Human immunodeficiency virus type 1 Vpr: functions and molecular interactions

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Human immunodeficiency virus type 1 (HIV-1) viral protein R (Vpr) is an accessory protein that interacts with a number of cellular and viral proteins. The functions of many of these interactions in the pathogenesis of HIV-1 have been identified. Deletion of the vpr gene reduces the virulence of HIV-1 dramatically, indicating the importance of this protein for the virus. This review describes the current findings on several established functions of HIV-1 Vpr and some possible roles proposed for this protein. Because Vpr exploits cellular proteins and pathways to influence the biology of HIV-1, understanding the functions of Vpr usually involves the study of cellular pathways. Several functions of Vpr are attributed to the virion-incorporated protein, but some of them are attributed to the expression of Vpr in HIV-1-infected cells. The structure of Vpr may be key to understanding the variety of its interactions. Due to the critical role of Vpr in HIV-1 pathogenicity, study of the interactions between Vpr and cellular proteins may help us to understand the mechanism(s) of HIV-1 pathogenicity.

Introduction

The virulence of a virus depends on the virus–host interactions, which are postulated by a variety of factors, such as route of virus entry, dose of the virus and the host’s age, sex, immune status and species. The host may suppress every stage of the virus replication cycle by posing different obstacles to the progression of virus replication. A successful virus must overcome these obstacles. Viruses have evolved proteins to overcome the defensive mechanisms of the host (Tungaturthi et al., 2004). Lentivirus genomes contain a number of accessory and regulatory genes in addition to the gag, pol and env genes that are commonly found in all retroviruses. Human immunodeficiency virus type 1 (HIV-1) encodes two regulatory genes in addition to the gag, pol and env genes that are commonly found in all retroviruses. Human immunodeficiency virus type 1 (HIV-1) encodes two regulatory proteins, Tat and Rev, and four accessory proteins [viral infectivity factor (Vif), viral protein R (Vpr), viral protein U (Vpu) and negative factor (Nef)]. The regulatory proteins are essential for virus replication by controlling HIV gene expression in host cells. In contrast, accessory proteins are often dispensable for virus replication in vitro (Anderson & Hope, 2004).

The HIV-1 genome encodes a 14 kDa accessory protein, Vpr, which is a versatile, virion-associated protein composed of 96 aa (Felzien et al., 1998; Müller et al., 2000; Muthumani et al., 2000). Vpr is incorporated into HIV-1 virions through direct interaction with the p6 domain of the Gag protein (Bachand et al., 1999; Lavallee et al., 1994). Vpr has a variety of roles in determining HIV-1 infectivity and the number of its newly identified roles is still increasing. Some of the functions proposed for this protein include modulation of transcription of the virus genome (Sawaya et al., 2000), induction of apoptosis, disruption of cell-cycle control, induction of defects in mitosis (Chang et al., 2004), nuclear transport of the HIV-1 pre-integration complex (PIC) (Vodicka et al., 1998), facilitation of reverse transcription (Rogel et al., 1995), suppression of immune activation (Ramanathan et al., 2002) and reduction of the HIV mutation rate (Jowett et al., 1999). In addition, the interactions of Vpr with a number of human proteins have been identified, but the functions of some of those interactions are not clear (Felzien et al., 1998; Ramanathan et al., 2002; Sawaya et al., 2000). Vpr contains a flexible N-terminal region, three $\alpha$-helical domains with amphipathic properties and a flexible C-terminal region (Fig. 1). Each function or interaction of Vpr is attributed to one or more of its domains. Identification of the functions of Vpr domains is usually carried out by mutagenesis studies (Tungaturthi et al., 2004).

