

Membrane Potential Depolarization as a Triggering Mechanism for Vpu-Mediated HIV-1 Release

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ABSTRACT Vpu, a component unique to HIV-1, greatly enhances the efficiency of viral particle release by unclear mechanisms. This Vpu function is intrinsically linked to its channel-like structure, which enables it to interfere with homologous transmembrane structures in infected cells. Because Vpu interacts destructively with host background K⁺ channels that set the cell resting potential, we hypothesized that Vpu might trigger viral release by destabilizing the electric field across a budding membrane. Here, we found that the efficiency of Vpu-mediated viral release is inversely correlated with membrane potential polarization. By inhibiting the background K⁺ currents, Vpu dissipates the voltage constraint on viral particle discharge. As a proof of concept, we show that HIV-1 release can be accelerated by externally imposed depolarization alone. Our findings identify the trigger of Vpu-mediated release as a manifestation of the general principle of depolarization-stimulated exocytosis.

INTRODUCTION

One major distinction between HIV-1 and the less virulent HIV-2 is the unique presence of *vpu* in the former. The main function of Vpu is to expedite the spread of viral progeny (1). Vpu is a small, versatile protein that is capable of interacting with a wide range of host proteins, including β TrCP (2), UBP (3), CD74 (4), BST-2/tetherin (5,6), CAML (7), and background two-pore K⁺ (K_{2P}) channel TASK (8). BST-2/tetherin and TASK are capable of suppressing HIV-1 release by direct interaction with Vpu (5–8). BST-2/tetherin has been proposed to counteract Vpu-mediated release by retaining, or gluing, the viral membrane to the host plasma membrane after fission. However, BST-2/tetherin also inhibits viral release in diverse classes of viruses that lack Vpu, so the mechanism of Vpu-mediated release is likely complex and involves multiple cellular/physiological processes (9–13).

On the other hand, Vpu has been noted to modify host membrane permeability with its channel-like transmembrane domain (8,14,15). The function of Vpu is linked to its ability to enhance the rate of viral release (1,8,16). Among a handful of channel-like viral proteins, or viroporins, recognized up to date (17–21), murine hepatitis virus E protein and SARS coronavirus 3a protein are also capable of accelerating virus egress (20–22). However, the mechanistic link between their viroporin activities and their ability to enhance viral release remains unclear (21).

We previously found that Vpu interacts destructively with a ubiquitously expressed K_{2P} channel, TASK (8). TASK channels exhibit noninactivating, background K⁺ currents (23,24). TASK conductance limits the rate of Vpu-mediated viral release, whereas Vpu oligomerization with TASK

exerts a dominant-negative effect on the normal assembly and function of TASK channels. Of note, the first transmembrane segment of TASK-1 (Ttm1) alone is capable of destroying the normal assembly of TASK and enhancing HIV-1 release just like Vpu. The fact that these two distinct activities can be solely elicited from the expression of a unique transmembrane structure shared by both Vpu and Ttm1 suggests a coupled mechanism (8). Because K_{2P} channels set resting potentials in many types of cells (25), we sought to determine whether membrane potential stability is detrimental to viral particle release, in which case Vpu could promote viral release by destabilizing the cell membrane potential.

Here, we found that the efficiency of HIV-1 release is inversely correlated with membrane potential stability. Vpu functions as a membrane depolarizer to dissipate the restricting transmembrane voltage on particle budding/fission. As a proof of concept, we demonstrate that the rate of HIV-1 release can be modulated by membrane potential depolarization alone. It is conceivable that other viroporins that partake in the process of fission or viral egress likely utilize the same triggering mechanism.

MATERIALS AND METHODS

Plasmids

The plasmids for TASK-1, Vpu, and HERG (pCGI-TASK1, pCCI-Vpu, pAdC8I-Vpu, and pCGI-HERG) were described previously (8). CGI (GFPIRS), CCI, and AdC8I are bicistronic vectors carrying reporter genes of GFP, CFP, and CD8, respectively (26,27). The TASK-1 mutants were constructed based on pCGI-TASK1 (8) at R38A and/or R40A (National Center for Biotechnology Information accession No. AAC39952) with the use of a QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). The plasmid for pNL4-3/Udel is a Vpu-deficient variant of the HIV-1 proviral clone, pNL4-3 (28). Both the pNL4-3 and pNL4-3/Udel plasmids were generously provided by S. Bour (National Institutes of Health (NIH)).

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Cells and transfection

HeLa cells were maintained in Eagle's minimum essential medium supplemented with 1X nonessential amino acids, 10% fetal bovine serum, and 5- $\mu\text{g}/\text{mL}$ plasmocin (InvivoGen, San Diego, CA). HEK-293 and K562 cells were maintained in Dulbecco's modified Eagle's medium containing the same fetal bovine serum and antibiotics. For patch clamp of HEK-293 cells, transient transfection was performed with 0.4 μg of one of the bicistronic channel plasmids (pCGI-TASK, pCGI-TASK_{R38A}, pCGI-TASK_{R40A}, or pCGI-TASK_{AQA}) alone, or together with 0.4 μg of the bicistronic pAdC81-Vpu in a six-well plate. CD8-positive cells were identified by 5–10 min of immunostaining using anti-CD8 Quantum Red conjugate antibody (clone UCHT-4; Sigma, St. Louis, MO). TASK-Vpu coexpression was determined by both green and red fluorescence. For knockdown of endogenous background K⁺ channels, TASK1/3/5 siRNA was transfected into cells using LipofectAMINE PLUS or AMAXA reagents. The effectiveness of TASK1/3/5 siRNA was assessed based on its ability to depolarize cell membrane potentials in a K⁺-dependent fashion. The siRNA sequences were as follows:

