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Review

HIV-1 Vpu — an ion channel in search of a job☆

Klaus Strebel *

Viral Biochemistry Section, Laboratory of Molecular Microbiology, NIAID, NIH Bldg. 4, Room 310, 4 Center Drive MSC 0460, Bethesda, MD 20892-0460, USA

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ABSTRACT

Vpu is a small membrane protein encoded by HIV-1 and some SIV isolates. The protein is best known for its ability to degrade CD4 and to enhance the release of progeny virions from infected cells. However, Vpu also promotes host-cell apoptosis by deregulating the NFκB signaling pathway and it assembles into cation-conducting membrane pores. This review summarizes our current understanding of these various functions of Vpu with particular emphasis on recent progress in the Vpu field. This article is part of a Special Issue entitled: Viral Membrane Proteins — Channels for Cellular Networking.

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Contents

1. Introduction

Viruses face a variety of obstacles when infecting a new host. Of particular importance is the host innate immune system that can form a potent barrier to viral infections. Viruses have adapted to this challenge in a variety of ways. Primate lentiviruses, including HIV-1, have acquired several genes whose primary if not exclusive function is to neutralize host innate immune defense mechanisms (for review see Refs. [1,2]). Vpu is an accessory protein unique to HIV-1 and its predecessor SIV strains. The initial functional property attributed to Vpu was to facilitate virus release from infected cells [3,4]. It became apparent early on that this function of Vpu was host cell-dependent [5,6], suggesting the involvement of host cell factor(s). This observation was further supported by experiments involving heterokaryon analyses [7]. However, not until 20 years after the initial

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* Tel.: +1 301 496 3132; fax: +1 301 402 0226.
E-mail address: kstrebel@nih.gov.

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discovery of Vpu and its effect on virus release was the involvement of BST-2 unraveled [8,9]. Prior to the identification of BST-2, different functional models of how Vpu regulates virus release had been proposed. These included a correlation of enhanced virus release with a Vpu ion channel activity or with the inactivation of a cellular background channel, TASK-1 [10–12]. However, the identification of BST-2 as an interferon-inducible host factor whose expression induced a severe but Vpu-sensitive restriction of virus release caused a major shift in research focus and made the earlier models fade into the background. It is fair to say that the identification of BST-2 and its effect on virus release has been a major advance in the field and investigating the role of Vpu in the antagonism of BST-2 is currently the most active aspect of Vpu-related research. Nevertheless, one should not ignore the fact that Vpu controls additional functions. These include degradation of the HIV-receptor CD4 or the inhibition of NFκB activation. These functions of Vpu are mechanistically relatively well understood although their functional significance for HIV replication is still under discussion. The purpose of this review is to provide a brief update on our current understanding of the role of Vpu in regulating virus release and to discuss its other known functions and their potential impact on virus replication

