouvenet et al., 2009; Kaletsky et al., 2009; Neil et al., 2007). As noted above, a number of models have been proposed over the years to explain how Vpu enhances virus release. However, the identification

of BST-2 has, at least temporarily, sidelined earlier models of Vpu function and focused the Vpu field on the functional inactivation of BST-2 by Vpu.

Vpu and BST-2 are both integral membrane proteins albeit with different membrane topologies. BST-2 has a short N-terminal cytoplasmic domain with the bulk of the protein comprising the C-terminal ectodomain. Vpu, on the other hand has virtually no ectodomain and essentially consists of an N-terminal TM domain and a C-terminal cytoplasmic domain (Figure 3). Recent data suggest that the BST-2 TM domain is critical for interference by Vpu (Douglas et al., 2009; Gupta et al., 2009a; McNatt et al., 2009; Mitchell et al., 2009; Perez-Caballero et al., 2009; Rong et al., 2009) consistent with our previous observation of the importance of the Vpu TM domain for the regulation of virus release (Schubert et al., 1996a). Indeed, physical interaction of Vpu and BST-2 and the critical importance of the BST-2 TM domain for this interaction was demonstrated in the course of co-immunoprecipitation studies (Douglas et al., 2009; Dube et al., 2010; Gupta et al., 2009a; Iwabu et al., 2009; Jia et al., 2009; McNatt et al., 2009; Rong et al., 2009). The exact cellular site where Vpu targets BST-2 remains unknown. Infection of cells by wt virus results in the redistribution of BST-2 from the plasma membrane to early endosomes (Habermann et al., 2010; Neil et al., 2006). This involves internalization of BST-2 through clathrin-dependent endocytosis although there is an ongoing debate whether this involves the AP-2 adapter complex (Mitchell et al., 2009; Rollason et al., 2007) or a direct interaction with α -adaptin (Masuyama et al., 2009). As for Vpu, the protein is predominantly localized to membranes of the Golgi and TGN (Klimkait et al., 1990; Schubert et al., 1996a; Varthakavi et al., 2006) and was found to colocalize with BST-2 in endosomes and the TGN (Douglas et al., 2009; Dube et al., 2009; Neil et al., 2008; Rollason et al., 2007; Van Damme et al., 2008). Furthermore, mutations affecting TGN localization of Vpu were unable to antagonize BST-2 suggesting that Vpu targets BST-2 in this compartment (Dube et al., 2009). However, Vpu was also identified at the cell surface (Bour et al., 1999a) and a direct effect of Vpu on cell surface BST-2 cannot be ruled out. In fact, one recent study proposed that Vpu targets BST-2 at the plasma membrane (Iwabu et al., 2009).

It is widely accepted that Vpu downregulates BST-2 from the cell surface and that the reduced surface expression of BST-2 accounts for the increased virus release (Douglas et al., 2009; Habermann et al., 2010; Le Tortorec and Neil, 2009; Mitchell et al., 2009; Miyagi et al., 2009; Pardieu et al., 2010; Sato et al., 2009; Schindler et al., 2010; Van Damme et al., 2008). Interestingly, Vpu does not seem to increase the rate of BST-2 internalization (Dube et al., 2010; Mitchell et al., 2009). This suggests that Vpu may affect resupply or surface delivery of BST-2, a function that could be exerted from an intracellular location such as the trans-Golgi compartment. Vpu was also found to reduce total cellular levels of endogenous as well as exogenously expressed BST-2 (Bartee et al., 2006; Douglas et al., 2009; Goffinet et al., 2009; Mangeat et al., 2009; Mitchell et al., 2009; Miyagi et al., 2009). How this is accomplished is still under debate and it is not clear yet whether the reduced BST-2 levels are a cause or consequence of BST-2 surface downmodulation. Several studies reported the involvement of a proteasomal degradation pathway (Goffinet et al., 2009) and suggested β -TrCP dependence (Mangeat et al., 2009). In contrast, other studies reported a β -TrCP-dependent endo-lysosomal pathway to be important for degradation (Douglas et al., 2009; Iwabu et al., 2009; Mitchell et al., 2009). The involvement of β -TrCP in the virus release activity of Vpu necessitates conservation of Vpu's TrCP binding motif. However, mutation of this motif was previously found to only partially affect Vpu's virus release activity (Schubert et al., 1995; Schubert and Strebel, 1994; Van Damme et al., 2008) and in more recent studies expression of a TrCP-binding mutant of Vpu (Vpu2/6) supported HIV replication with wild type kinetics in a variety of cell types including PBMC (Miyagi et al., 2009). Expression of the TrCP binding-deficient Vpu2/6 mutant had no effect on total cellular BST-2 levels and appeared to stabilize or even increase BST-2 surface expression (Miyagi et al., 2009). Importantly, degradation of BST-2 was not essential for Vpu to enhance virus release (Goffinet et al., 2010; Miyagi et al., 2009).

