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Review Article

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.

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 down-modulates 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 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 multi-functional 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

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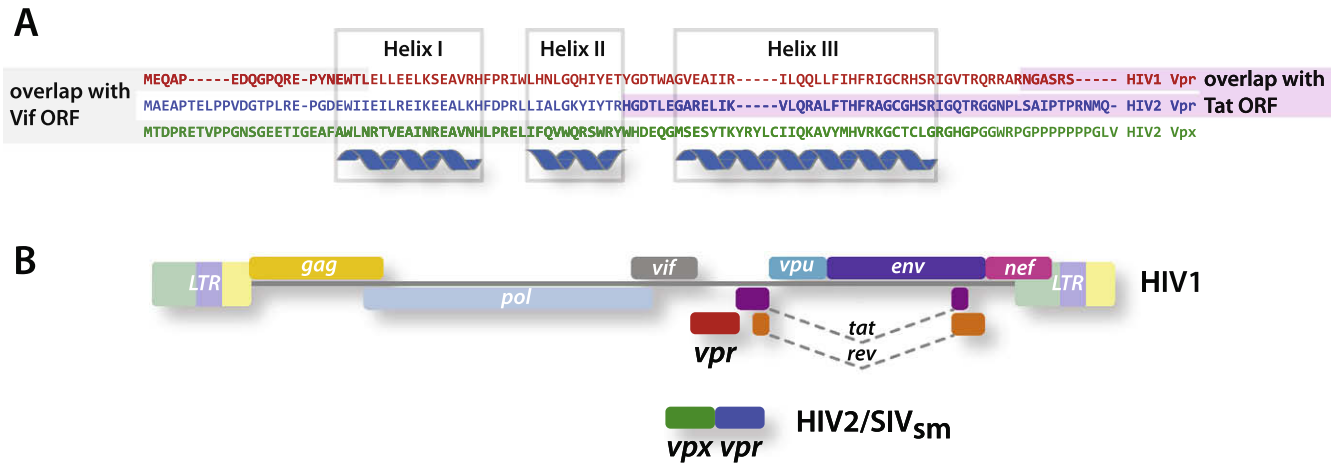

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Functions		HIV2 Vpx	HIV1 Vpr	HIV2 Vpr
G2 cell cycle arrest		✘ [30]	✔ [28,29,44]	✔ [30]
Enhance Macrophage infection	Nuclear import of pre-integration complex	✔ [30]	✔ [26, 27, 30, 32-35]	✘ [30]
	Reverse transcription	✔ [30, 39-43]	✘ [115]	✘ [30]
Cell 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 gray. The amino acids that are predicted to form three α-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.

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 afore-mentioned gene duplication event allowed it to become functionally superior to a multi-functional 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.