2. Functional properties of HIV-1 Vpu

2.1. Vpu induces degradation of CD4

The Vpu open reading frame is located upstream of and overlapping the viral *env* gene. Interestingly, Vpu and the envelope precursor glycoprotein, gpl60, are expressed from a single bicistronic mRNA while all other HIV products are produced from a complex pattern of differentially spliced mRNAs or via the proteolytic processing of the Gag and Gag-Pol polyproteins. This unusual utilization of viral transcripts suggested a requirement for the coordinate action of Vpu and Env. Indeed, it was known at the time that CD4, the receptor for HIV-1 had a propensity to interact with gpl60 in the endoplasmic reticulum (ER), resulting in the accumulation of trafficking-incompetent complexes of CD4 and Env in the ER [13–15]. This led to the investigation of a possible role of Vpu in the dissociation of such Env-CD4 complexes and the subsequent identification of Vpu-induced degradation of CD4 [16,17]. Indeed, in Vpu-expressing cells, *de novo* synthesized CD4 is rapidly degraded in the ER reducing its normal half-life of about 6 h to approximately 15 min (for review see Ref. [18]). It is well accepted that ER-associated degradation of CD4 by Vpu requires a physical interaction of the cytoplasmic domains of the two proteins that in CD4 has been mapped to a short membrane-proximal motif [19–23] and in Vpu was more recently shown to involve both cytoplasmic α-helices [24] (Fig. 1A). Some groups reported an additional contribution of the transmembrane (TM) domains of CD4 and Vpu as prerequisite for CD4 degradation [25–27]. Of particular interest is the observation that a conserved tryptophan residue (W22) in the Vpu TM domain, when mutated to leucine (W22L) did not prevent Vpu–CD4 interaction but almost completely abolished Vpu-induced degradation of CD4 [27]. This finding is intriguing as it implies a contribution of the Vpu TM domain to CD4 degradation that goes beyond the mere stabilization of Vpu–CD4 complexes. Other reports, however, demonstrated that replacing the TM domain of CD4 by that of CD8 [28] or scrambling the sequence of the Vpu TM domain, which included a repositioning of W22 [29] had no impact on the ability of Vpu to induce CD4 degradation. Furthermore, replacing the entire Vpu TM domain by TM domains from heterologous transmembrane proteins did not affect the ability of Vpu to induce CD4 degradation ([30] and Strebel, unpublished). It is unclear why an individual amino acid change in the Vpu TM domain has a more drastic effect than global replacement of the entire TM domain in either Vpu or CD4. However, structural changes in the membrane helix are a likely cause. It is conceivable that relatively non-specific interactions between the hydrophobic membrane helices of CD4 and Vpu or its chimeras stabilize Vpu–CD4 interactions and contribute to Vpu-induced CD4 degradation.

Aside from sequences required for the physical interaction of Vpu and CD4, a conserved di-phosphoserine motif located between the two cytoplasmic α-helices in Vpu is critical for the assembly of a β-TrCP-containing SKP1, Cullin, F-box protein (SCF) E3 ubiquitin ligase complex (SCF^{TrCP}) [31] that ultimately leads to the ubiquitination of CD4 followed by proteasomal degradation (reviewed in [18,32] (Fig. 1A). The role of the Vpu phosphoserine residues in the induction of CD4 degradation became apparent when yeast two-hybrid assays as well as coimmunoprecipitation studies revealed an interaction of Vpu with human β-TrCP [31]. The interaction of Vpu with β-TrCP leads to the assembly of a ubiquitin ligase complex that targets the cytoplasmic domain of CD4 [31,33,34]. Interestingly, Vpu itself is not ubiquitinated in the process and therefore assumes the role of an inert adapter molecule. This characteristic of Vpu likely accounts for the effect of Vpu on NFκB deregulation described below. The cytoplasmic domain of CD4 contains four lysine residues as potential targets for ubiquitination. Interestingly, mutation of all four lysines neither abolished Vpu-induced CD4 ubiquitination nor did it prevent Vpu-induced degradation [33]. An explanation for this phenomenon may be provided by the recent observation that degradation of CD4 by Vpu may involve not only ubiquitination of lysines but also serine/threonine residues in its cytoplasmic domain [34].

2.2. Vpu regulates detachment of virions

2.2.1. BST-2 inhibits virus release

Recent efforts in the Vpu field have almost exclusively focused on understanding its role in regulating virus release. It is now generally accepted that the regulation of virus release by Vpu involves the functional inactivation of BST-2 (also known as CD317, HM1.24, or tetherin). BST-2 was originally identified as a cellular transmembrane protein in terminally differentiated human B cells of patients with multiple myeloma [35,36] and was later identified as the host factor responsible for the Vpu-sensitive inhibition of virus particle release [8,9]. BST-2 was identified as a target for Vpu already two years earlier in a proteomics study that also identified the Kaposi sarcoma-associated herpesvirus K5 protein as a BST-2 antagonist. In fact, both Vpu and K5 when overexpressed in HeLa cells reduced the levels of endogenous BST-2 [37].