5'-GUAGAACAUUGCAGAACACCUU-3' (TASK1/3/5 sense)
 5'-GGUGUUCUGCAUGUUCUACUU-3' (TASK1/3/5 antisense)
 5'-AGGUAGUGUAAUCGCCUUGUU-3' (control sense)
 5'-CAAGGCGAUUACACUACUUU-3' (control antisense)

Electrophysiology

Voltage-clamp experiments were performed on day 1–2-transfected cells at room temperature (~25°C). Gene expression was assessed by reporter GFP or CD8. Whole-cell voltage clamp was performed using an Axonpatch 200B patch-clamp amplifier (Axon Instruments, Sunnyvale, CA) and filtered at 2 or 5 kHz. Holding at 0 mV, the step protocol began from –100 to 80 mV in a 20-mV increment, with each step lasting 100 ms. The ramp protocol covered the same voltage range in 100 or 400 ms. The bath solution contained (in mM) NaCl, 140; KCl, 5; Ca(OH)₂, 2; Mg(OH)₂, 1; glucose, 10; and HEPES, 10 (pH 7.4). The internal pipette solution contained (in mM) NaCl, 5; KCl, 120; MgCl₂, 1; EGTA, 2; HEPES, 10; and Mg-ATP, 2.5 (pH 7.2). All buffers were 0.22 μm -filtered and monitored for osmolarity. Leak current was not subtracted. The currents recorded at each voltage step were averaged among the mid-40 ms and divided by individual cell capacitance to express current densities (pA/pF).

Single-channel recordings used patch pipettes of 20–30 M Ω pulled from 1.5 mm outer diameter quartz glass capillaries (model No. Q150-110-10; Sutter Instruments, Novato, CA). The seal resistances were 10–50 G Ω . Data were sampled at 50 kHz and low-pass filtered (four-pole Bessel, –3 dB at 5 kHz). The threshold for detecting channel openings was set at 50%. The number of channels per patch was determined by the maximum number of stacked openings in response to depolarization at 80 mV in long runs (>100 sweeps). I-V relationships were plotted using the mean peak values from amplitude histograms at different membrane potentials. The pipette and bath solutions contained (in mM) KCl, 120; NaCl, 5; MgCl₂, 1; EGTA, 2; HEPES, 10; and Mg-ATP, 2.5 (pH 7.2).

To compare the effects of TASK1/3/5 knockdown on the resting membrane potentials, transient transfection of the nonspecific siRNA or TASK1/3/5 siRNA was performed. For visual identification of the transfected cells, 0.05 μg of green fluorescent protein EGFP was coexpressed as a reporter. Measurements of the resting membrane potentials in transfected cells were obtained using the current-clamp mode of whole-cell patch clamp. Patch electrodes were prepared from 8161 Corning glass (Dow-Corning, Midland, MI). The resistance of the electrodes was <2 M Ω . Voltage was recorded and filtered at 1 kHz. The intracellular solution contained (in mM) KCl, 125; KOH, 25; CaCl₂, 1; MgCl₂, 1; K-ATP, 4; EGTA, 10; and HEPES, 10 (pH 7.2 with KOH). The external solution contained (mM): NaCl, 140; KCl, 5; Mg(OH)₂, 1; Ca(OH)₂, 2; glucose, 10; and HEPES, 10 (pH 7.35 with KOH).

High K⁺-induced depolarization induced by elevated extracellular [K⁺] was verified by DiBAC₄(5) or Di-8-ANEPPS staining, and the degrees of depolarization were quantified by flow cytometry (BD FACSCalibur). For experiments with HIV-1, the degrees of depolarization were quantified by a Typhoon Imager (Amersham, Piscataway, NJ). Data were analyzed with Student's unpaired *t*-test and expressed as the mean \pm SE.

Virus release assay

HeLa cells at 50% confluency in P25 flasks were transiently transfected with 2–3 μg of one of the HIV-1 proviral constructs (pNL4-3, pNL4-3/Udel) and 0.7 μg of a channel plasmid or 120 ng of the siRNA. One-tenth of the culture media was retrieved from each sample and 0.22- μm -filtered at each time point. Cells were collected at the end of the experiment and subjected to whole-cell lysis with 1% sodium dodecyl sulfate. The viral content in culture media and in cell lysates was determined by enzyme-linked immunosorbent assay of HIV-1 p24 antigen. Viral release efficiencies were compared with the control set (cotransfection of pNL4-3 and pCGI).

To assess the effects of externally imposed depolarization on viral release, we varied K⁺ concentrations ([K]_o = 5, 31, 65, 85, and 105 mM) in culture media by substituting Na⁺ with K⁺. The in-house-made media were 0.22- μm -filtered and tested for osmolarity consistency. On day 1 post-transfection, culture media were replaced with fresh media containing elevated [K⁺]. An aliquot of culture media was retrieved immediately after change of media (*t*₀). High K⁺ challenges lasted for a maximum of 2 h, and a fraction of the media was collected again (*t*₁). One-tenth of the media was retrieved and filtered at each time point. Viral release was compared with the control set (100%).