The cytopathic effects induced by Vpr are mostly attributed to the N terminus, which is able to form ion channels in cell membranes. These effects are unrelated to the reported activities of Vpr, including virion association, G2 arrest, induction of apoptosis, etc. (Piller et al., 1999; Somasundaran et al., 2002). Incorporation of Vpr into HIV-1 virions is blocked by a range of mutations distributed in different regions of the protein, indicating that different domains of Vpr are involved in its
HIV-1 Vpr enhances the ability of HIV-1 to replicate in terminally differentiated macrophages, which is attributed to the activity of Vpr in active nuclear import of the virus PIC (Jenkins et al., 1998; Le Rouzic et al., 2002; Schang, 2003; Suzuki et al., 2009). The HIV-1 PIC is composed of viral proteins, such as Vpr, reverse transcriptase (RT), integrase (IN), nucleocapsid (NC) and matrix (MA), in addition to viral nucleic acids. Although the precise role(s) of some of these proteins has not been described, their importance for nuclear import of the PIC has been demonstrated. The nuclear-localization signals (NLSs) in both MA and IN have been identified, and it is believed that both utilize the classical nuclear-import pathway that includes interaction with importins. In contrast, a canonical NLS has not been identified in Vpr, but it displays karyophilic properties. It is believed that the nuclear localization of Vpr is mediated by an unknown pathway that is distinct from the classical NLS- and M9-dependent pathways. In addition, it has also been suggested that Vpr utilizes the cellular machinery that regulates nucleocytoplasmic shuttling to transfer the proviral DNA to the nucleus. For example, in the presence of importin-α, the nuclear localization of Vpr increases. It has been suggested that interaction of Vpr with importin-α facilitates its nuclear localization (Gallay et al., 1996, 1997; Nitahara-Kasahara et al., 2007).

To identify the role of Vpr in the nuclear import of PIC, green fluorescent protein (GFP) has been fused to Vpr. By using the GFP–Vpr-labelled PIC, it has been demonstrated that the PIC is associated closely with cytoplasmic microtubules that direct it toward the nucleus, where it localizes in the perinuclear region close to centrosomes. It has also been shown that the PIC utilizes cytoplasmic dynein to travel toward the nucleus. It has not still been identified whether Vpr plays an active role during the transfer of the PIC along microtubules, or whether it is only associated with PIC in this step and starts its role during the next steps (Le Rouzic & Benichou, 2005).

Selective trafficking between the nucleus and cytoplasm is carried out through nuclear-pore complexes (NPCs), which form aqueous channels in the nuclear envelope. NPCs are huge protein complexes composed of 30 distinct nuclear-pore proteins, called nucleoporins (Nups). Some Nups that contain phenylalanine–glycine (FG) repeats are found in the filamentous structures emanating from both sides of NPCs, thereby provide docking sites for various transport factors. Importin-α is a common floating receptor that binds to the NLSs of cargo proteins. FG repeats on the cytoplasmic filaments and cytoplasmic ring moiety of NPCs recognize the importin receptor. It has been shown that HIV-1 Vpr binds to the FG repeats of several Nups, including human p54 and p58. HIV-1 Vpr also binds to human CG1 (hCG1), but this interaction is not mediated by the FG repeat of this Nup. Interaction of Vpr with the N-terminal region (which does not contain FG repeats) of hCG1 is essential for docking of Vpr to the nuclear envelope (Le Rouzic & Benichou, 2005; Vodicka et al., 1998; Zeitler & Weis, 2004). It has been demonstrated that the α-helical domains of Vpr are essential for its interaction with hCG1. This interaction results in Vpr accumulation in the nuclear envelope, which is believed to be involved in active nuclear import of the PIC in non-dividing cells, such as macrophages. It may also be involved in targeting the PIC to the NPC before its translocation into the nucleus. In addition to the conventional routes suggested for nuclear import of the PIC, it has also been reported that Vpr mediates transient, localized herniations in the nuclear envelope, resulting in mixing of cytoplasmic and nuclear components. These herniations probably contribute to the G2-arrest activity of Vpr and may also provide an unconventional route for nuclear import of the PIC. In fact, the interaction between Vpr and hCG1 could cause misassembly of the NPC, resulting in alternations of the architecture of the nuclear envelope that facilitate nuclear import of the PIC (de Noronha et al., 2001; Le Rouzic et al., 2002).
Induction of G2 arrest

