Diversity of HIV-1 Vpr Interactions Involves Usage of the WXXF Motif of Host Cell Proteins

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Targeting protein or RNA moieties to specific cellular compartments may enhance their desired functions and specificities. Human immunodeficiency virus type I (HIV-1) encodes proteins in addition to Gag, Pol, and Env that are packaged into virus particles. One such retroviral-incorporated protein is Vpr, which is present in all primate lentiviruses. Vpr has been implicated in different roles within the HIV-1 life cycle. In testing a new hypothesis in which viral proteins are utilized as docking sites to incorporate protein moieties into virions, we used the peptide phage display approach to search for Vpr-specific binding peptides. In the present studies, we demonstrate that most of the peptides that bind to Vpr have a common motif, WXXF. More importantly, we demonstrate that the WXXF motif of uracil DNA glycosylase is implicated in the interaction of uracil DNA glycosylase with Vpr intracellularly. Finally, a dimer of the WXXF motif was fused to the chloramphenicol acetyl transferase (CAT) gene, and it was demonstrated that the WXXF dimer-CAT fusion protein construct produces CAT activity within virions in the presence of Vpr as a docking protein. This study provides a novel potential strategy in the targeting of antiviral agents to interfere with HIV-1 replication.

Human immunodeficiency virus type 1 (HIV-1)1 is a member of the lentivirus family. HIV-1 has a complex viral life cycle and utilizes multiple cellular and virally encoded regulatory proteins to tightly control its replication (1). The essential retroviral enzymes, reverse transcriptase, ribonuclease H, protease (PR), and integrase, lack cellular counterparts and have been used as targets for developing agents that inhibit virus replication (2–4). Despite considerable advances in anti-reverse transcriptase and PR therapy, it is obvious that ongoing genetic changes of the virus can confer drug resistance (5). This article must therefore be hereby marked "advertisement." The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement.

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Specific cellular compartment localization of therapeutic moieties influences their desired functions. Cytoplasmically localized anti-HIV-1 integrase single chain variable fragments inhibit HIV-1 infection of T-lymphocytes more potently than anti-integrase single chain variable fragments that are concentrated in nuclei (6). As well, functional ribozymes have also been tested by targeting these moieties into murine leukemia virus virions in comparison with cytoplasmically localized ribozymes (7). The concept of incorporation of foreign proteins into retrovirus particles has previously been reported, utilizing fusion with HIV-1 Gag and Vpr (8–12). Sorting of most cellular proteins into specific cellular compartments is determined by protein-protein interactions through specific domains (7). Membrane protein docking with target protein signal domains can incorporate target proteins into either the Golgi apparatus or mitochondria (7). Applying this target protein docking model for further molecular analyses is now possible due to the development of the phage display system.2 Short peptide libraries can be generated, and panning against any target protein for searching specific binding peptides can be accomplished. By engineering the target protein binding peptide into specific protein moieties, it should be possible to deliver the binding peptide protein fusion moieties into specific cellular or viral compartments.

HIV-1 encodes proteins in addition to Gag, Pol, and Env that are packaged into virus particles. These include Vpr, which is present in all primate lentiviruses (14). The virion-associated protein, Vpr, has been studied extensively with respect to understanding its role in lentivirus infection. Vpr is expressed relatively late in the viral life cycle and encodes a 14-kDa protein (15) that is predominantly localized in the nucleus of infected cells (16). Vpr has been reported to be incorporated into viral particles at molar quantities (16, 17). The carboxyterminal domain (p6 region) of the Gag polypeptide precursor, p55, plays a role in the packaging of Vpr into virions (18–21). None of these publications have shown evidence for a direct interaction between Vpr and p6 proteins. Recently, several groups have shown a direct interaction between Vpr and the nucleocapsid protein, p7 (22, 23). Roques and co-workers (23) show that the interaction between Ncp7 and Vpr occurs in vitro by a recognition mechanism requiring the zinc fingers of Ncp7 and the last 16 amino acids of Vpr (23). The authors suggest that Ncp7 cooperates, possibly with p6, to induce Vpr encapsidation in mature HIV-1 particles (23). Several biological functions of Vpr have been defined. It has been shown that Vpr is essential for optimal infection of macrophages (24–26). Vpr has been reported to influence the nuclear transport of the viral preintegration complex (27). Vpr activates transcription from the HIV-1 long terminal repeat (28–30), influences terminal

1 The abbreviations used are: HIV-1, human immunodeficiency virus type I; PR, protease; UDG, uracil DNA glycosylase; bp, base pair; CAT, chloramphenicol acetyltransferase; dWF, WXXF dimer; PCR, polymerase chain reaction; GST, glutathione S-transferase; WT, wild type; CMV, cytomegalovirus.