2.2.2. Structural considerations

BST-2 is a 30–36 kDa type II transmembrane glycoprotein consisting of 180 amino acids [38]. Rat BST-2 is predicted to have an N-terminal transmembrane (TM) region and a C-terminal glycosylphosphatidylinositol (GPI) anchor [39]. However, recent evidence suggests that the C-terminal membrane anchor, at least in human BST-2, could be a second transmembrane domain rather than a GPI anchor [40]. The two TM domains are separated by approximately 120 residues that constitute the protein's ectodomain, which is predicted to form a rod-like α-helical structure of about 16–17 nm [41–44]. The BST-2 ectodomain contains two N-linked carbohydrates [35,39,45,46] whose functional importance is not clear [45,46]. Three cysteine residues in the BST-2 ectodomain contribute to the formation of covalently linked dimers [35,36,45,46]. Interestingly, only one cysteine residue is necessary and any one of the three cysteine residues is sufficient for the assembly of BST-2 into functional covalently linked dimers [45,46].

A current model suggests that BST-2 tethers mature virions to the cell surface by means of its N- and C-terminal TM domains. Interestingly, artificial BST-2 consisting of the N-terminal TM region of transferrin receptor, a coiled-coil ectodomain of the cytoplasmic dimeric protein dystrophia myotonica protein kinase, and a GPI anchor signal derived from urokinase plasminogen activator receptor was capable of inhibiting the release of HIV-1 virions tethered to the cell surface