The role of HIV-1 Vpr in inhibition of normal cell growth is well established. It is believed that the interruption of cell division by Vpr increases virus replication and induces programmed cell death. Vpr mediates cell-cycle arrest at the G2/M transition in various mammalian cells. The cell-cycle G2 arrest provides a replication advantage for the virus, because the transcription level of the provirus is higher during G2 (Belzile et al., 2007; Elder et al., 2001; Goh et al., 1998). As the pathways regulating the cell cycle are highly conserved in eukaryotic cells (Krylov et al., 2003; Warbrick & Fantes, 1988), transfection of yeast cells by vectors expressing Vpr also interrupts the cell cycle. Therefore, in studies of cell-cycle arrest induced by Vpr, the use of yeast cells is common, because they are easy to handle (Benko et al., 2007; Elder et al., 2001; Huard et al., 2008).

The eukaryotic cell cycle is controlled by a complex network of proteins and genes. Cyclin-dependent protein kinases (CDKs) initiate the crucial events of the cell cycle by phosphorylating specific protein targets. The phosphorylation activity of CDKs is tightly dependent on binding to cyclins. Binding of cyclins to CDKs results in CDK–cyclin complexes. As there are always excess amounts of CDKs in eukaryotic cells, it is the availability of cyclins that determines the number of CDK–cyclin complexes in eukaryotic cells. CDK–cyclin complexes can be down-regulated either by inhibitory phosphorylation of the CDK subunit or by binding to inhibitory molecules designated cyclin-dependent kinase inhibitors (Csikasz-Nagy et al., 2006). G2 arrest is characterized by low levels of cyclin B1–p34Cdc2 and inhibitory phosphorylation of p34Cdc2. It has been shown that Vpr directly inhibits the in vitro activity of a phosphatase, Cdc25C, which normally activates cyclin B1–p34Cdc2 (Fig. 2). Although the Vpr-binding site on Cdc25C is not its catalytic site, Cdc25C is inactivated by binding to Vpr, resulting in inhibition of Cdc25C phosphatase activity. In the absence of Cdc25C phosphatase activity, cyclin B1–p34Cdc2 remains in its phosphorylated form, which is inactive (Goh et al., 2004; He et al., 1995). 14–3–3 proteins are also involved in this pathway. These proteins normally regulate cell-cycle progression by changing the activities of cyclins, including Cdc25C. DNA damage results in Cdc25C phosphorylation, providing the active binding site for 14–3–3. It has been shown that the C-terminal region of Vpr interacts with the C-terminal region of 14–3–3, leading to the association of 14–3–3 with Cdc25C. This complex is not able to activate cyclin B1–p34Cdc2, therefore the cell cycle is arrested (Fig. 2) (Kino et al., 2005).

Inactivating Cdc25C is not the only pathway utilized by Vpr to arrest the cell cycle. Several studies have documented the association of HIV-1 Vpr with a cellular protein that now is called DCAF-1 (DDB1- and CUL4-associated factor 1), also known as VprBP (Vpr-binding protein). Further research demonstrated the role of this protein in DNA replication and embryonic development. It has recently been demonstrated that Vpr exploits DCAF-1, damaged DNA-binding protein 1 (DDB1) and the cullin 4A (CUL4A) ubiquitin ligase complex to interfere with the DNA replication machinery of infected cells, resulting in cell-cycle arrest (Fig. 3). The pathway exploited by Vpr implies that it functions as an adaptor protein in protein networks to interfere with the cell cycle and replication machinery (Giaccia & Kastan, 1998; Gieffers et al., 2000; Hrecka et al., 2007; Wen et al., 2007). Vpr exploits DCAF-1 as an adaptor to engage DDB1 as a component of the CUL4A ubiquitin ligase complex. As this complex is involved in proteasomal degradation, it was suggested that G2 arrest is induced by Vpr through degradation of an unidentified protein that is needed for progression of cells from G2 arrest to mitosis (Wen et al., 2007). In fact, the CUL4A ubiquitin ligase complex acts as a pivotal step, regulating different cellular pathways by targeting proteins for ubiquitin-dependent degradation. It has recently been demonstrated that, in this complex, DDB1 interacts with

