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The Functions of the HIV1 protein Vpr and its action through the DCAF1•DDB1•Cullin4 ubiquitin ligase

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Abstract

Among the proteins encoded by human and simian immunodeficiency viruses (HIV and SIV) at least three, Vif, Vpu and Vpr, subvert cellular ubiquitin ligases to block the action of anti-viral defenses. This review focuses on Vpr and its HIV2/SIV counterparts, Vpx and Vpr, which all engage the DDB1•Cullin4 ubiquitin ligase complex through the DCAF1 adaptor protein. Here, we discuss the multiple functions that have been linked to Vpr expression and summarize the current knowledge on the role of the ubiquitin ligase complex in carrying out a subset of these activities.

Keywords

Vpr; DCAF1; ubiquitin ligase; cell cycle arrest; macrophage infection

1. Introduction

1.1 Proteins unique to complex retroviruses

Complex retroviruses express a number of gene products in addition to the Gag, Pol and Env proteins expressed by all retroviruses (Fig. 1). The general function of these additional proteins is to prepare infected cells for virus production. The first of these proteins encoded by HIV are expressed from highly spliced messages. Tat functions to enhance RNA polymerase II processivity of transcription from the HIV LTR [1,2] and Rev expedites RNA export from the cell nucleus and thereby decreases the extent to which viral RNA is spliced [3]. Nef, another protein expressed early after infection, down-modulates CD4 to control super infection [4–7]. Other functions have, however also been ascribed to this protein and contribute to HIV biology [8–10]. Vpu similarly downmodulates CD4 expression at the cell surface [11] and importantly counteracts the cellular protein tetherin, which retains newly produced virions at the cell surface [12,13]. The *vif* gene product redirects a cellular ubiquitin ligase to target the cellular cytidine deaminases, APOBEC3G and APOBEC3F, for proteasomal degradation [14–19]. In the absence of Vif, APOBEC3G and APOBEC3F have highly effective anti-viral activity [20–23].

Several functions have been identified for the protein Vpr. While all of these may ultimately impact the virus, the host cells or both, identification of the primary functions that Vpr evolved

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will both further our understanding of HIV biology and help in the identification of new options for therapeutic intervention.

1.2 Vpr function is evolutionarily conserved among primate lentiviruses

HIV1-encoded Vpr is a 14-kDa, virion-associated protein that has two widely accepted biological effects. One is to promote infection of non-dividing cells, specifically those of the myeloid lineage [24–27], and the other is to trigger G2 cell cycle arrest in dividing cells [28, 29]. In HIV2 and SIV that infects sooty mangabey monkeys and macaques (SIV_{smm} and SIV_{mac}), two separate proteins, designated Vpx and Vpr, carry out these functions respectively [30] (Fig. 1). Phylogenetic analysis, interestingly, shows that the coding sequences for the two Vpr-like proteins in HIV2 and its SIV counterparts likely arose from the duplication of a single HIV1-*vpr*-like precursor [31]. It is likely that the burden of carrying extra nucleic acid is offset by the functional refinement of the proteins that the duplication allowed. In addition to being a multifunctional protein HIV1 Vpr is further constrained from evolving because its coding sequences overlap *vif* at the amino-terminus and *tat* at the carboxy-terminus (Fig. 1). In HIV2/$SIV_{smm/mac}$, *vpx* overlaps *vif* at its amino-terminus and *vpr* overlaps *tat* at its carboxy-terminus (Fig. 1). Thus in each instance evolution of one end is not limited by the overlap with another reading frame. The constraints on HIV1 Vpr may prevent both further functional optimization and easy escape from immune responses or therapeutic interventions. HIV2/$SIV_{smm/mac}$ Vpx, on the other hand, has evolved to reduce functional overlap and thus may have been optimized to act more efficiently and to shed any “off-target” effects that could be detrimental to the virus. The reduced functional overlap may similarly aid viral immune evasion.