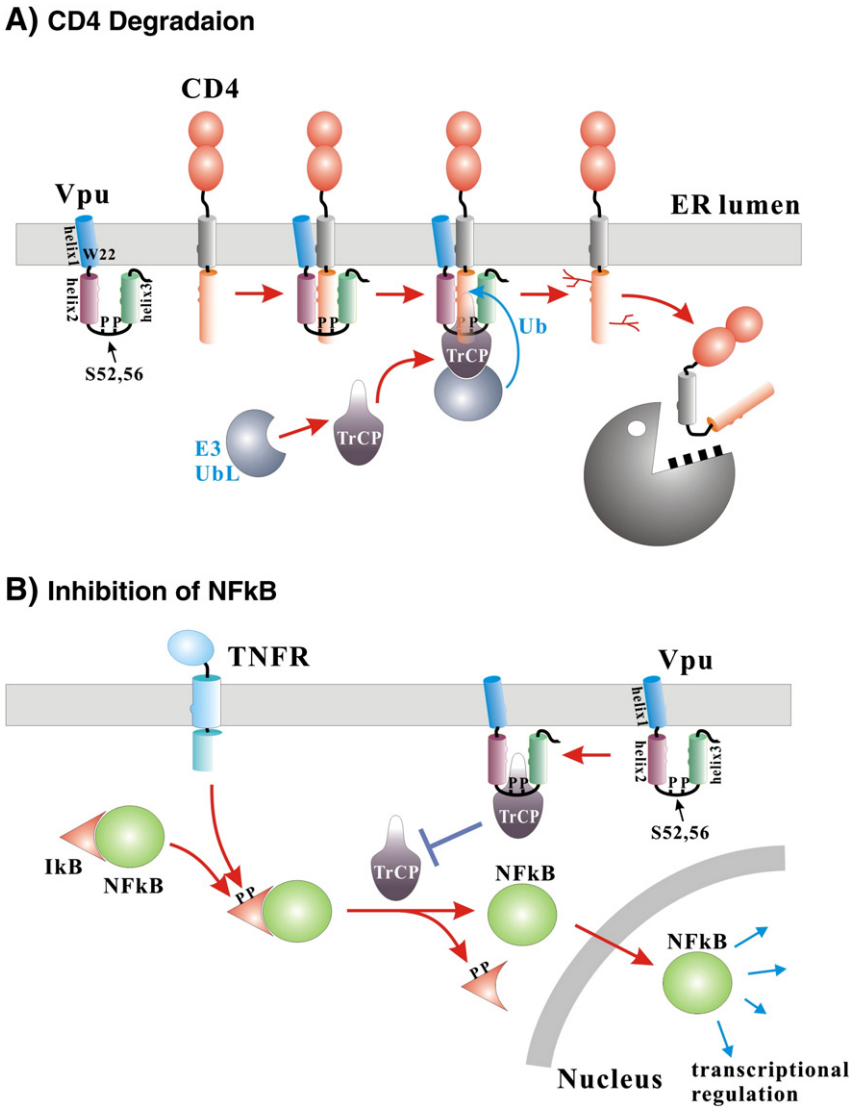

Fig. 1. CD4 degradation and deregulation of the NFkB pathway involve Vpu-β-TrCP interactions. (A) Vpu can bind newly synthesized CD4 in the endoplasmic reticulum (ER) through interactions mediated by the cytoplasmic helices as well as the transmembrane (TM) helices. The role of W22 in the Vpu TM helix is discussed in the text (Section 2.1.). Vpu is constitutively phosphorylated at serines 52 and 56. This motif constitutes a binding motif for β-TrCP and leads to the assembly of an E3–Ub ligase complex that results in ubiquitination and proteasomal degradation of CD4. (B) NFkB resides in the cytoplasm in an inactive form bound to its inhibitor IkB. Upon receptor stimulation, IkB is phosphorylated and degraded following β-TrCP induced ubiquitination. This releases NFkB, which then traffics to the nucleus and activates gene transcription. Deregulation of the NFkB pathway by Vpu is due to the competitive binding of β-TrCP. Unlike IκB, Vpu is not degraded upon binding to β-TrCP. This leads to a depletion of free β-TrCP in the cell and inhibition of NFkB activation following receptor stimulation.

[46]. This suggests that secondary structure rather than primary amino acid sequence of BST-2 is important for tethering function. This conclusion is supported by the observation that alanine scanning across much of the ectodomain failed to affect BST-2 function [47] and there is significant flexibility in the size of the BST-2 ectodomain [48]. Extending or substituting the BST-2 ectodomain using a heterologous coiled-coil motif maintained tethering function [48]. In fact, adding heterologous coiled-coil sequences to the existing BST-2 coil-coil thereby almost doubling the size of the BST-2 ectodomain had no impact on BST-2 function [48]. Conversely, approximately half of the ectodomain, including a block of seven heptad motifs believed to be important for coiled-coil formation, could be deleted without losing tethering function. Taken together, these data indicate that the size and primary sequence of the BST-2 ectodomain can be altered to a significant degree without loss of function.

Electron microscopic images not only revealed *vpu*-defective virions tethered to the cell surface; in many instances, virus particles appear to be tethered to each other [8,49,50]. If BST-2 were responsible for the formation of virus aggregates, it would be expected that BST-2 is present in such virions. Indeed, defective BST-2 variants lacking either N- or C-terminal TM domain were readily detected in cell-free virus preparations [46] suggesting that BST-2 is not actively excluded from virions. Interestingly, immune-electron microscopy identified the presence of wild type BST-2 in both wild type and Vpu-defective virions [51,52] and similar results were obtained using a bead-based virion capture assay [52]. Furthermore, immunoblot analysis of concentrated virus preparations revealed the presence of BST-2 in both wild type and Vpu-defective virions. Indeed, Vpu-defective virions contained significantly higher levels of BST-2 than wild type virus, when normalized for capsid levels [52]. This result is surprising if one accepts the popular tethering model of BST-2, which proposes that BST-2 physically tethers virions to the virus-producing cell. If one assumes that the association of BST-2 with nascent virions follows a Gaussian distribution model, one might predict that even in the absence of Vpu, a certain fraction of virions will be spontaneously released due to insufficient levels of BST-2 associated with these virions.