![Fig. 2. Cell-cycle arrest through inactivation of Cdc25C.](http://vir.sgmjournals.org)
multiple WD40-repeat (WDR) proteins, which serve as the substrate-recognition subunits of the CUL4–DDB1 ubiquitin ligase. More than 150–300 WDR proteins have been identified in the human genome. Considering the variety of WDR proteins, the impact of biological processes through CUL4 ligase-mediated proteolysis can be understood. It has been reported that CDT2 interacts with the CUL4A ubiquitin ligase complex, enabling it to target CDT1 for degradation. CDT1 is a replication factor, depletion of which prevents DNA replication. Although cell-cycle arrest cannot be attributed to the degradation of a single protein through CUL4 ligase-mediated proteolysis, resulting in G2 arrest, CDT1 can be addressed as a key protein that is ubiquitinated by the CUL4A ubiquitin ligase complex, activated by Vpr. Indeed, many other proteins may play similar roles in parallel with CDT1 (Higa & Zhang, 2007; Jin et al., 2006; Rialland et al., 2002).

**Induction of apoptosis**

Although a variety of stimuli have been described as causes of apoptosis, apoptosis is regulated through one of two known cell death-signalling pathways: the extrinsic and intrinsic pathways. Both pathways share similar molecules (especially caspases) and features. The extrinsic pathway is initiated by external stimuli that are sensed by cell-death receptors on the cell membrane. Mitochondria play the central role in the intrinsic pathway by releasing molecules that trigger apoptosis (Budihardjo et al., 1999; Green, 2000). Several studies have shown that HIV-1 Vpr is able to induce the intrinsic pathway of apoptosis in a number of human cell lines and to promote apoptosis during HIV-1 infection. Not only does Vpr induce apoptosis in infected cells, but it has also been suggested that induction of apoptosis in uninfected bystander cells is caused by Vpr. However, neither HIV-2 nor simian immunodeficiency virus Vpr induces apoptosis in mammalian cell lines. Induction of apoptosis by several HIV-1 proteins, including Nef, Vif, Vpr, Vpu, Tat and Rev, has also been reported (Andersen et al., 2008; Chang et al., 2000; Conti et al., 2000; Stewart et al., 1999; Yedavalli et al., 2005).

Apoptosis studies indicate that mitochondrial intermembrane proteins, including adenine nucleotide translocator (ANT), apoptosis-inducing factor (AIF), cytochrome c, procaspases and heat-shock proteins (HSPs), are released during apoptosis and are essential for the activation of caspases and DNases. Several studies have demonstrated that HIV-1 Vpr permeabilizes the mitochondrial membrane by binding to the permeability transition pore complex (PTPC); lack of PTPC results in resistance to the apoptotic effect of Vpr. It has also been demonstrated that, in the presence of Vpr, cytochrome c is released from mitochondria, resulting in apoptosis. In fact, the mechanism responsible for mitochondrial-membrane permeabilization involves a number of proteins, including PTPC, proapoptotic members of the Bcl-2 family, ANT and the voltage-dependent anion channel (VDAC) (Jacotot et al., 2000).

The impact of Vpr on mitochondrial-membrane permeability is also attributed to its interaction with ANT in the inner mitochondrial membrane. It is believed that the interaction between Vpr and ANT cooperatively forms large conductance channels in the inner membrane. These results suggest that the interactions of Vpr with PTPC and ANT lead to the formation of channels in the outer and inner mitochondrial membranes, respectively. These events result in release of a number of molecules, such as cytochrome c from mitochondria (Jacotot et al., 2001; Yedavalli et al., 2005). The released cytochrome c molecules bind to Apaf-1 (apoptotic peptidase-activating factor 1) and form apoptosome complexes that become activated by caspase 9. Activated apoptosome complexes trigger the caspase cascade and apoptosis (Fig. 4) (Kim et al., 2005; Muthumani et al., 2002b; Zou et al., 2003).