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Vpr and WXXF Motif

differentiation of some cell types (31), plays a role in the reactivation of viral gene expression, as demonstrated by addition of exogenous Vpr to cultures of latently infected T cell lines (32, 33), and causes blockage of cells in the G2 stage of the cell cycle (34–37). Recently, Chen and co-workers (38) showed that Vpr is capable of inducing apoptosis after cell cycle arrest. Other recent studies indicate that the Vpr protein can also associate with cellular proteins, such as glucocorticoid receptors (39), the transcription factors Sp1 and TFIIB (28, 30), or the uracil DNA glycosylase (UDG) enzyme involved in cellular DNA repair (40).

Since the highly conserved HIV-1 Vpr protein can be packaged in quantities within virions similar to those of the major structural proteins, this accessory protein may be used as a docking target to deliver anti-viral agents or other foreign proteins to progeny virus. In an attempt to understand how Vpr can interact with different viral and cellular proteins that are implicated in diverse virological and cell biological pathways, we used the “peptide phage-display” methodology to determine whether any common binding motif is shared among those proteins. We demonstrate that most of the peptides that bind to Vpr contain a common motif, WXXF. In this report, the domain-specific intracellular interactions of the WXXF with Vpr were further confirmed by utilizing a variety of complementary systems. Finally, to evaluate the feasibility of this peptide-based docking strategy, we have constructed a WXXF-CAT fusion protein and analyzed the ability of this fusion construct to be packaged into HIV-1 particles through interaction with Vpr. Our results show that the WXXF-CAT fusion protein is incorporated into HIV-1 particles through a Vpr-dependent docking mechanism. This study represents a new approach in the targeting of anti-viral agents with the ability to potentially interfere with HIV-1 replication.

MATERIALS AND METHODS

Plasmids—The HIV-1 molecular clones used in this study included pNL4-3 (41) and pNL4-3ΔVPR. These strains were obtained from the AIDS reagent repository (NIH). pNL4-3ΔVPR was created by deletion of a 1.1-kb EcoRI-BamHI fragment encoding the full-length Vpr reading frame. The murine leukemia virus-based retroviral expression vector, pSLXCMV-CAT, was used in these experiments and has been described previously (42). pSLXCMV-VPR-CAT was constructed by inserting a 304-bp BamHI-MluI fragment containing the vpr gene via BamHI-MluI sites of the polylinker region of the pSLXCMV vector. The stop codon of the vpr gene was removed and replaced by a MluI site to permit the fusion of the 677-bp MluI-BamHI fragment containing the CAT gene into pSLXCMV-VPR via MluI-BglII sites. The pSLXCMV-dWF-CAT was constructed by inserting a 76-bp BamHI-MluI fragment containing the WXXF dimer (dWF) into pSLXCMV-VPR-CAT in the BamHI-MluI sites. The dWF fragment was synthesized by the polymerase chain reaction (PCR) using WF-dimer-1 as a sense primer and WF-dimer-2 as an antisense primer. The construction of plasmids, GAL4 DNA binding domain (pGBT10), and GAL4 activation domain (pGADGH) have been described elsewhere (43). The UDG gene was used as a PCR template, corresponding to the cDNA UDG described previously (44). This gene was amplified by PCR using a 5′-primer, UDG2, containing EcoRI and SalI restriction sites, respectively, and cloned in-frame into the corresponding sites of pGBT10 to obtain the plasmid pGβ-UDG. Mutagenesis of the UDG gene was performed with complementary PCR oligonucleotides that contained the region of the gene with the desired mutations. Two rounds of PCR using the primers UDGD222, UDGD225, and UDGD152, were used to construct pUDGD222G, pUDGD225F, and pUDGD152L, respectively. The PCR products were then cloned back into EcoRI and SalI restriction sites of pGBT10. All the DNA fragments that corresponded to the peptides (peptides 1–10) were digested by PCR from purified phage DNA and then cloned in-frame into the corresponding sites of pGADGH (EcoRI-BglII) to obtain the plasmids pGAD-peptides (1–10). All new constructs were sequenced to verify reading frames and mutations.