RESULTS AND DISCUSSION

Sequence analyses of the K_{2P} channel family revealed a patch of the conserved dibasic residues, R-X-R, immediately after the first transmembrane region (K. Hsu, J. Han, and E. Marban, unpublished) (Fig. 1). Several point mutations in this region of TASK-1 (R38A, R40A, and AQA (R38A and R40A)) resulted in different levels of protein expression and channel activities (Fig. S1 in the Supporting Material and Fig. 2 A). TASK_{R38A} had very low protein expression

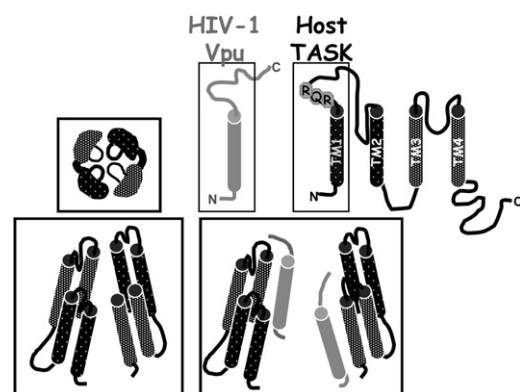


FIGURE 1 Scheme for the protein-protein interaction between HIV-1 Vpu and host TASK channels. Top: The primary structures of HIV-1 Vpu and the host TASK channel. The regions of their sequence homology are boxed. The conserved RRR residues in TASK-1 are marked in small circles. Bottom: A model for their protein-protein interaction. Left: TASK is a dimeric channel (shown *top* and *side* views). Right: Vpu probably interacts with its homologous region in TASK, resulting in destruction or dysfunction of the host channel.

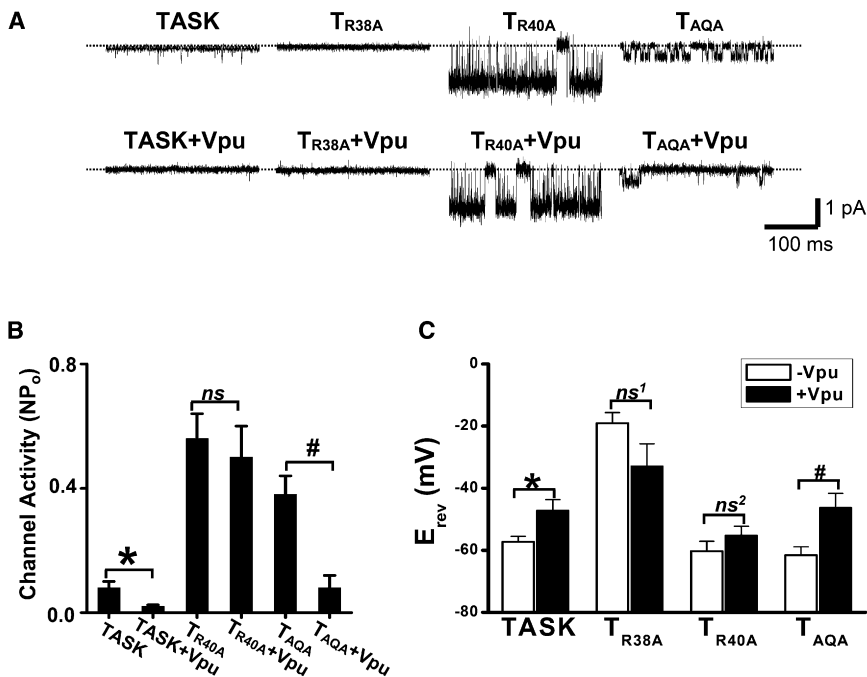


FIGURE 2 Vpu differentially affects the channel activities of TASK and its three functionally distinct point mutants. (A) Single-channel recordings of TASK and its mutants from inside-out patches in symmetrical 120 mM KCl at -40 mV. The dotted line indicates zero currents. (B) Channel activities (NP₀) were determined from individual inside-out patches. $*P < 0.05$ vs. TASK-1; $^{ns}P > 0.05$ vs. TASK_{R40A}; $^{\#}P < 0.05$ vs. TASK_{AQA}. Data are expressed as the mean \pm SE from 10–15 cells per group. (C) Vpu exerted different degrees of inhibition on the membrane potentials of the cells expressing different TASK mutants. Reversal membrane potentials in a Ringer-like solution were determined from whole-cell recordings using a ramp protocol. Vpu depolarized TASK and TASK_{AQA}-expressed cells ($*P = 0.01$ vs. TASK alone; $^{\#}P = 0.008$ vs. TASK_{AQA} alone), but not TASK_{R38A} or TASK_{R40A}-expressed cells ($^{ns1}P = 0.1$ vs. TASK_{R38A} alone; $^{ns2}P = 0.25$ vs. TASK_{R40A} alone). Nonfunctional TASK_{R38A} did not alter the cell membrane potentials. Data are expressed as the mean \pm SE from seven to 31 cells per group.