It has been documented that a conserved motif in the C-terminal region of Vpr is responsible for the induction of apoptosis. Interestingly, the C-terminal peptides containing the conserved sequence at aa 71–82 can even induce apoptosis. Extracellular addition of C-terminal peptides to human CD4+ cells causes membrane permeabilization, DNA fragmentation and formation of apoptotic bodies, which are all signs of apoptosis. Similar effects can also be
observed by adding the polypeptides to yeast, indicating that Vpr targets fundamental cellular pathways common to many eukaryotic cells (Arunagiri et al., 1997).

HS1-associated protein X-1 (HAX-1) has also been demonstrated as a target for Vpr in the induction of apoptosis. HAX-1 is a proapoptotic factor that is found mainly in mitochondria. Current findings indicate that overexpression of HAX-1 inhibits Vpr-induced apoptosis. The number of molecules that have been found to be involved in Vpr-induced apoptosis is still increasing, suggesting that additional mechanisms and molecules may be utilized by Vpr for induction of apoptosis (Vafiadaki et al., 2006, 2009; Yedavalli et al., 2005).

Modulation of gene expression

Although the first reported function for Vpr was transactivation of the virus long terminal repeat (LTR), the mechanism has not yet been elucidated completely. The ability of Vpr to induce G2 arrest has also been linked to transactivation of the LTR. Transactivation activity of the LTR during G2 is five to ten times higher than that during G1; therefore, the reproduction level of wild-type HIV-1 is five to ten times higher than that of Vpr-mutated viruses, indicating the importance of transactivation by Vpr (Zhu et al., 2001). Indeed, Vpr transactivation activity is moderate and that from the HIV-1 Tat protein is stronger (Kino & Chrousos, 2004; Poon et al., 2007). Vpr affects not only virus replication, but also cellular gene expression, proliferation and differentiation. As Vpr circulates in the blood of HIV-1-infected individuals, it may also affect the gene expression of non-infected cells (Balasubramanyam et al., 2007; Xiao et al., 2008).

As already mentioned, Vpr enhances the activity of the HIV-1 promoter located in the LTR. This region is also a target for the p53 and Sp1 transcription factors. The ability of Vpr to stimulate the HIV-1 promoter is affected negatively in the presence of p53. Sp1 is a cellular transcription factor essential for the transcriptional activation of the LTR by Vpr (Pauls et al., 2006; Sawaya et al., 1998). Vpr specifically activates HIV-1 LTR-directed transcription on a minimal promoter containing a TATA box and the binding motifs for Sp1. Studies indicate that Vpr contains a leucine zipper-like domain, aa 60–81 in -helix III, which interacts with Sp1 when it is bound to the Sp1 motifs on the HIV-1 LTR (Wang et al., 1995, 1996). It has been reported that Vpr cooperates with nuclear-receptor coactivators p300/CBP and SRC-1a to activate the glucocorticoid-responsive mouse mammary tumor virus promoter. Several studies have also reported that Vpr transactivates the HIV-1 LTR promoter by direct interaction with the p300 coactivator. It has been suggested that Vpr recruits p300/CBP to transactivate the HIV-1 LTR (Kino et al., 2002).

Direct interaction between HIV-1 Vpr and the glucocorticoid receptor (GR) has also been reported to affect virus replication and gene expression. Human Vpr-interacting protein (hVIP) is also critical for the interaction of HIV-1 Vpr with GR. Glucocorticoids exert anti-inflammatory and immunosuppressive effects by interacting with their specific intracellular receptors, such as GR. Recent studies suggest that Vpr mimics some of the effects of glucocorticoids through interaction with GR (Ramanathan et al., 2002). Vpr has also been suggested to bind to the LTR through interaction with GR. Vpr shares the LXLL motif, aa 64–68, with steroid-receptor coactivators. This motif enables Vpr to bind to ligand-activated GR. These results suggest that Vpr functions as an adaptor molecule to...
connect the different molecules required for high promoter activity (Kino et al., 2002; Shrivastav et al., 2008).