Thus, the level of BST-2 in spontaneously released Vpu-defective virions should be lower than or equal to that observed in wild type virions but certainly not higher. Consistent with that we found that preparations of *vpu*-defective virions that had been removed from BST-2 positive HeLa cells by physical force lacked detectable levels of BST-2 [53]. While the reasons for this apparent discrepancy remain unclear, it is fair to say that endogenous wild type BST-2 is packaged into both wild type and Vpu-defective HIV-1 virions at levels that are not inhibitory.

2.2.3. How does Vpu antagonize BST-2?

While Vpu expression ultimately leads to the cell-surface downmodulation of BST-2 [9,53–59], we found that in productively infected T cell cultures, Vpu can antagonize BST-2 to yield efficient virus release without apparent effect on its cell surface expression [53]. Similar observations were reported for the antagonism of BST-2 by the Ebola virus glycoprotein [60]. This raises the question of how it is possible that the tethering activity of BST-2 can be inhibited without removal of the protein from the cell surface. One possible solution is that Vpu and/or Ebola glycoprotein alter the surface distribution of BST-2, which is normally associated with membrane raft structures [39,40,60–64]. However, Lopez et al failed to see an effect of Vpu or Ebola glycoprotein on BST-2's distribution within lipid rafts [60]. BST-2 rapidly cycles between the cell surface and internal membranes [56,58,65] (Fig. 2; (1)); however, Vpu does not increase the rate of BST-2 internalization but rather seems to affect the resupply of newly synthesized BST-2 to the cell surface [65,66] (Fig. 2; (4)). Such a function 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 [37,53,56,57,67,68]. How exactly 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 [67] and suggested β-TrCP dependence [68]. In contrast, other studies reported a β-TrCP-dependent endo-lysosomal pathway to be important for degradation [56,57,59]. 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 [9,69,70] and in more recent studies expression of a TrCP-binding mutant of Vpu (Vpu26) supported HIV replication with wild type kinetics in a variety of cell types including PBMC [53]. Finally, expression of a β-TrCP binding-deficient Vpu26 mutant did not reduce total cellular BST-2 levels but, to the contrary, appeared to stabilize or even increase BST-2 surface expression [53]. This phenomenon could explain the partial effect of Vpu26 on virus release, which could be the result of increased levels of BST-2 rather than partial antagonism of BST-2 by Vpu26. Importantly, degradation of BST-2 was not essential for Vpu to enhance virus release [53,71] suggesting it may be a downstream consequence of the Vpu-induced BST-2 surface downmodulation.

2.2.5. BST-2: A host-restriction factor or a modulator of viral dissemination?

BST-2 can be neutralized by three different lentiviral proteins: HIV-1 employs Vpu, HIV-2 uses its Env glycoprotein, and SIV uses Nef. All three proteins are either integral membrane proteins (Vpu, Env) or are membrane-associated by means of a myristic acid modification (Nef) and are thought to interfere with BST-2 function via direct physical interaction [55–59,68,72–85]. While their molecular mechanisms of action may vary, the fact that lentiviruses have evolved three proteins to independently target BST-2 highlights the importance of controlling BST-2 function. It remains a matter of debate however, exactly why control of BST-2 is of such importance to HIV. The expression of BST-2 on human target cells has been credited with the limited transmission of HIV-1 from its predecessor SIV [86]. This is because Vpu from SIVcpz, while capable of degrading CD4, is unable to target chimpanzee or human BST-2 [75]. In these viruses, Nef has assumed the role of Vpu to enhance virus release. However, Nef cannot target human BST-2 because its target sequence (G/D)DIWK) located in the cytoplasmic domain of BST-2 has been lost in human BST-2 more than 800,000 years ago [87]. It is not quite clear how or even why Vpu acquired its anti-BST-2 activity. For instance HIV-2, which lacks a *vpu* gene, has learned to use its Env glycoprotein to antagonize human BST-2 [55,88–90]. Of note, even some HIV-1 isolates, such as the R5-tropic AD8 isolate, can make use of their