2. What are the functions of Vpr and why are they important for viral replication and pathogenesis?

2.1 Vpr enhances macrophage infection

The function of Vpr relevant to viral replication has been enigmatic but clues are slowly beginning to emerge. Boosting infection of myeloid lineage-derived cells positively impacts HIV and SIV in the most obvious manner. Older experiments showed that Vpr facilitates nuclear import of viral pre-integration complexes in non-dividing cells [26,27,30,32–35]. This function is shared between HIV1 Vpr and HIV2/$SIV_{smm/mac}$ Vpx and was attributed to nuclear import signals which are found on both. Interestingly however, there are multiple nuclear import signals in the pre-integration complex, including one each in Gag and integrase and one in a triple-helix reverse transcription intermediate (reviewed in [36]). This of course implies that there is redundancy for the indispensable nuclear import process; however, other work [37,38] suggests that none of these signals are required for infection of non-dividing cells.

A number of recent reports suggest that the block that restricts $SIV_{smm/mac}$ and HIV2 infection in macrophages is not at the level of nuclear import [39–43]. These reports provide evidence that an as yet unidentified cellular factor interferes with efficient reverse transcription of the viral genomes. None of the work that introduced this new macrophage anti-viral factor focused specific attention on HIV1 Vpr and its role in facilitating macrophage infection. Therefore, it is not known whether HIV1 Vpr functions like HIV2/$SIV_{smm/mac}$ Vpx to facilitate macrophage infection. Vpx from both $SIV_{smm/mac}$ and HIV2 had such a profound effect on the infectivity of SIV and even that of HIV1 in macrophages that the previously well established role of HIV1 Vpr in promoting macrophage infection was overshadowed [42]. It is possible that the optimization of Vpx after the aforementioned gene duplication event allowed it to become functionally superior to a multifunctional HIV1 Vpr-like precursor. Intriguingly, if HIV1 Vpr blocks the function of the same macrophage anti-viral factor, its weaker action may make it easier to defeat with therapeutic interventions. Further, HIV1 Vpr may be less able to evade an immune response or a Vpr-directed therapeutic agent due to its overlapping function(s). The

role of HIV1 Vpr in macrophage infection must therefore be re-examined in light of these more recent studies.

2.2 Vpr causes G2 cell cycle arrest

Expression of HIV1 or HIV2/SIV$_{smm/mac}$ Vpr causes G2 cell cycle arrest [28,29]. This function has been demonstrated with expression of Vpr alone, in the context of the virus and even in primary cells from infected patients [28,44]. The biological significance of this G2 arrest, however, isn't well understood.

2.2a Does G2 arrest itself benefit viral replication?—G2, the pause between the duplication of cellular chromatin and cell division, provides a favorable environment for virus production. During G2 phase, unlike in S-phase and in mitosis, chromatin is transcribed and mRNA is actively translated. This environment allows a 2–3-fold enhancement in virus production over asynchronous cell populations [45]. While this increase appears modest, the cumulative effect over several replication cycles could boost virus production significantly.

2.2b Is G2 arrest the function for which Vpr evolved or is it a by-product of another process?—Vpr appears to aid HIV replication most in terminally differentiated macrophages, which of course do not divide. Further, in a typical experiment, not every cell that is transfected with an HIV1 Vpr expression vector responds by arresting in G2. That percentage is significantly lower in cells transfected with Vpr from HIV2 (de Noronha unpublished observations and [46]). Assuming that HIV2 Vpr is less restricted than HIV1 Vpr to evolve its function, more efficient arrest is expected if that is the prime function of Vpr. Of note, HIV2 Vpr is present in smaller quantities in total cell lysates than HIV1 Vpr [46]. Finally, the HIV1 protein, Vif, when expressed at high levels, also causes cells to accumulate in G2 [47]. This effect also requires the action of an ubiquitin ligase, albeit a different one than Vpr engages. Thus, it appears possible that G2 arrest can result from the over-engagement of ubiquitin ligases rather than from a specific Vif- or Vpr-mediated function.

2.3 What are the other functions of Vpr?

2.3a Vpr causes cell death—Vpr has been linked to other functions that may be direct or indirect consequences of Vpr action. For example expression of HIV1 Vpr leads to cell death, but it's not clear whether it is by apoptosis [48] or by necrosis [49]. Further unclear is whether cell death is a function of Vpr that is advantageous for the virus or merely again a consequence of another process; does holding cells in G2 for extended periods contribute to cell death? Neither HIV2/SIV$_{smm/mac}$ Vpr nor Vpx induce apoptosis in nonhuman and human cell lines [50].