HIV-1 Vpr has been demonstrated to bind specifically to the general transcription factor TFIIIB. The interacting motif of TFIIIB overlaps the domain of TFIIIB that is involved in the intramolecular bridge between its N and C termini. Binding of Vpr may induce a conformational change in TFIIIB that could possibly affect its activity (Agostini et al., 1996, 1999).

Nuclear factor κB (NF-κB) is one of the main mediators of the HIV-1 LTR. The N-terminal region of NF-κB mediates DNA binding, dimerization and interaction with inhibitory proteins. Activator protein 1 (AP-1) is also a transcription factor that has been suggested as a mediator for Vpr. Some studies have shown that the extracellular form of synthetic Vpr can stimulate the HIV-1 LTR via both AP-1 and NF-κB activation. Extracellular Vpr is commonly found in the blood of HIV-positive individuals and may be involved in the stimulation of virus transactivation. Although no receptor has been yet identified for extracellular Vpr, studies using confocal microscopy indicate that it can enter cells easily, and it is believed that even this form of the protein enhances transcription of the promoters that interact with NF-κB and AP-1 (Varin et al., 2005).

Upregulation of human survivin at the transcriptional level by HIV-1 Vpr has also been reported. Human survivin is involved in inhibition of apoptosis and regulation of cell division. The survivin gene is regulated in a cell cycle-dependent manner. Vpr specifically upregulates survivin expression through a cell cycle-regulated mechanism. It is still not clear what elements on the survivin gene are used in survivin upregulation by Vpr (Zhu et al., 2003).

The transactivation activity of Vpr is related to the cis-acting elements within the U3 region of the HIV-1 LTR, and the binding sites for several proteins, including Sp1, NF-κB, AP-1 and GRE, also lie in this region. The impact of Vpr on Nef expression from unintegrated HIV-1 has recently been demonstrated, suggesting that Vpr increases Nef expression before integration (Poon et al., 2007; Varin et al., 2005).

**Suppression of immune activation**

This function of Vpr is related closely to the effect of Vpr on gene expression but, because suppression of immune activation could be a consequence of gene expression, it is considered an independent function. Although many studies indicate that HIV-1 Env, Tat and Nef affect immune responses (Muthumani et al., 2005), the immune suppression observed in HIV-1-infected patients is also partly attributed to Vpr. This protein suppresses antigen-specific CD8+ mediated cytotoxic T-lymphocyte (CTL) and T-helper type 1 (Th1) immune responses. The molecular mechanisms of the suppression of CTL and antibody production by Vpr are still under debate, but it has been suggested that Vpr may prevent production of antibodies against the virus by inhibiting T-cell clonal expansion through inducing G2 arrest and suppressing T-cell proliferation. Suppression of the host inflammatory responses by Vpr has also been reported. Vpr down-regulates proinflammatory cytokines and chemokines, resulting in inhibition of the host inflammatory responses (Muthumani et al., 2004a; Zhao et al., 2005). A recent study also suggests that Vpr may alter sensitivity to insulin and thereby play a role in the development of lipodystrophy and insulin resistance (Shrivastav et al., 2008).

*In vitro* studies have implicated the ability of Vpr to downregulate the expression of several immunologically important molecules, such as CD40, CD80, CD83 and CD86, on macrophages and dendritic cells. Vpr reduces the capacity of monocytes to mature to dendritic cells in tissue culture (Muthumani et al., 2004b). A recent study indicates that Vpr impairs natural killer (NK) cell function in *vitro*. Production of gamma interferon is reduced in these impaired NK cells (Majumder et al., 2008).

Many of the experiments indicating that Vpr suppresses immune-cell activation and cytokine production have been performed *in vitro*. *In vitro*, Vpr targets and suppresses NF-κB activity, which is critical for T-cell activation and cytokine production. Similar results have been obtained from several *in vivo* studies (Ayyavoo et al., 2002; Muthumani et al., 2005). In a plasmid vaccine model using Vpr-expressing vectors, Vpr altered the induction of the CD8+ T-cell response. The mechanism of immune suppression could be complex and may be related to G2 arrest in T cells, suppression of NF-κB, induction of apoptosis in T cells and the GR pathway. Even in the absence of the effect of Vpr on CD8+ T cells, the immune response is affected by targeting antigen-presenting cells and T-helper cells, indicating the complexity of immune suppression by Vpr (Muthumani et al., 2002a).