His-tag Vpr Purification—Baculovirus expression vector, pACHis-SV-A-Vpr, was used to generate the recombinant Vpr-baculovirus.2 SF9 cells were infected with Vpr baculoviruses (1 × 10⁶ colony-forming units/ml). SF9 cells expressing His-tag Vpr were harvested by centrifugation. The cells were suspended in binding buffer (20 mM Tris-HCl, pH 7.9, 0.5 mM NaCl, 5 mM imidazole). After sonication, the cellular debris, which contains most of the expressed Vpr, was collected by centrifugation (4,000 g for 20 min at 20,000 × g for 5 min) and washed with binding buffer. The pellet was then dissolved in binding buffer containing 6 M guanidine and incubated at room temperature for 1 h. Insoluble material was removed by centrifugation (20,000 × g for 20 min), and the supernatant was mixed with 1 ml of pre-equilibrated His-Brin resin (Novagen) and incubated at 4 °C for 1 h with gentle rotation. Nonbound substances were washed from the resin with binding buffer containing 6 M guanidine and the binding protein was eluted with binding buffer containing 6 M guanidine and 0.5 M imidazole. After dialysis against water, the eluted purified His-tag Vpr was precipitated and redisolved in phosphate-buffered saline.

GST Fusion Protein Expression and Purification—The pGEX-GST-Vpr was constructed by inserting a 304-bp BamHI-HindIII fragment containing the vpr gene via BamHI-HindIII sites of pGEX-KG (44). The expression of the GST-Vpr fusion protein was performed as described previously (45). Protein expression was induced with 0.1 mM isopropyl-1-thio-β-D-galactopyranoside for 1 h. Cells were harvested by centrifugation and suspended in 10 ml of PBST (20 mM phosphate buffer, pH 7.3, 150 mM NaCl, 1% Triton X-100) containing 2 mM EDTA, 0.4 mM phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin, 5 μg/ml 1-chloro-3-ethyl-7-amino-2-heptanone, and 100 mM NaCl. The supernatant was applied to 1 ml of glutathione agarose column (Sigma) pre-equilibrated with TBST (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.5% Tween 20). After washing with phosphate-buffered saline, the bound GST-Vpr was eluted by 5 mM glutathione in 50 mM Tris-HCl, pH 8.0, 150 mM NaCl.

Phage-display Peptide Screening—A phage-display peptide library kit (New England Biolabs, Beverly, MA) was used to screen binding peptides. The kit contained a heptapeptide phage-display library. For phage panning, 50 mg of purified GST or GST-Vpr fusion protein was added directly to 50 ml of glutathione-agarose gel. After a 30-min incubation at 4 °C, the gel was washed with TBST. The phage library (2 × 10¹³ plaque-forming units in 250 ml of TBST) was added to the GST-containing gel, mixed, and incubated at room temperature for 1 h, then centrifuged at low speed for 2 min. Supernatant was washed with 250 ml of TBST. The nonbound phage was added to GST or GST-Vpr fusion protein at equal amounts. The mixtures were incubated at room temperature for 1 h then centrifuged and washed extensively with TBST to prepare the nonbinding phage. The binding phage was eluted with 100 ml of 5 mM reduced glutathione in TBS (50 mM Tris-HCl, pH 7.5, 150 mM NaCl). The binding specificity between GST and GST-Vpr was titered, as suggested by the protocol from this kit.

An alternative screening method will be described in detail elsewhere. Briefly, purified and denatured His-tag Vpr was dissolved in 6 M guanidine, and the His-Vpr protein was coated into a 12-well plate at 7 mg/ml in 100 mM Tris and 1 mM EDTA at room temperature overnight at 4 °C. The sample was washed thoroughly with TBST. The binding phages were eluted with a solution of the free His-tag Vpr protein. Binding specificity was compared by the titer between target protein Vpr-coated wells and non-target wells.