and thus did not conduct currents in the transfected cells, whereas TASK_{R40A} and TASK_{AQA} exhibited protein levels comparable to that of wild-type TASK. However, the latter two mutants showed functionally distinctive profiles. Therefore, we used these three mutants to test our hypothesis for the mechanism of Vpu-mediated release. These point mutations did not affect the expression of Vpu or the protein-protein oligomerization between TASK and Vpu in the heterologously expressed HEK-293 cells (Fig. S1). However, the interaction between Vpu and each mutant led to very different functional consequences. Both TASK_{R40A} and TASK_{AQA} exhibited fivefold higher single-channel activities than wild-type TASK, but Vpu inhibited only TASK_{AQA} (70–80% inhibition, the same as for the wild-type channel in Fig. 2 B). TASK_{R40A}, with a single-channel conductance threefold that of TASK_{AQA} or the wild-type, maintained its currents and the cell membrane potential upon Vpu coexpression (Fig. 2). TASK_{R40A} also outperformed TASK and TASK_{AQA} in restricting Vpu-mediated viral release (80% vs. 50% inhibition in Fig. 3). On the other hand, TASK_{R38A}, with very little protein expression (Fig. S1), did not exhibit any detectable channel activities (Fig. 2) or the ability to suppress Vpu-mediated release (Fig. 3). We observed that when TASK currents remained substantial, Vpu-assisted release was stagnant. There appears to be an inverse relationship between the magnitude of background K⁺ conductance and the efficiency of Vpu-mediated release. Conceivably, suppression of the stabilizing background K⁺ currents could unleash viral particle release.

The sustaining background K⁺ conductance stabilized cell membrane at the polarized potentials: TASK_{R40A} main-

tained cell membranes at -60.3 ± 3.2 mV ($-Vpu$) and -55.2 ± 2.9 mV ($+Vpu$). But for TASK-1 and TASK_{AQA}, their cell potentials were depolarized 10–20 mV from ~ -60 mV upon Vpu coexpression (Fig. 2 C). Nonetheless, their potentials remained more polarized than those cells bearing little or no background K⁺ conductance (i.e., -45 mV vs. -20 mV for TASK_{R38A}-expressing cells). These differences in membrane potentials ($\Delta\psi$) conceivably could account for the observed 50–60% release inhibition by TASK and TASK_{AQA}, and the 80% inhibition by TASK_{R40A} (Fig. 3). Nonconducting TASK_{R38A} did not

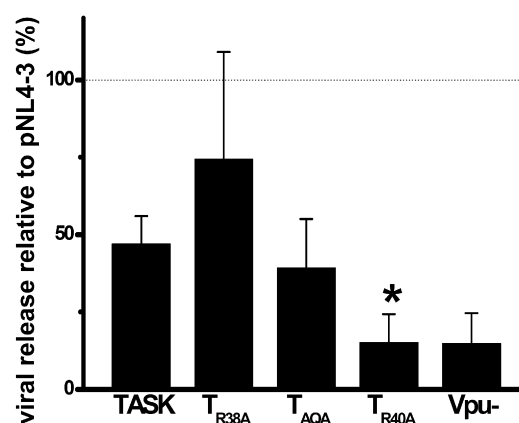


FIGURE 3 TASK and its three point mutants exert different degrees of inhibition on Vpu-mediated viral particle release. The effects of TASK and its mutants on particle release were compared with the single expression of HIV-1 proviral NL4-3 (set at 100%). TASK_{R40A} inhibited HIV-1 release more substantially than TASK-1 ($*P = 0.036$). Vpu- (NL4-3/Udel) represents the level of HIV-1 release lacking Vpu assistance. Data are expressed as the mean \pm SE from four to five independent experiments.

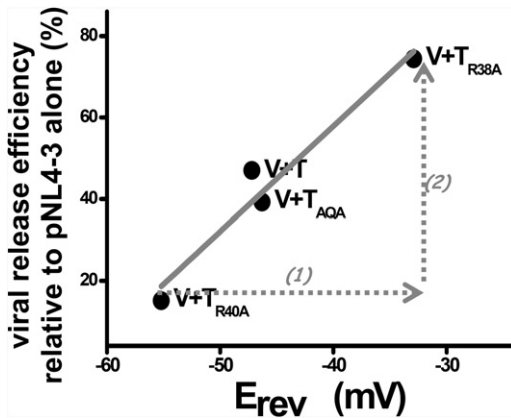


FIGURE 4 The efficiency of Vpu-mediated HIV-1 release is directly proportional to the degree of membrane potential depolarization. The plot combines data from Figs. 2 C and 3 and shows a direct relationship between residual cell potentials and HIV-1 release efficiencies. Each dot represents an averaged value. The observed relation can be dissected into two consecutive vectors: 1), membrane potential depolarization; and 2), release acceleration.

impose such a voltage barrier against virus discharge (Figs. 2 and 3). Thus, the efficiency of Vpu-mediate release was directly proportional to the degree of membrane potential depolarization (Fig. 4).

In various physiological processes, background K^+ conductance stabilizes the electrochemical membrane potential and hence reduces excitability and secretion (30–33). Our results suggest that the process of HIV-1 release was

similarly affected by $\Delta\psi$ (Fig. 4). To dissipate the restricting electric field across a budding membrane, Vpu probably acts like a membrane depolarizer that diminishes the stabilizing background K^+ currents. Indeed, when heterologously expressed in IL-2-activated, human CD4+ T cells, Vpu induced 15–20 mV of membrane potential depolarization. This Vpu activity functionally resembled that of TASK siRNA in dissipating the endogenous background K^+ conductance in T lymphocytes (34): both were capable of depolarizing transmembrane potentials and reducing membrane sensitivity to extracellular K^+ (Fig. 5). Others have shown that Vpu heterologously expressed in *Xenopus* oocytes also inhibits K^+ permeation (15). Thus, the function of Vpu is intrinsically linked to its propensity to oligomerize destructively with endogenous K^+ channel subunits such as TASK (8,17).