Another explanation for the possible mechanism of immune suppression by Vpr focuses on the GR pathway. As already mentioned, the direct interaction between Vpr and GR is well established. Glucocorticoids are known to control inflammation and to induce immunosuppressive signals by binding to GR (Andersen & Planellès, 2005). Interestingly, the GR–Vpr complex is also able to induce similar effects and is suppressed by GR-pathway inhibitors. Apparently, Vpr mimics the interaction of glucocorticoids with GR to suppress immune activation (Moon & Yang, 2006; Muthumani et al., 2006).

**Fidelity of reverse transcription**

HIV-1 RT is an error-prone enzyme that misincorporates approximately one nucleotide in every 2000–5000 polymerized nucleotides (Basavapathruni & Anderson, 2007; Li et al., 1997). Reverse transcription of the HIV-1 genomic RNA is initiated in the virus core, where NC is associated with the RNA genome. The reverse-transcribed genome is directed toward the nucleus by the viral and cellular...
proteins forming the PIC (Gao et al., 2007; Sun et al., 1997). A number of studies have confirmed that Vpr co-localizes with IN and virus nucleic acids within the PIC, and remains associated with the viral DNA within 4–16 h after infection (Le Rouzic & Benichou, 2005).

HIV-1 Vpr interacts with a cellular protein, uracil-DNA glycosylase 2 (UNG2), which is a DNA-repair enzyme involved in nucleotide-excision repair. Uracil can be introduced into DNA either by cytosine deamination or by misincorporation of dUTP (Chen et al., 2004; Mansky et al., 2000). If the uracil is not repaired by UNG2, after the next round of replication, a C→T transition mutation occurs in that DNA strand and a G→A transition mutation occurs in the opposite strand of DNA. In the absence of Vpr, a fourfold increase in the rate of G→A mutations has been reported in each round of HIV-1 replication (Chen et al., 2002). These G→A mutations are the main mutational force in the evolution of drug-resistant HIV-1 strains (Berkhout & de Ronde, 2004). On the other hand, accumulation of these mutations can result in non-functional genomes (Keele et al., 2008; Priet et al., 2003). Vpr may play a role in virus evolution by balancing the level of mutations.

Several studies have established that the interaction of Vpr with UNG2 in order to incorporate it into HIV-1 virions correlates with the influence of Vpr on the HIV-1 mutation rate (Chen et al., 2002). The interaction between Vpr and UNG2 has been demonstrated both in vitro and ex vivo in Vpr-expressing cells. Vpr has been found to incorporate specifically the nuclear form of UNG2 into HIV-1 virions.

HIV-1 IN may also participate in the virion incorporation of UNG2, but the role of Vpr in the virion incorporation of UNG2 seems to be more essential and is correlated with the ability of Vpr to alter the mutation rate of HIV-1 (Chen et al., 2004; Mansky, 1996).

dUTPases are another class of enzymes that inhibit uracil incorporation into DNA during DNA synthesis, but utilize a mechanism distinct from that utilized by UNG2. Both UNG2 and dUTPase are encoded by some DNA viruses, such as poxviruses and herpesviruses. Some retroviruses encode only dUTPase. The genomes of non-primate lentiviruses, such as equine infectious anemia and feline immunodeficiency viruses, contain a dUTPase-encoding sequence as an integral part of the pol gene, lying between the RNase H and IN sequences. In contrast, the genomes of primate lentiviruses do not contain gene sequences encoding either dUTPase or UNG2. It is believed that the interaction of HIV-1 Vpr with cellular UNG2 compensates for the lack of a viral dUTPase to correct uracil misincorporation into virus DNA (Bouhamdan et al., 1996).