2.3b Vpr regulates gene expression—Vpr expression can modify both viral and host gene transcription. As a virion-incorporated protein, Vpr is poised to activate immediate-early HIV1 gene expression prior to Tat production. Indeed, one of the first functions attributed to Vpr was transactivation of the HIV1 long terminal repeat (LTR) [51]. This transactivation function is conserved among primate lentivirus Vpr proteins but is not shared by Vpx [52]. Subsequent studies showed that Vpr and Tat have an additive effect on HIV1 LTR activation [53,54]. However, this function has been linked to the cell cycle, suggesting that LTR transactivation is a by-product of G2 arrest. In support of this hypothesis, expression from the HIV1 LTR is highest in cells in the G2 phase [45], and Vpr mutants that fail to induce G2 cell cycle arrest also fail to activate an HIV1 LTR luciferase reporter [45,53]. However, Vpr has also been shown to regulate transcription from the LTR in a manner that is not linked specifically to Vpr-mediated cell cycle arrest [51].

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In addition to regulating the HIV1 LTR, Vpr also modulates host gene expression. Vpr activates expression of the cyclin-dependent kinase inhibitor, p21WAF1, in a variety of cell types [55, 56]. Changes in the levels of various cytokines and chemokines have also been associated with Vpr expression. These include IL-12 [57–59], TGF-beta [58], IL-8 [60], CD28, CTLA-4, IFN-gamma [61], RANTES, MIP-1-alpha and MIP-1-beta [62,63], and IL2, IL4, IL10, and TNF-alpha [57].

Vpr may alter gene expression directly through cellular factors that modulate transcription or by direct DNA-protein interactions. For example, Vpr physically engages transcription factors/co-activators such as SP-1 [64], the glucocorticoid receptor [65], p300/CREB-binding protein [54] and TFIIB [66]. These interactions can modify both the amplitude of virus expression as well as the host response to the virus. Further, Vpr has been shown to activate the HIV1 LTR by directly binding to specific DNA sequences within the LTR [67,68].

2.3c Vpr signals a DNA damage response—Expression of Vpr triggers a DNA damage response that depends on the activation of ATR but not ATM [44,69]. The signals that trigger the response have not been resolved. Vpr has been shown to cause double strand breaks in DNA [70]. Since Vpr has no apparent enzymatic function, recruitment of other proteins would be necessary. Other work demonstrated that Vpr expression causes disruptions of the nuclear envelope architecture [71]. The nuclear envelope is important for the elongation phase of DNA replication [72] and stalled replication activates signaling through ATR. The mechanism through which Vpr disrupts the nuclear envelope structure and the impact that this effect has on HIV remains to be resolved.

2.4 Insights into Vpr function in vivo

The impact of Vpr on HIV and SIV may not be fully revealed in *in vitro* infections. The effect of deletion of Vpr from HIV1 or deletion of Vpx from HIV2/SIV is evident in *in vitro* infections only in non-dividing cells whereas in dividing cells, HIV1 Vpr and HIV2/SIV Vpx seem to be dispensable for infectivity [24,25,30,73,74]. Deletion of Vpr from HIV2/SIV has little effect on *in vitro* infections regardless of the cell type [30]. It is clear however, that Vpr/Vpx benefits the virus *in vivo*. In a rhesus macaque infection experiment where the SIV_{mac293} Vpr start codon was mutated to TTG, three of five test animals showed reversion to ATG. The remaining two maintained low, persistent levels of virus and did not develop disease during the observation period [75]. In another study, also using SIV_{mac293}, deletions were introduced into the Vpr or Vpx reading frames, or into both [76]. Reversion was not possible in this scenario. The most notable decrease in virus replication and pathology was in the animals infected with Vpx(−) Vpr(−) virus, followed by Vpx(−) virus [76]. Thus Vpr appeared to act synergistically with Vpx but curiously deletion of Vpr alone seemed to have little effect on viral pathology.