Because Vpu coexpression did not alter the unitary current amplitudes of TASK or its mutants (Fig. 2 A), its inhibitory activity did not involve the formation of dysfunctional K_{2P} -Vpu hybrid channels on the cell surface. Instead, Vpu probably inhibited by reducing the number of functional background K^+ channels on the plasma membrane, and this process likely took place intracellularly. Because Vpu is a connector to the ubiquitin-proteasome degradation pathway, it is capable of accelerating degradation of interfering host proteins and channels (2,8). In this way, Vpu, and perhaps other viroporins as well, could remotely enhance virus discharge on the plasma membrane while being predominantly localized in the perinuclear regions (21,35).

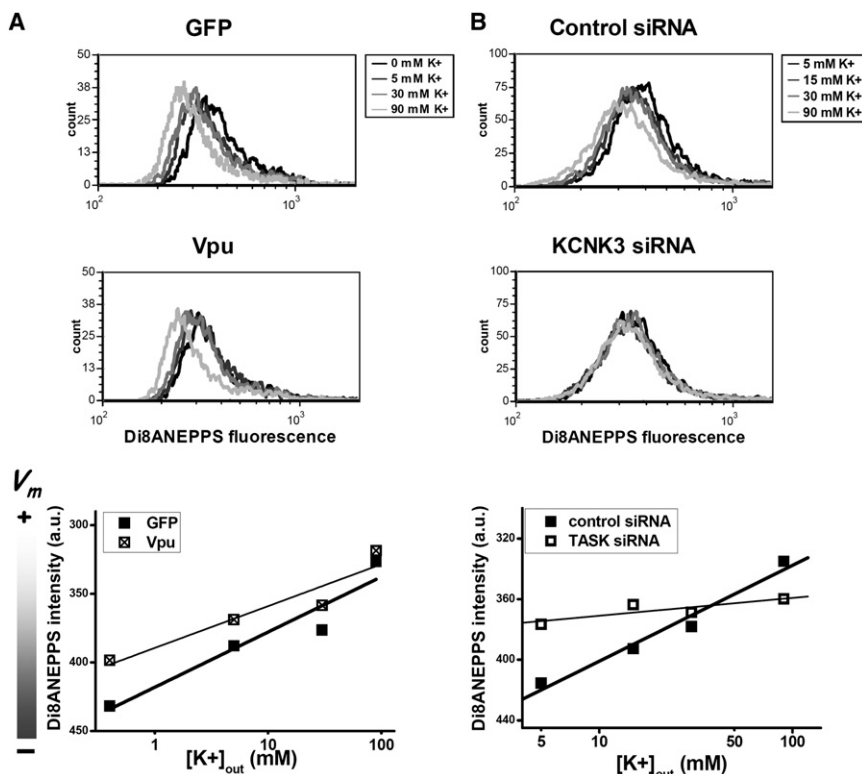


FIGURE 5 Knockdown of endogenous background K^+ channels by Vpu or TASK siRNA results in membrane potential depolarization in activated human CD4+ T cells. (A and B) Cell membrane potentials (V_m) in different extracellular $[K^+]$ were recorded with the polarization-sensitive fluorescent dye Di-8-ANEPPS. Plasmid- or siRNA-transfected cells were gated by GFP coexpression and their transmembrane potentials were resolved using other fluorescent channels. The Di-8-ANEPPS histograms from representative flow cytometry experiments are shown. There is a direct correlation between extracellular $[K^+]$ and membrane potential polarization in the control cells, indicating the presence of K^+ conductance. The correlation is significantly reduced in cells expressing Vpu (A) or TASK siRNA (B). The different slopes in A and B indicate that TASK siRNA imposes a stronger $\Delta\psi$ inhibition on the endogenous K^+ currents than Vpu.

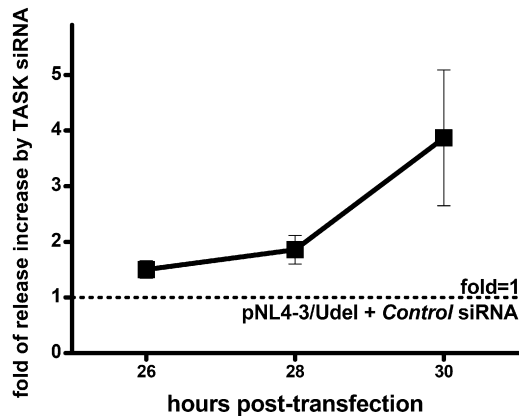


FIGURE 6 Knockdown of endogenous TASK channels in HeLa cells enhances HIV-1 release. Viral release efficiencies in the absence of Vpu expression (NL4-3/Udel) increased with treatment of TASK1/3/5 siRNA. Data are expressed as the mean \pm SE. The dashed line (fold = 1) refers to the control cells expressing both pNL4-3/Udel and control siRNA.