Vpr has been reported to interact through its N terminus with Lys–tRNA synthetase. This interaction has been observed both in vitro and ex vivo. Several cellular and viral proteins interact with Vpr and induce conformational changes that expose the N-terminal region of Vpr and thus regulate its affinity for Lys–tRNA synthetase. During the early stages of HIV replication, Vpr binds to NCp7, which may expose the N-terminal region of Vpr, resulting in a high affinity for Lys–tRNA synthetase. During the later stages of infection, the N-terminal region of Vpr interacts with the HIV-encoded p6, which may also affect Vpr interaction with the Lys–tRNA synthetase. As tRNA_Lys acts as a primer for initiation of reverse transcription of the HIV-1 genome, it suggests that this mechanism is utilized by Vpr to potentiate HIV-1 reverse transcription (Stark & Hay, 1998).

Additional interactions and possible functions of Vpr

HIV-1 Vpr has been observed to form ion channels in planar lipid bilayers, resulting in an inward sodium current followed by cell death in cell cultures. Mutagenesis studies have shown that the N-terminal region of Vpr is responsible for the above functions. A 40 aa peptide in the N-terminal region of Vpr is sufficient to form ion channels that are able to cause cell death. It is believed that this region is responsible for Vpr’s ion-channel activity and cytotoxic effects. These effects could be caused by the extracellular form of Vpr that is found in the serum and cerebrospinal fluid of AIDS patients (Piller et al., 1998, 1999). Extracellular Vpr can also disturb neuronal communication, resulting in neuronal dysfunction (Rom et al., 2009). It also inhibits neuronal development through the induction of mitochondrial dysfunction (Kitayama et al., 2008). Apparently, extracellular Vpr induces different levels of cytopathogenicity depending on the cell type (Huang et al., 2000).

The interaction of HHR23A, a protein involved in nucleotide-excision repair, with HIV-1 Vpr has been reported. It has been demonstrated that a C-terminal, 45 aa region of HHR23A binds to Vpr. Overexpression of HHR23A results in partial alleviation of the G2 arrest induced by Vpr. It has been suggested that the interaction between Vpr and HHR23A could be involved in the G2 arrest induced by Vpr (Engler et al., 2001; Withers-Ward et al., 1997).

HIV-1 Vif expression has recently been implicated in the inhibition of Vpr-mediated G2 arrest, and deletion of Vif from the HIV-1 genome results in an increase in G2 arrest induced by Vpr. In addition, T cells infected with Vif-deleted HIV-1 express higher levels of Vpr than cells infected with the wild-type virus. It has been suggested that inhibition of Vpr by Vif is mediated by proteasomal degradation, similar to the other proteins that are directed toward proteasomal degradation by Vif (Wang et al., 2008).

Concluding remarks

Interaction of a single protein with a variety of different proteins may seem surprising. Some proteins, such as...
proteinases, chaperones and ubiquitin, interact with different proteins and direct them toward one pathway, e.g. proteinases only degrade proteins. The amazing property of Vpr is that this small polypeptide interacts with a variety of proteins and directs them toward different pathways. Several hypotheses have been suggested to explain the capability of Vpr to exert so many effects through direct protein–protein interactions. One hypothesis suggests that Vpr possesses structural features similar to those of HSP70, a cellular chaperone, enabling Vpr to bind to many proteins with sufficient energy to cause changes in the activity of target proteins (Basanez & Zimmerberg, 2001).

In an attempt to find a common characteristic for the proteins that interact with Vpr, the WXXF motif (where X is unknown) was identified as an important criterion in some Vpr-interacting proteins. The WXXF motif of UNG2 was implicated in its interaction with Vpr. The N-terminal region of TFIIIB, which binds specifically to Vpr, also contains the WXXF motif. Mutant forms of TFIIB that have a point mutation in the WXXF motif are not able to interact with Vpr. Interestingly, it was demonstrated that, by attaching the WXXF motif to a non-human protein, chloramphenicol acetyltransferase, it was incorporated into HIV-1 virions in the presence of Vpr (Agostini et al., 1999; Bouhamdan et al., 1998; Yao et al., 2002). Although the main functions of Vpr have apparently been described, there are still several interactions between Vpr and proteins of which the functions remain to be elucidated.

References


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