A third trial, using SIV_{smm} with deletions of Vpr, Vpx, or both in pig-tailed macaques, showed the importance of Vpr in viral spread and disease [77]. In this experiment, half of the animals infected with Vpr(−) virus survived despite first reaching peak virus levels that were comparable to those in animals that died. Possible interpretations of this observation are that the virus population was able to expand initially but was then inefficient at evading a later, more refined, immune response or that it was impaired in transitioning to a new host cell population after the first had been saturated or depleted.

The possibility that Vpr contributes to viral immune-evasion is supported by two papers showing that expression of Vpr hinders effective cellular immune responses against Vpr itself as well as against co-expressed viral antigens [78,79]. The role of Vpr could thus be to blunt cellular immune responses directed against HIV. This function may also be carried out by HIV2/$SIV_{smm/mac}$ Vpr, Vpx or both together. Cellular immune responses play an important role in keeping HIV levels in check, especially in elite HIV controllers who can maintain low

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viral loads without HAART therapy. CTLs however play a major role in helping to suppress virus until late in infection. Perhaps Vpr influences the quality of the CTL response that is proving to be a critical part of elite controllers' antiviral armamentarium.

The second interpretation of the observations from the *in vivo* experiments is that Vpr broadens the range of cell types permissive to infection. SIV Vpx expands the cellular host-range of the virus into macrophages and perhaps also into as yet unidentified cell-types that are required to maintain the virus later in infection. Vpx may do this by defeating an antiviral host defense like that which it blocks in macrophages. This capacity may be less pronounced in HIV1.

3. Vpr and the ubiquitin ligase complex

Dissecting cause and effect for Vpr will be difficult. For example, G2 cell cycle arrest can be triggered by numerous processes that can elicit various changes within the cell. Recently our lab and others identified a DCAF1•DDB1•Cul4-containing ubiquitin ligase complex as a partner for HIV1 Vpr, HIV2 Vpr or for HIV2/$SIV_{smm/mac}$ Vpx [80–86] (Fig. 2). Ubiquitin ligases attach 76-amino-acid-long ubiquitin peptide side chains to proteins, usually at lysine residues. These ubiquitinations can be simple or further branched from lysine on ubiquitin itself. The modifications can direct changes in function or subcellular distribution. Most commonly however polyubiquitination marks proteins for destruction by proteasomes.

A number of targets have been discovered for the DDB1•Cul4-containing ubiquitin ligase complex in the absence of Vpr. The association between DDB1 and Cul4 was first described in the context of DNA damage resulting from cellular exposure to ultraviolet light [87]. More recent studies showed that DDB1 links various other proteins, either directly or indirectly to the Cul4 E3 ubiquitin ligase component. These ubiquitination targets include histones [88, 89], STAT1 [90], XPC [91,92], Chk1 [93], CDT1 [94–96], Merlin [97], c-jun [98], TSC2 [99] and $p27^{kip}$ [100].

The DDB1•Cul4 ubiquitin ligase complex is required for at least three Vpr-associated phenotypes. These include *(1)* HIV1 and HIV2 Vpr-mediated G2 cell cycle arrest, *(2)* facilitation of macrophage infection by HIV2/$SIV_{smm/mac}$ Vpx and *(3)* HIV1 Vpr-mediated degradation of the uracil-DNA glycosylases UNG2 and SMUG1 [101]. In addition, HIV1 Vpr function in triggering a DNA damage response has also been linked to the DDB1•Cul4 ubiquitin ligase. While HIV1 Vpr has also been shown to promote degradation of the interferon response factor IRF3, this particular Vpr function has not been linked to a specific ubiquitin ligase complex [102].

Several labs analyzed cellular partners of HIV1 Vpr to determine how and why this viral protein engages cellular processes (reviewed in [103]). Vpr has been shown, using mostly yeast 2-hybrid screens, to interact with a number of cellular proteins. Recent searches for Vpr partners however relied on mass-spectrometry to identify proteins that co-immunoprecipitate with Vpr. Some studies further identified proteins that co-purify with HIV2/SIV_{mac} Vpr and Vpx, which share about 50% and 25% protein sequence identity with HIV1 Vpr respectively [80,83].