When highly specific binding phages were obtained, individual phages were isolated for reamplification by re-infection of Escherichia coli, and extracted phage DNA was sequenced using oligonucleotide 96gIII and autofluorescent-labeled DNA sequencing (ABI377 model).

Yeast Two-hybrid System—The yeast reporter strain, HFR7 (40) containing two GAL4-inducible reporter genes, HIS3 and LacZ, was co-transformed with plasmids, pGBUDG and pGDA-Vpr. Double transformants were plated on tryptophan-, leucine-, and histidine-deficient synthetic medium, and growth continued for 3 days. A liquid culture assay for β-galactosidase activity was performed as described (40) using SFY526 as a yeast reporter strain (46) and O-nitrophenyl-β-D-galactoside as a substrate. Each assay was performed in triplicate.

Transfections—293T cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum at 37 °C. Transfections were performed by standard calcium phosphate transfection (Promega, Inc.) 1 × 10⁶ 293T cells were co-transfected with 10 μg of pNL4-3 or pNL4-3ΔVPR DNA and 10 μg of pSLXCMV-CAT, pSLXCMV-VPR-CAT, or pSLXCMV-dWF-CAT, and then incubated in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum (growth medium) for 7 h post-transfection. This medium was then removed and replaced with fresh Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, and viruses...
containing supernatants and cells were collected at 48 h. The amount of virus present in transfected cell supernatants was determined by measuring HIV-1 p24 antigen levels using an enzyme-linked immunosorbent assay (Dupont).

**CAT Assays—**CAT assays were performed as described previously (39). Briefly, 1 × 10⁶ transfected cells were harvested by centrifugation. The pelleted cells were lysed in 0.9 ml of CAT lysis buffer (Promega, Inc.) and 50 μl of supernatants normalized for protein content and used for standard CAT assays. Virions obtained from transfected 293T supernatants were concentrated by ultracentrifugation (20,000 × g for 3 h in a A-621 Sorval rotor) and purified on 20%, 65% sucrose gradients (40,000 × g for 16 h in a TH-441 Sorval rotor). Each fraction was quantified by measuring HIV-1 p24 antigen levels. Virions from the peak were normalized for HIV-1 p24 antigen and finally lysed with 0.3 ml of Promega CAT lysis buffer at room temperature for 15 min then at 65 °C for 15 min. Lysed virions were assayed for CAT activity. CAT activity was detected by thin-layer chromatographic separation of [14C]labeled ampicillin from its acetylated derivatives and was quantitated by radioactivity counting in liquid scintillation. One hundred microliters of the lysed virions (p24 in ng/ml) was used in each CAT assay, with a fixed time of 3 h.

**Western Blot Analysis—**Yeast cells growing with similar density were lysed in radioimmunoprecipitation assay buffer, and immunoprecipitation of lysates was performed with a mouse anti-GAL4-DNA binding domain antibody (Tebu) and protein A-Sepharose beads. After washes in radioimmunoprecipitation assay buffer, bound proteins were subjected to 12% SDS-polyacrylamide gel electrophoresis, electrophoresis transferred to a polyvinylidene difluoride membrane (Amersham Pharmacia Biotech), and immunodetected with a rabbit anti-GAL4 binding domain antibody (Tebu) followed by horseradish peroxidase-linked swine antirabbit immunoglobulin (Dako). Antibody binding was demonstrated with ECL Western blotting detection reagents (Amersham). Underlined regions indicate base changes from point mutations.