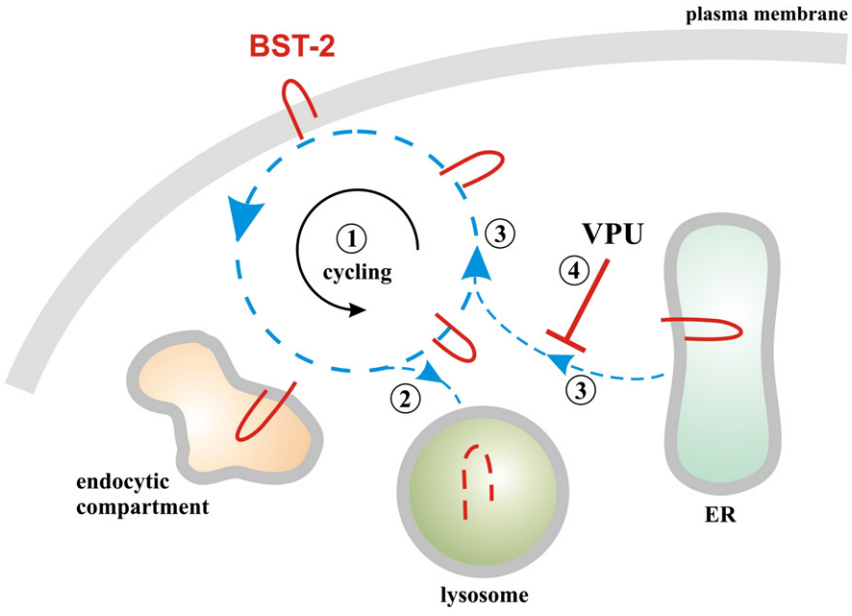

Fig. 2. Vpu antagonizes BST-2 by inhibiting the resupply of BST-2 from the ER. (1) Existing BST-2 rapidly cycles between the cell surface and endocytic compartments. During each cycle a fraction of the protein is routed to lysosomes for degradation (2). Steady state levels of surface BST-2 are maintained by resupply of degraded BST-2 from the endoplasmic reticulum (ER; (3)). Vpu interferes with the resupply of surface BST-2 presumably by redirecting BST-2 directly to the endocytic/lysosomal compartment (4).

Env protein to regulate virus release from human cells [91] raising the interesting question of whether following the initial zoonosis, the Env protein temporarily assumed Vpu-like function. At any rate, the ability to target BST-2 is not a feature of Vpu that arose only as a result of the transmission of SIV into humans. Indeed, Vpus encoded by SIVgsn, SIVmon, SIVmus, and SIVden are active against the BST-2 of their native hosts but not against human BST-2 [75,92].

Inhibition of virus release should not be mistaken for inhibition of virus replication. Even though in short-term analyses, BST-2 seemed to delay the cell-to-cell transmission of *vpu*-defective HIV-1 [93], and even though BST-2 potently inhibited replication of Mo-MLV in interferon-stimulated NIH 3T3 cells or in mice treated with polyinosinic:polycytidylic acid, no difference in plasma viremia was observed under physiological conditions between untreated normal or BST-2 knockout MoMLV-infected mice [94]. Also, in long-term multi-round replication analyses, the phenotype of a *vpu* defect in HIV-1 was evident by reduced levels of cell-free virus but not by reduced viral replication kinetics [3,4]. This indicates that *vpu*-defective virus can employ a cell-to-cell mode of transmission that, at least in tissue culture, is as effective as cell-free transmission by wild type virus. Thus, we prefer to look at BST-2 as a modulator of the mode of viral transmission (i.e. cell-free versus cell-to-cell) rather than a restriction factor inhibiting virus replication. If virus spread from person to person occurs through transmission of cell-free virus, viruses unable to target BST-2 will certainly have a selective disadvantage. On the other hand, the fact that a number of HIV-1 isolates, including HXB2, MAL, and AD8, carried mutations in the *vpu* translation initiation codon [95,96], suggests that Vpu expression may provide selective advantages or disadvantages and may be turned on or off depending on the environmental pressures provided by the host.