As a proof of concept, we verified the direct involvement of $\Delta\psi$ in HIV-1 release by manipulating membrane potentials. We adopted two methods to test the effects of depolarization on release. In the first approach, we depolarized cell potentials by knocking down the endogenous TASK channels in HeLa cells expressing *vpu*-inactivated HIV-1 (NL4-3/Udel), and found that this alone promoted viral release up to sevenfold (Fig. 6). Knockdown of endogenous TASK1/3/5 elevated the rates of virus release more effectively in the absence of Vpu than in the presence of Vpu. Since HIV-1 particles are heterogeneous, the induced particles by membrane potential depolarization were also examined for infectivity. The viral particles released from the cells treated with TASK1/3/5 siRNA showed infectivity comparable to the particles from the cells treated with the nonspecific siRNA (Fig. S2). Thus, depolarization-induced viral particles are functional. This result is reminiscent of an early finding that particles derived from *vpu*-inactivated HIV-1 NL4-3/Udel are as infectious as those derived from NL4-3 (28), reinforcing the notion that membrane potential depolarization by either TASK siRNA or Vpu primarily affects the final fission step and not the earlier steps (e.g., packaging).

In the second approach, we depolarized the cell membrane potential with high K^+ culture media and observed whether HIV-1 release could be accelerated accordingly (as dissected in the two consecutive vectors in Fig. 4 (1 \rightarrow 2)). Cell culture media were replaced with depolarizing high K^+ media 1 day after transfection with the HIV-1 proviral plasmids (NL4-3 versus NL4-3/Udel lacking *vpu* expression). In HeLa cells expressing NL4-3/Udel, high K^+ -imposed depolarization enhanced virus release up to sevenfold (Fig. 7). Depolarization-induced viral release was significantly less pronounced in the cells expressing complete HIV-1 (NL4-3). The high- K^+ effect was more evident in the absence of Vpu (Fig. 7), pointing to an occlusive mechanism involving membrane potential depolarization. The results again support the notion that there exists a transmembrane voltage constraint for viral release that can be sufficiently dissipated by HIV-1 Vpu. To the extent that Vpu may form homo-oligomers, the channels thus formed may further attenuate $\Delta\psi$ to promote viral fission and release (16,17,36).

In addition to the mechanism involving $\Delta\psi$ presented here, BST2/tetherin was recently shown to be an important restriction factor for Vpu-mediated release (5,6). This discovery was based on earlier findings that the effect of Vpu on release enhancement is cell-type-dependent (37). For example, the efficiency of viral release in HeLa and human CD4+ T cells is greatly enhanced by Vpu, whereas the efficiency in HEK-293T cells is Vpu-independent. Furthermore, because Vpu is capable of facilitating the release of widely divergent retroviruses, the mechanisms used by Vpu to promote release are likely intrinsic to various types of host cells (38). Studies that examined differences in gene expression between Vpu-dependent (e.g., HeLa) and Vpu-independent (e.g., HEK-293T) cell lines showed that BST-2 is expressed in Vpu-dependent cells but not in Vpu-independent cells, and is an endogenous restricting factor against viral release (5,6,9). It has been suggested that the rate of release in Vpu-independent (permissive) cells is not affected by Vpu expression because their endogenous BST-2 is too low to counteract Vpu effectively to limit the release. In contrast, in Vpu-dependent (restrictive) cells, viral release is limited in the absence of Vpu due to the comparably higher endogenous BST-2 levels. However, the

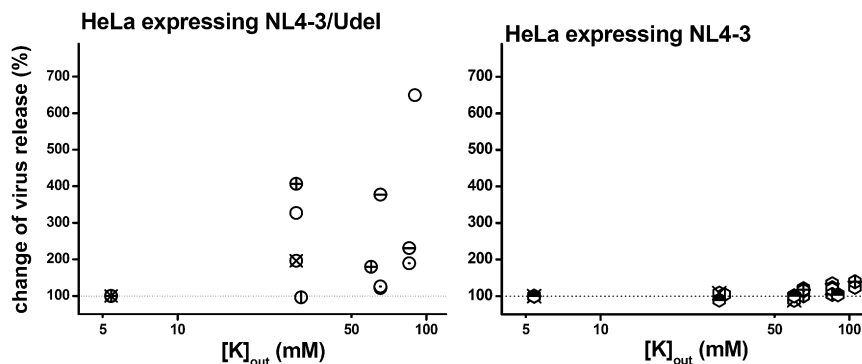


FIGURE 7 High K^+ -induced depolarization enhances viral release in HeLa cells. Left: The depolarization effects on HIV-1 release are significant in the absence of Vpu (NL4-3/Udel). Right: The depolarization effects are comparably smaller ($\leq 50\%$) in HeLa cells expressing NL4-3. The effects of depolarization were compared with those observed in cells immersed in media containing 5.4 mM K^+ (set fold = 1). Different symbols represent independent experiments. Cell depolarization by elevated external K^+ concentrations ($[K^+]_{out}$) was validated using a membrane depolarization-sensitive fluorescent dye, DiBAC₄(5).

protective effect of endogenous BST-2 in the restrictive cells can be diminished by Vpu expression, which allows acceleration of viral release (5).

Of note, overexpression of BST-2 in the permissive HEK-293T cells can induce massive retention of the NL4-3/Udel virions on the cell surface, thereby changing HEK-293T cells from Vpu-independent to Vpu-dependent (5). It has been proposed that Vpu antagonizes BST-2 by accelerating degradation of BST-2 through its interaction with β TrCP and other components of the ubiquitin-proteasome degradation system (39,40). Thus, whether a cell type is permissive or restrictive appears to be influenced by the expression levels of the endogenous restriction factors. This notion also fits our mutant data, in which the degree of release enhancement by Vpu was directly proportional to the magnitude of inhibition Vpu imposed on background K^+ conductance (Fig. 4).