One analysis revealed that like HIV1 Vpr, HIV2 Vpr and to a much lesser extent HIV2 Vpx also engage the DCAF1•DDB1•Cul4 ubiquitin ligase complex [80]. Another study found that HIV2 and SIV_{mac} Vpx assembled with DCAF1 [86]. This interaction connects Vpr specifically to the DDB1•Cul4 ubiquitin ligase complex as depletion of DCAF1 reduced the quantity of DDB1 that was recovered after immunoprecipitation of HIV1 Vpr. Interestingly, the association between Vpr and DCAF1 had been made a decade ago, before contemporary technology and knowledge could allow the discoverers to appreciate the significance of this finding [104]. Of note, Angers *et al.*, looking for adaptors between DDB1 and unspecified target proteins, found that DCAF1 is one such protein [105]. Further confirmation that DCAF1

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acts as an adaptor between DDB1 and putative target proteins came through the findings of other labs [105,106].

3.1 The role of the ubiquitin ligase in Vpr-mediated G2 arrest

The involvement of the DCAF1•DDB1•Cul4 ubiquitin ligase complex in G2 arrest was confirmed by several independent means. First, severing the connection between Vpr and the rest of the complex using siRNA or shRNA attenuated Vpr-mediated arrest in a dose-dependent manner. Disruption of the complex by depleting DDB1, over-expressing DDB1 or over-expressing a dominant negative version of cullin4A led to the same outcome. Importantly, none of the modifications to the ubiquitin ligase complex that blocked Vpr-mediated G2 arrest interfered with the cells capacity to arrest in G2 in response to chemically induced DNA damage [80]. A subsequent publication of experiments investigating the *in vivo* function of DCAF1 showed that cells lacking the capacity to express this protein were slowed in their transition through S-phase over a 5-day time course [107]; however, this did not appear to be the case in analyses examining Vpr function on a shorter time-scale.

3.2 The role of ubiquitin ligase in HIV1 Vpr and HIV2/SIV Vpx-mediated facilitation of macrophage infection

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HIV1 Vpr is a karyophilic protein, localizes to the nuclear periphery, and associates with the viral pre-integration complex (PIC). As such, it is easy to envision a role for Vpr in nuclear import of the PIC. Support for this model was provided by the following results: mutation of nuclear localization signals (NLS) in Vpr abrogated HIV1 infection of growth-arrested cells, *vpr* defective PICs cannot translocate to the nucleus in an *in vitro* nuclear import assay, disruption of the interaction between Vpr and nuclear envelope or import factors reduces HIV1 infectivity in macrophages and *vpr(−)* virus resulted in lower levels of Gag transcripts but did not affect total proviral levels as measured by semi-quantitative PCR in macrophages [34, 108–113]. However, the model that Vpr is required for nuclear entry of the PIC in macrophages is controversial since redundant nuclear localization signals found in HIV integrase, matrix and in the viral DNA have been shown to be critical for nuclear import (reviewed in [114]). Furthermore, there is no evidence that Vpr participates directly in nuclear transport of the PIC in macrophages. Finally, for HIV2/SIV infection, the function of enhancing macrophage infection segregates to HIV2 Vpx [30] and recent work re-examining the role of Vpx in macrophage infection found that it acts by blocking a restriction to reverse transcription rather than by enhancing nuclear import [39,42,43,115].