3. About ion channels and virus release

3.1. Vpu forms ion conductive membrane pores

Based on the structural similarity of Vpu with the influenza virus M2 ion channel protein it was speculated that homo-oligomeric complexes of Vpu might possess pore-forming abilities [97]. Indeed, Vpu ion channel activity was experimentally demonstrated as current fluctuations across an artificial lipid bilayer containing either full-length recombinant Vpu protein or synthetic peptides corresponding to the cytoplasmic domain of Vpu [10,11,98,99]. The Vpu channel activity is selective for monovalent cations such as sodium and potassium. Of note, the channel activity of the Vpu TM domain was specific as scrambling the sequence abolished channel activity [29]. This intriguing correlation between the ability of Vpu to form ion conductive channels and to enhance viral particle release *in vivo* led to the proposal that regulation of virus release by Vpu might involve an alteration of the plasma membrane potential. This model was supported by the observation that membrane depolarization could affect the rate of HIV-1 virus release [100] and that expression of Vpu in yeast caused membrane depolarization resulting in cytotoxicity and impaired cell growth, an effect that could be minimized by increasing extracellular K(+) concentration [101]. Additional support for the ion channel model of Vpu came from animal studies in which scrambling the Vpu TM domain in the context of a SHIV virus significantly reduced viral load and lowered viral pathogenicity [102]. Despite all of that, exactly how ion channel activity of Vpu might regulate viral particle production had remained unclear. Indeed, more recent studies argue against an involvement of Vpu ion channel activity in Vpu-enhanced virus release. These new findings are based on the observation that some mutations in the Vpu TM domain affecting channel activity (e.g. S23A or S23L) did not impair the ability to enhance virus release; conversely, some mutations affecting virus release (e.g. A14N, A18N) retained ion channel activity [54,103–105]. Thus, while there is no doubt that Vpu can assemble into ion conducting membrane pores, the functional significance of Vpu ion channel activity for HIV replication remains unclear.

3.2. Vpu inhibits the cellular ion channel TASK-1

A different model for Vpu function was proposed by a study suggesting that Vpu affects the activity of the mammalian background K(+) channel TASK-1 [12]. The N-terminal domain of TASK-1 exhibits significant structural homology to Vpu. Indeed, Vpu was found to interact with TASK-1 and coexpression of Vpu inhibited the TASK-1 ion channel activity [12]. Also, coexpression of TASK-1 with the N-terminal fragment exhibiting homology to Vpu (Ttm1) inhibited TASK-1 ion channel function. Interestingly, expression of Ttm1 increased release of Vpu-defective virus from HeLa cells to a similar extend than wt Vpu [12]. Thus, it was proposed that TASK-1 was a cellular inhibitor of virus release whose activity has to be neutralized by Vpu. If this were true, one would expect that TASK-1 is expressed in non-permissive cell types such as HeLa or Jurkat but is absent in permissive cells, e.g. Cos-7 or 293T. This has not been observed, however. Alternatively, one could argue that TASK-1 itself functions in a cell-type independent manner but indirectly regulates virus release by affecting BST-2 function. At this point, there is, however, no experimental evidence for such a function of TASK-1. Thus, based on current knowledge BST-2 is the sole contender for the role of regulator of viral particle release.