To integrate the BST-2 model with the proposed depolarization mechanism, we investigated whether the effects of background K_{2P} channel activities on HIV-1 release were also cell-type-dependent. We first determined the endogenous TASK levels in HEK-293 (permissive) versus HeLa (restrictive) cells, and then assessed their impact on Vpu-mediated release. Current clamp experiments revealed that HeLa cells had more polarized resting membrane potentials than did HEK-293 cells (Fig. 8 A). Based on the degrees of membrane potential depolarization by TASK siRNA, HeLa cells expressed significantly more TASK than HEK-293 cells (Fig. 8 A). Knockdown of endogenous TASK depolarized both cell lines to similar voltages, indicating that TASK1/3/5 channels were primarily responsible for setting cell membrane potentials in these cell lines (Fig. 8 A). Because HEK-293 cells expressed smaller background K^+ conductance than HeLa cells, they were likely to impose smaller restricting $\Delta\psi$ on particle release. Indeed, membrane potential depolarization induced by elevated $[K^+]_{out}$ enhanced NL4-3/Udel release up to 2.5-fold in HEK-293 cells (Fig. 8 B), and the enhancement was not as large as in HeLa cells (Fig. 7). On the other hand, release enhancement by depolarization was much smaller ($\leq 50\%$) in the presence of Vpu for both cell lines (Figs. 7 and 8 B), again suggesting depolarization as a triggering mechanism for Vpu-mediated release. Because elevation of extracellular K^+ drove different cells to the same depolarized potentials, particle release from HEK-293 cells, which had smaller restricting $\Delta\psi$, was affected less by depolarization than release from HeLa cells (Figs. 7 and 8 B). A comparison of the results from HEK-293 versus HeLa cells (Figs. 7 and 8) shows that their different degrees of NL4-3/Udel release enhancement by depolarization reflect the different degrees of membrane potential polarization (or, in essence, TASK expression) of the two cell lines. Conceivably, the size of background K^+ conductance could also help determine whether a particular cell type is permissive or restrictive.

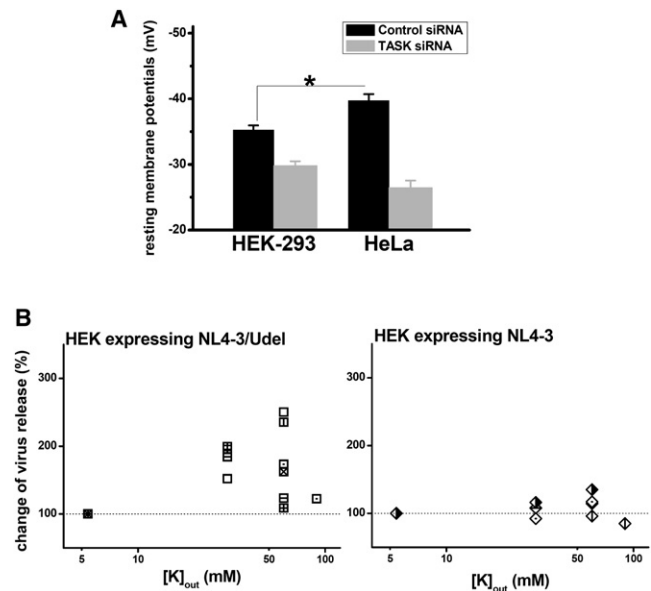


FIGURE 8 Endogenous TASK expression in HEK293 cells stabilizes the cell membrane potentials and allows viral release efficiencies to be enhanced by depolarization. (A) Knockdown of endogenous TASK1/3/5 revealed different levels of TASK1/3/5 expression in HEK-293 and HeLa cells. Because the main function of K_{2P} channels is to set cell resting potentials, the endogenous expression of TASK1/3/5 was assessed in terms of the degree of membrane potential depolarization (mV) by TASK siRNA. TASK1/3/5 expression was significantly higher in HeLa than in HEK-293 cells. The resting membrane potentials of HeLa cells were also more polarized than those of HEK-293 cells, indicating that TASK channels are pivotal for membrane potential stability in HEK-293 and HeLa cells. (B) To study the impact of depolarization on viral release in HEK-293 cells, the cells expressing NL4-3 or NL4-3/Udel were depolarized in high K^+ media for 2 h. Left: Depolarization by elevated $[K^+]_{out}$ enhanced particle release up to 2.5-fold in the absence of Vpu. Right: In the presence of Vpu, there was little increase of release ($\leq 50\%$) by membrane potential depolarization. Different symbols represent independent experiments.

How do the two proposed mechanisms (membrane potential depolarization and tetherin/tethering) fit into the release process mediated by Vpu? To ascertain whether the two mechanisms are linked at the protein level, we sought to determine whether the endogenous expressions of TASK and BST-2 were correlated. Knockdown of endogenous TASK did not affect the protein level of BST-2 in HeLa cells, indicating that the endogenous levels of TASK and BST-2 were independent of each other (Fig. S3). Moreover, BST-2 was not expressed in HEK-293 cells at all (Fig. S3), whereas TASK channels were expressed in both HEK-293 and HeLa cells, with a higher level in the latter (Fig. 8 A). The endogenous expression of TASK channels in HEK-293 cells allowed viral release to be enhanced by depolarization through high K^+ media (Fig. 8 A) or TASK knockdown (data not shown). Therefore, the mechanisms used by BST-2 and TASK to counteract Vpu-mediated release were independent of each other.