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The most recent findings regarding Vpx function call into question the previously accepted model in which HIV2/SIV$_{smm}$ Vpx, like HIV1 Vpr, enhanced macrophage infectivity by facilitating nuclear entry of the PIC [30,116]. HIV2/SIV Vpx, like HIV1 Vpr, contains a nuclear localization signal, is incorporated into virions, and associates with the PIC. However, an experiment in which heterokaryons were formed between permissive cells and macrophages [42] show that Vpx defeats a macrophage-specific restriction. The restriction occurs during reverse transcription; real-time PCR of viral DNA at different stages early during infection showed that early, late, and post-nuclear imported transcripts are decreased in macrophages infected with HIV-2/SIV *vpx(−)* virus [39,42,43,117]. This is supported by another study demonstrating that Vpx is critical for completion of reverse transcription in macrophages [40]. Interestingly, Vpx neutralizes a restriction to reverse transcription in macrophages of not only complex lentiviruses such as HIV2, SIV and HIV1, but also of gammaretroviruses such as murine leukemia virus (MLV) [118]. Importantly, the role of HIV2/SIV$_{smm/mac}$ Vpx in counteracting macrophage restriction is dependent on its association with the same ubiquitin ligase complex that interacts with HIV1 Vpr [39,42,43] (Fig. 2). Experiments using siRNA directed against components of the DCAF1•DDB1•Cul4 ubiquitin ligase complex, a Vpx mutant, Q76R, that fails to interact with DCAF1, or proteasome inhibitors, decreased the

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capacity of Vpx to neutralize the macrophage restriction factor [39,42,43]. It is interesting that even though HIV1 Vpr interacts with the DCAF1•DDB1•Cul4 ubiquitin ligase complex HIV-2/SIV Vpx enhances macrophage infectivity more than HIV1 Vpr [42,117]. This is evident from the observation that Vpx supplied in *trans* by either pre-infection with SIV or by co-packaging of Vpx with HIV1 virions enhances the infectivity of HIV1 in macrophages [42,117]. Perhaps the interaction between HIV-2/SIV Vpx and the ubiquitin ligase complex is optimized to target the macrophage restriction. Alternatively, due to its multifunctional nature, HIV1 Vpr may engage more targets than HIV2/SIV Vpx and thus neutralize the macrophage restriction factor less effectively. Whether the interaction between HIV1 Vpr and the DCAF1•DDB1•Cul4 ubiquitin ligase complex is required to enhance macrophage infectivity is an issue for further study.

Finally, while there is consensus among published studies that HIV1 Vpr and HIV2/SIV Vpx function to enhance infection of human macrophages [24–27,34,41,119,120], the mechanism employed by HIV1 Vpr and HIV2/SIV Vpx remains unknown.

3.3 The role of ubiquitin ligase in HIV1 Vpr-mediated protein degradation

To date only a few proteins have been identified that are degraded in the presence of HIV1 Vpr. Okumura *et al.* found that expression of either HIV Vif or Vpr caused a decrease in IRF3 protein levels [102]. This was attributed to proteasomal degradation; however no specific ubiquitin ligase was implicated in targeting the degradation. The decrease in IRF3 could, in the context of a natural infection benefit the virus by thwarting host immune defenses.

The interaction between HIV1 Vpr and UNG2 was discovered over a decade ago using yeast 2-hybrid technology [121]. These experiments showed that Vpr recruits UNG2 into virions [122,123]. This association was intriguing because non-dividing cells, like terminally differentiated macrophages, harbor skewed nucleotide pools favoring RNA synthesis rather than the DNA synthesis. Tapping into UNG2 function, particularly by recruiting it into virions, could target misincorporated uridines excision. Further, viruses that lack Vpr-like open reading frames but infect non-dividing cells minimize uracil misincorporation by encoding dUTPase as a part of the *pol* gene.

More recently the discovery that APOBEC3G, a cytidine deaminase, attacks the HIV genome during reverse transcription re-ignited interest in the association between Vpr and UNG2 [124]. Schroefelbauer *et al.* discovered that expression of Vpr rather than recruiting UNG2 into virions, promoted not only its degradation, but also that of SMUG1 [101]. They further demonstrated that in the presence of APOBEC3G, Vpr-mediated degradation of UNG2 enhanced viral infectivity. Fenard *et al.* recently demonstrated that UNG2 exerts a negative effect of transcription from the HIV1 LTR [125]. Work from another group however showed that UNG2, in association with integrase, is vital for HIV propagation [126]. Yet other groups showed that UNG2 has little or no effect on HIV replication [127,128]. Finally, the link between Vpr and UNG2 can also be dissociated from G2 cell cycle arrest [129]. Thus, the significance of this function for HIV infection and pathogenesis remains unclear.