4. Other functions of Vpu

4.1. Vpu induces apoptosis by deregulating the NFkB pathway

As noted above (Section 2.1.), Vpu-induced degradation of CD4 involves the recruitment of β-TrCP via a conserved di-phosphoserine motif in the Vpu cytoplasmic domain. Interestingly, the recognition motif on all known cellular substrates of β-TrCP consists of a pair of conserved phospho-serine residues similar to those present in Vpu [31]. Serine-phosphorylation plays the major regulatory role in the stability of SCF target proteins. For example, activation of the IκB kinase complex (IKK) by external stimuli such as TNFα induces the serine-phosphorylation of IκBα followed by rapid TrCP-mediated proteasome degradation [106]. More relevant to Vpu, BST-2 was recently shown to activate NFκB in a Vpu-sensitive manner [107,108]. Intriguingly, Vpu differs from normal cellular substrate of β-TrCP by its resistance to proteasome degradation. Indeed, while the SCF^{TrCP} usually degrades the serine-phosphorylated protein directly bound to the β-TrCP WD domains (i.e. Vpu), CD4 – bound to the Vpu cytoplasmic domain – is degraded instead. This phenomenon has serious implications for the regulation and availability of the SCF^{TrCP} in cells that express Vpu. Indeed, due to the fact that Vpu is constitutively phosphorylated [109], binds β-TrCP with high affinity [31], and is not released from the complex by degradation [110], Vpu expression in HIV-infected cells was likely to perturb the physiological function of the SCF^{TrCP} through competitive trapping of β-TrCP (Fig. 1B). Indeed, the deregulation of IκB by Vpu was shown to lead to inhibition of both HIV- and TNF-α-induced activation of NFκB [110]. The deregulation of NFκB in Vpu expressing cells has far-reaching consequences since NFκB is a central transcription factor that regulates the expression of key cellular genes involved in cell proliferation, cytokine production and the induction of apoptosis [111,112]. Inhibition of NFκB activity by Vpu might therefore contribute to the induction of apoptosis in HIV-1 infected cells [113,114]. This was confirmed experimentally by showing that in a population of Jurkat cells expressing wild-type HIV-1, twice as many cells underwent apoptosis than in cells infected with a Vpu-defective virus [115]. Mechanistically, Vpu was shown to inhibit the NFκB-dependent expression of anti-apoptotic genes such as Bcl-2 family proteins, leading to enhanced intracellular levels of the apoptosis-promoting caspase-3 [115]. Active caspase-3 then triggers a reaction that results in the cleavage of a

number of target proteins including Bcl-2 family proteins and leads to cell death [115].

4. Conclusions

The HIV-1 Vpu protein is a small transmembrane protein with several independent functions that are controlled from different cellular compartment. Degradation of CD4 occurs in the endoplasmic reticulum and involves the ERAD pathway. Vpu's function is that of a molecular adaptor that connects CD4 to the SCF^{TrCP} E3 ubiquitin ligase complex ultimately resulting in the degradation of CD4. Sequences in the Vpu cytoplasmic domain are critical for this process but a specific contribution of the TM domain is being proposed as well. Enhanced virus release is accomplished by down-modulating BST-2 from the cell surface. The precise mechanism of BST-2 antagonism by Vpu is not yet understood. However, the Vpu TM domain is critical for this process to enable the physical association with BST-2. Finally, Vpu deregulates the NFκB pathway due to two unusual characteristics: (i) Vpu is constitutively phosphorylated and thus capable of continuously binding β-TrCP; (ii) unlike normal cellular substrates of β-TrCP, Vpu is not degraded as a result of β-TrCP engagement and is thus capable of depleting the cellular pool of free β-TrCP. Since β-TrCP is a critical regulator of the NFκB inhibitor IκB, depletion of free β-TrCP prevents NFκB activation. Structurally, Vpu assembles into homo-oligomeric complexes with the ability to form ion conductive membrane pores. While regulation of virus release by Vpu was initially attributed to ion channel activity, experimental evidence no longer supports such a model. It is therefore unclear at this point, which – if any – of the described functions of Vpu require oligomerization of Vpu. Thus, while nothing has changed on the fact that Vpu can form ion conducting membrane pores, its job description remains undefined.

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