The mechanisms of Vpu-mediated release involve at least these two independent physical parameters: 1), $\Delta\psi$

elimination (noncontact); and 2), contact/tethering. TASK channels, and perhaps other background K^+ channels as well, stabilize the electric field across a budding membrane, thereby counteracting Vpu-induced depolarization in a non-contact fashion. In comparison, BST-2 is proposed to bind both the viral and the host cell membranes, thereby restricting viral release through direct physical contact (5).

Because electric potential and contact/tethering have distinct physical properties, these two types of barriers are expected to play important roles during different stages of Vpu-mediated release. Before fission is completed, the viral and the cell membranes are continuous. Any significant changes in the membrane potentials of the host cells (such as depolarization, as presented here) could affect the fission process. Upon completion of virus-cell fission, their membranes become separate, so $\Delta\psi$ in the host cells no longer has any impact on the viruses. After membrane fission, according to the tetherin/tethering model, endogenous BST-2/tetherin physically links the viral and the host cell membranes to prevent particle egress.

CONCLUSIONS

As summarized in our model (Fig. 9), Vpu may trigger depolarization (i.e., from -60 mV to -45 mV in human CD4⁺ T cells) to dissipate the stabilizing transmembrane potential that is inhibitory for membrane fusion/fission (gray shade). We hypothesize that dissipation of this energy barrier essentially reduces the contact repulsion between the two apposing membranes to be fused. Imposing the depolarization further (i.e., high K^+ stimulation for Vpu⁺ cells, or up from the gray-shaded transmembrane voltage barrier in the model) may be redundant, since after fission the restricting force for viral particle release is of a different nature. Others have suggested that after fission, BST-2/tetherin may still retain, or glue, the viral membrane to the host cell membrane (5,6) (Fig. 9).

The proposed mechanism through which Vpu promotes viral release by membrane potential depolarization accentuates the key role of K_{2P} channels in cellular physiology (25). Activation of background K_{2P} channels such as TASK leads to hyperpolarization and suppression of the excitatory drive and secretion (31,41). In motoneurons, activation of native TASK-like current using the inhalational anesthetic halothane also leads to hyperpolarization and suppression of neuronal firing (33). In a study of adrenal glands, inactivation of native TASK depolarizes the membrane potentials, leading to enhancement of aldosterone secretion (32). Analogous inhibition of another background K_{2P} channel, TREK-1, by corticotropin, promotes cortisol secretion in bovine adrenal fasciculate cells (30). Although HIV-1 release differs importantly in its mechanistic details from secretion/exocytosis processes, they can all be induced by a destabilized electric field. The channel-like structure of Vpu enables it to depolarize the budding membrane,

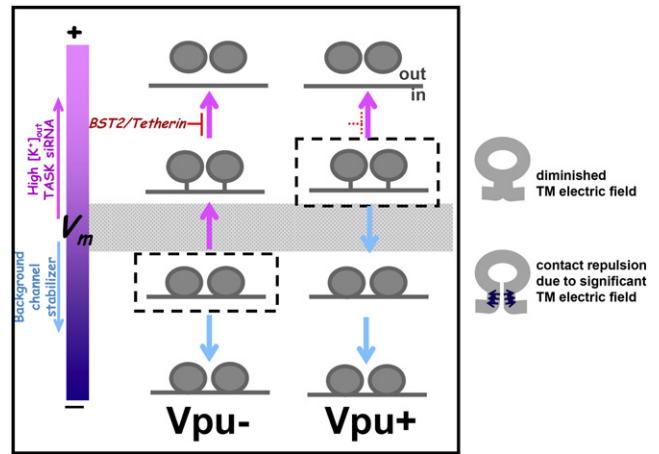


FIGURE 9 A model depicts virus release as a function of membrane potentials (V_m). In/Out refers to the position of budding viruses (circles) inside or outside of the plasma membrane (horizontal lines). Dashed boxes mark the resting states of V_m . Vpu depolarizes the resting V_m . The gray shade represents the proposed transmembrane voltage barrier for particle budding. Red arrows point to membrane potential instability/depolarization (+), and blue arrows point to potential stability/hyperpolarization (-). Vpu-induced depolarization likely diminishes the electric and/or contact repulsion as two opposing membranes are about to fuse. After membrane fusion/fission occurs, BST-2/tetherin may restrict viral release by gluing viral membranes to the cell surface. The resting membrane potentials of HeLa cells (restrictive) are more polarized than that of HEK-293 cells (permissive). According to the proposed membrane potential model, the energy barrier for release in HeLa cells may be significantly larger than the barrier in HEK-293 cells. According to the tetherin/tethering model for restrictive cells, such as HeLa, endogenous BST-2 may limit particle release by physically binding to both viral and host cell membranes in the absence of Vpu (as shown in the upper left panel). In the presence of Vpu, endogenous BST-2 is not sufficient to limit the release (as shown by dashed lines in the upper-right panel).

making particle release an energetically favorable process. Its unique presence in HIV-1 conceivably contributes to the particularly high transmissibility and disease progression of HIV-1 as compared to HIV-2 or other lentiviruses.

SUPPORTING MATERIAL

Three figures are available at [http://www.biophysj.org/biophysj/supplemental/S0006-3495\(10\)00899-4](http://www.biophysj.org/biophysj/supplemental/S0006-3495(10)00899-4).

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