3.4 The role of the ubiquitin ligase complex in HIV1 Vpr-mediated DNA damage

Schröfelbauer *et al.* were the first to propose a model for Vpr-mediated DNA damage that incorporates the association between Vpr and the ubiquitin ligase complex [85]. The model suggested that by usurping the ubiquitin ligase complex Vpr hinders its normal function in DNA repair. This effect however is revealed primarily in response to UV irradiation and thus does not account for G2 cell cycle arrest that is encountered upon Vpr expression.

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Regardless of how the initial DNA damage signal is generated, recent work demonstrated that activation of ATR depends on the interaction between Vpr and the ubiquitin ligase complex [130]. The work further showed that Vpr, likely through the DNA-damage signal promotes expression of NKG2D ligands on HIV-infected cells. Cell surface expression of NKG2D ligands makes infected cells targets for killing by natural killer cells [130,131]. How expression of this cell surface molecule benefits HIV by enhancing killing of infected cells is still unclear. Like many other experimental observations, this one raises more interesting questions and holds the promise for additional means of therapeutic intervention.

4. Future directions for Vpr research

After years of study, the role of HIV1 Vpr and its HIV2 and SIV paralogs in viral infection and pathogenesis has not been fully revealed. Recent progress, including the discovery that a number of Vpr functions depend upon its assembly with an ubiquitin ligase complex that includes DCAF1, DDB1 and Cul4, has opened new opportunities to learn about and perhaps to block Vpr function.

Does Vpr target one or many proteins for ubiquitination? UNG2 assembles with HIV1 Vpr through a WXXF motif [132]. This motif is not uncommon and is also found on SMUG1. Vpr requires a tryptophan at position 54 for its interaction with UNG2, and a glutamine at position 65 for UNG2 degradation, yet the former residue is dispensable for Vpr-mediated G2 arrest [101]. This suggests despite its small size Vpr can target proteins for ubiquitination with at least two different motifs. Vpr-mediated ubiquitination can of course be modulated by the spatial and temporal availability of putative targets and the subcellular targeting signals of Vpr may further influence their distribution.

Identification of the biologically relevant substrates for Vpr-mediated ubiquitination will be a key step for revealing how and why Vpr is important for HIV infection and pathogenesis. This will help to determine, for example, whether HIV1 Vpr acts, albeit less efficiently, like HIV2/ $SIV_{smm/mac}$ Vpx to block an anti-viral factor. Identification of a Vpr-dependent ubiquitination substrate will also help to determine whether Vpr evolved to cause G2 cell cycle arrest or whether this is a by-product of another process. Finally, identification of the substrates may reveal new functions for this protein that were not apparent in previous studies.

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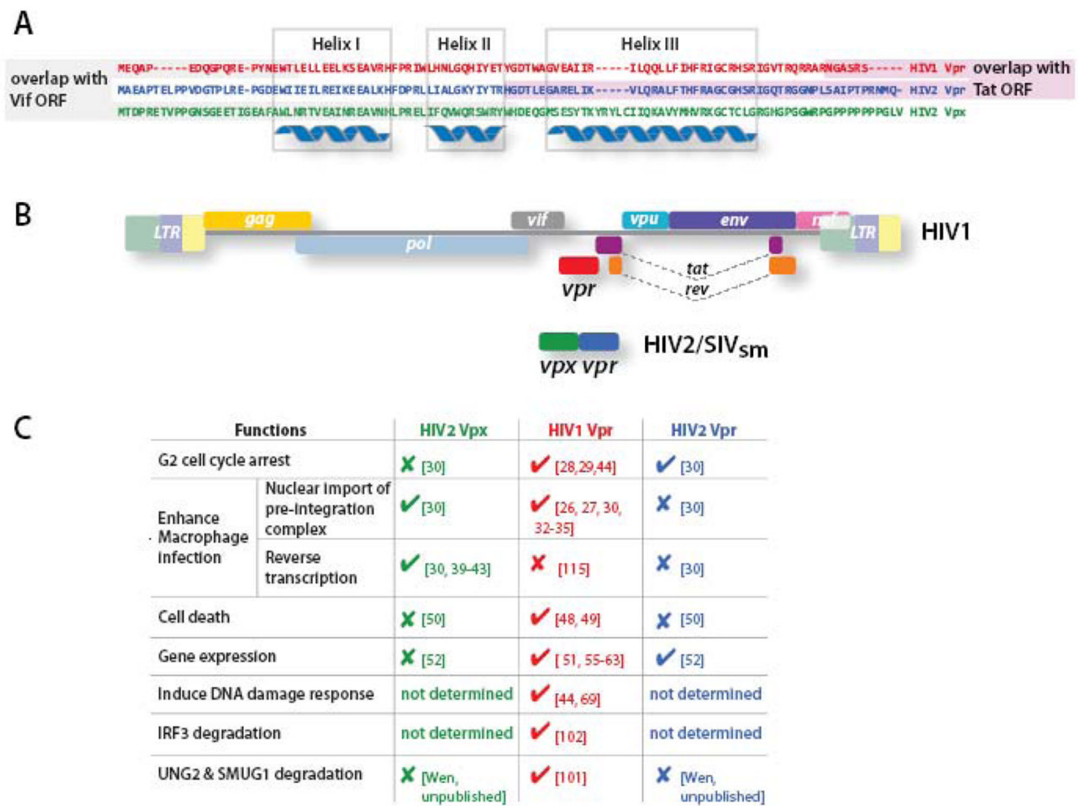