suggesting it may be a downstream consequence of the Vpu-induced BST-2 surface downmodulation. Clearly, more work needs to be done to sort out, which of the effects of Vpu on BST-2, i.e. surface expression, degradation, or intracellular sequestration, are critical for the enhancement of virus release.

9. Other viruses encode Vpu-like factors

The effects of BST-2 are not restricted to lentiviruses but were shown to affect the release of other retroviruses, including alpha-, beta-, delta-, spumaviruses as well as , and lentiviruses, filoviruses (Ebola, Marburg), herpesviruses (KSHV), and arenaviruses (Gottlinger et al., 1993; Jouvenet et al., 2009; Kaletsky et al., 2009; Mansouri et al., 2009; Sakuma et al., 2009). Furthermore, inhibition of HIV-1 virus release is not limited to human BST-2 but was observed for mouse, rat, and monkey BST-2 (Goffinet et al., 2009; McNatt et al., 2009). However, only human BST-2 is sensitive to Vpu.

Viral proteins other than Vpu have been shown to affect BST-2 restricted virus release. For instance the Env proteins of certain HIV-2 isolates and at least one R5-tropic HIV-1 isolate can functionally replace Vpu for efficient virus release (Abada et al., 2005; Bour et al., 1996; Bour and Strebel, 1996; Bour et al., 1999b; Noble et al., 2006; Ritter et al., 1996; Schubert et al., 1999). More recently, the Nef proteins encoded by certain SIV isolates were associated with a Vpu-like activity (Jia et al., 2009; Sauter et al., 2009; Yang et al., 2010; Zhang et al., 2009). Interestingly, the ability of HIV-2 Env and SIV Nef to antagonize BST-2 appears to involve cell surface downmodulation of BST-2 (Douglas et al., 2009; Gupta et al., 2009b; Jia et al., 2009; Le Tortorec and Neil, 2009; Yang et al., 2010); however, unlike Vpu, neither Nef nor HIV-2 Env seem to affect the stability of BST-2 (Douglas et al., 2009; Gupta et al., 2009b; Jia et al., 2009; Le Tortorec and Neil, 2009; Yang et al., 2010). Also, while Vpu interacts with BST-2 through its TM domain, HIV-2 and SIVtan Env presumably interact with BST-2 through their ectodomains and Nef was found to interact with the BST-2 cytoplasmic domain (Figure 3) (Gupta et al., 2009b; Jia et al., 2009; Le Tortorec and Neil, 2009; Yang et al., 2010; Zhang et al., 2009). This is consistent with our previous finding that the Vpu-like activity of HIV-2 Env can be controlled by a single amino acid change in its ectodomain (Bour et al., 2003).

Aside from these retroviral Vpu-like factors, the K5 protein of Kaposi sarcoma-associated herpesvirus (KSHV) and the Ebola glycoprotein (GP) were shown to downregulate BST-2 and to enhance virus release (Bartee et al., 2006; Kaletsky et al., 2009; Lopez et al., 2010; Mansouri et al., 2009; Neil et al., 2007; Pardieu et al., 2010). Functionally, the Ebola GP has much in common with the other BST-2 antagonists already discussed. For instance, Ebola GP physically interacts with BST-2 (Kaletsky et al., 2009) but, like HIV-2 and SIVtan Env does not affect steady state levels of BST-2 (Kaletsky et al., 2009). Unlike Vpu, Ebola GP is broadly active and able to overcome the inhibitory effects of multiple tetherins, including an artificial tetherin molecule (Lopez et al., 2010). K5 mediated reduction of BST-2 levels, on the other hand, involves ubiquitination of lysine 18 in the BST-2 cytoplasmic domain followed by endosomal degradation (Mansouri et al., 2009; Pardieu et al., 2010).