Functions		HIV2 Vpx	HIV1 Vpr	HIV2 Vpr
G2 cell cycle arrest		✗ [30]	✔ [28,29,44]	✔ [30]
Enhance Macrophage infection	Nuclear import of pre-integration complex	✔ [30]	✔ [26, 27, 30, 32-35]	✗ [30]
	Reverse transcription	✔ [30, 39-43]	✗ [115]	✗ [30]
Cell death		✗ [50]	✔ [48, 49]	✗ [50]
Gene expression		✗ [52]	✔ [51, 55-63]	✔ [52]
Induce DNA damage response		not determined	✔ [44, 69]	not determined
IRF3 degradation		not determined	✔ [102]	not determined
UNG2 & SMUG1 degradation		✗ [Wen, unpublished]	✔ [101]	✗ [Wen, unpublished]

Fig. 1.

A. Amino acid sequence alignment of HIV1 Vpr and HIV2 Vpr/Vpx. HIV1 Vpr shares about 50% and 25% protein sequence identity with HIV2/SIV$_{mac}$ Vpr and Vpx, respectively [78,81]. The region of HIV1 Vpr and HIV2 Vpr that overlaps with Tat is shaded in purple and the region of HIV1 Vpr and HIV2 Vpx that overlaps with Vif is shaded in grey. The amino acids that are predicted to form three alpha-helices are indicated. **B. Proviral genome structure of HIV**. Vpr is encoded by a reading frame in the center of the HIV1 genome that overlaps both *vif* and *tat* reading frames. HIV2 and the closely related virus SIVsmm/mac encode two vpr-like genes, *vpx* and *vpr*. **C. The functions of Vpr.** HIV1 Vpr is a multi-functional protein whose functions segregate to HIV2 Vpr or Vpx.

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Fig. 2.

A. Overview of DCAF1•DDB1•Cul4 ubiquitin ligase complex. Cullin 4 assembles with a multi-subunit complex to ubiquitylate targeted proteins. Cul4 C terminus associates with a small RING protein, ROC, that recruits and activates an E2 enzyme, while the N terminus of Cul4 interacts with a linker protein, DDB1 that binds the adaptor protein, DCAF1 that interacts with HIV1 Vpr, HIV2 Vpr or HIV2/SIV Vpx. **B. The role of the ubiquitin ligase complex in Vpr function.** Evidence supports a model in which Vpr/Vpx directs the DDB1•Cul4 ubiquitin ligase complex to ubiquitinate (1) a cellular uracil N glycosylase, UNG2, (2) a cellular protein required for cell cycle progression into mitosis, (3) a cellular protein that is degraded to activate ATR signaling and increases cell surface NKG2D ligands to enhance NK cell-

mediated killing, and (4) a cellular antiviral factor that inhibits viral reverse transcription in macrophages.

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