10. Is BST-2 a viral restriction factor?

A final point concerns the question of whether or not BST-2 deserves the label of viral restriction factor. It is true that BST-2 can potentially inhibit the release of viral particles, especially if the protein is over-expressed. Also, the fact that multiple viral proteins such as HIV-1 Vpu, HIV-2 Env, SIV Nef, or KSHV K5 have evolved to target BST-2 could suggest that this protein presents a problem that these viruses need to control. On the other hand, one could argue that BST-2 does not so much represent a threat to virus spread as it offers an opportunity for the virus to control its mode of transmission. There are several arguments to

support such a view. First of all, BST-2 does not actually restrict replication of Vpu-deficient HIV-1 but simply shifts virus spread from a cell-free to a cell-to-cell mode (Schubert et al., 1995). Indeed, Vpu-deficient HIV-1 replicates in tissue culture with the same kinetics as wild type virus except that the levels of cell-free virus are lower (Friborg et al., 1995b; Klimkait et al., 1990; Strebel et al., 1988; Terwilliger et al., 1989). It is also noteworthy that the effect of Vpu on virus replication in human PBMC is quite modest consistent with the low BST-2 levels in these cells (Miyagi et al., 2009; Schindler et al., 2010; Schubert et al., 1995). The effect of BST-2 on virus secretion from monocyte-derived macrophages is more pronounced (Schindler et al., 2010; Schubert et al., 1995). However, even in these cultures Vpu-deficient HIV-1 was able to spread via cell-cell mode (Schubert et al., 1995). Another reason to believe that viruses can use BST-2 to their advantage is that some HIV-1 isolates, including the ADA clone AD8 (Theodore et al., 1996) or the LAV Mal (Genbank accession A07116) and Yu2 isolates (Genbank accession HIVYU2X) carry a point mutation in the vpu initiation codon that disables expression of Vpu. Vpu expression in these isolates can easily be restored by a single nucleotide change. Similarly, the HIV-2 Env protein has the ability to turn its Vpu-like activity on or off through a single point mutation in the TM subunit (Abada et al., 2005; Bour et al., 2003). This allows the virus to adapt to changes in the host milieu by switching between cell-free and cell-to-cell modes of transmission. Based on current knowledge we would argue that BST-2 is not a viral restriction factor in the sense that it limits virus spread. Rather, BST-2 is a modulator that affects the mode of viral transmission and may, in fact, help the virus avoid the host's immune system. However, this issue may have to be revisited in light of a recent report suggesting that BST-2 restricts not only virus release but also cell-to-cell transmission of Vpu-deficient HIV-1 at least in a short term single-round assay (Casartelli et al., 2010).

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Figure 1.

Vpu secondary structure models as deduced from available NMR and modeling data. Two separate models are proposed. Model (I) is based on data presented in references (Park et al., 2003; Park and Opella, 2005; Sharpe et al., 2006). Model II is based on data presented in reference (Lemaitre et al., 2006). There is general consensus that the Vpu transmembrane helix is tilted. In model I, the two cytoplasmic helices are aligned with the lipid bilayer with the two phospho-serine residues protruding into the cytoplasm. Model II proposes that the two cytoplasmic helices are oriented almost parallel to each other.

Figure 2. Vpu-mediated proteasomal degradation of CD4

Vpu acts as an adaptor to connect the CD4 cytoplasmic domain to an (SCF^{TrCP}) E3 complex. Binding of Vpu to CD4 involves membrane-proximal α -helices and possibly the TM domains as well. Interaction of Vpu with the (SCF^{TrCP}) E3 complex is dependent on two conserved phosphoserine residues located in an unstructured region between the two cytoplasmic helices. This results in the ubiquitination of lysine residues in the cytoplasmic tail of CD4 and subsequent degradation by proteasome complexes. Heavy red zigzag line represents the interaction domains in the Vpu and CD4 cytoplasmic domains. Thin zigzag line represents the proposed interaction of Vpu and CD4 through their TM domains. TMD: transmembrane domain

Figure 3.

Interaction of BST-2 with HIV-1 proteins. Vpu interacts with BST-2 through its transmembrane domain. The interaction of HIV-2 Env with BST-2 has not been mapped in detail but involves the ectodomain. Interaction of BST-2 with Nef involves a G/DDIWK motif in the cytoplasmic domain. This motif is absent in Nef-insensitive BST-2 variants (including human BST-2). Proposed interaction points are indicated by red zig-zag lines. Myr: Nef myristoylation; GPI: glycosyl-phosphatidylinositol anchor; TMD: transmembrane domain