5′ primer UDG1: 5′-AAAGAATTCCCCCTCTCCTCGGCGTGATGGCC-3′.
3′ primer UDG2: 5′-CCCCATGTAGGAGAAGGCCGTTAGTCG-GACTAATAC-3′. Mutated complementary primers UDG225: 5′-GGCTGGGAGCCGGGACTGTGAGTT-3′ and 3′-CCGGACCTGTGTCGTCGTTGACCCAGCTGCAA-5′. Mutated complementary primers UDG222: 5′-GAGCGGAGGCGGGAGCCTGTC-3′ and 3′-CTGGCTCCGCCGCCTGGTCA-3′. Mutated complementary primers UDG152: 5′-CATGAGCCATCCTAGCTGCAAGGCTC-3′ and 3′-GTACCTGGATTAGATCGAGT-3′. Mutated complementary primers UDG2: 5′-GAATTCCTCTGTACGAGGCG-3′.
WF-dimer 1: 5′-GGGATCCATCAGCGGCGGCCTGGTGTGCTTTTTTGGC-3′.
WF-dimer 2: 5′-ACGCGTGGGGCCATGAAAGACCCACCAAATGCTGCTCCGCCCG-3′.
CC-3′ 96gIII sequencing primer: 5′-CCCTCATAGTGGAGCTGTAACG-3′.

**RESULTS**

**Mapping of Vpr-specific Binding Peptides—**Phage display was used to identify peptides that bind to the HIV-1 Vpr protein. Purified and denatured His-tag Vpr produced from baculovirus was used by coating plates as the target protein to screen the binding phage for Vpr interactions. The His-tag Vpr showed a high titer of bound phage compared with bovine serum albumin alone after second round panning (data not shown). To increase the binding specificity of phage to Vpr, a serum albumin alone after second round panning (data not shown) was used by coating plates as the target protein to screen the binding phage for Vpr interactions. The His-tag Vpr was further tested by using the yeast two-hybrid system, yielding binding to Vpr. The yeast strain HF7c was co-transformed with a plasmid expressing the Vpr protein fused to GAL4DB and plasmids expressing each of the peptides (1–10) fused to GAL4AD. Double transformants were screened for the ability to grow on His-deficient medium. As shown in Fig. 2, nine peptides were positive for interaction with Vpr. The peptides 3, 6, 7, and 9 showed a strong interaction, and peptides 4 and 5 demonstrated a moderate interaction, as illustrated by their ability to grow on His-deficient medium. The results of this analysis with all peptides indicated that the

![Fig. 1. Biopanning a heptapeptide library against His-tag Vpr and GST-Vpr. Three rounds of selection/amplification were carried out at constant stringency (0.5% Tween 20).](http://www.jbc.org/content/jbc/80/15/8011/fig/1.jpg)
peptides that contained the WXXF motif can bind to Vpr. No interaction was detected with peptide 2, which didn’t contain the WXXF motif.

**Binding of Vpr and UDG Mutants**—We recently reported that Vpr was able to bind tightly to and coimmunoprecipitated with UDG (40), the major uracil DNA glycosylase in human cells (47). Examination of the human UDG amino acid sequence indicates that this protein contains a WXXF motif (amino acids 222–225). The region of UDG protein containing the WXXF motif is conserved between different species, as shown in Table I by alignment of protein sequences of uracil DNA glycosylases.

To test the possible correlation between the WXXF motif of UDG and the interaction with Vpr, we analyzed the abilities of UDG mutants to bind to Vpr. Point mutations were constructed in the WXXF motif of UDG by PCR as described under “Materials and Methods.” All of these mutants were fused to GAL4BD and then assayed in yeast cells (HF7c) for interaction with the GAL4AD-Vpr hybrid. These mutants were first tested for protein stability in yeast cells by Western blotting of transfected cells with a Gal4BD antibody (Fig. 3A). All of the mutants were expressed at approximately similar levels as the wild-type UDG. The yeast strain, HF7c, was co-transformed with a plasmid expressing the Vpr protein fused to GAL4AD and plasmids expressing UDG mutants fused to GAL4BD. Double transformants were screened for the ability to grow on selective medium without histidine. GAL4AD alone was used as a negative control. ++, strong interaction; +, moderate interaction; + or −, weak interaction.

**Table I**

| Protein | WXXF Motif | Histidine |
|---------|------------|-----------|
| HUM     | WXXF       | +         |
| YEA     | WXXF       | −         |
| HSV1    | WXXF       | +         |
| HSV2    | WXXF       | +         |
| EBV     | WXXF       | +         |

**Fig. 2.** Specific interaction of Vpr with peptides containing the WXXF motif in the yeast two-hybrid system.

The HF7c reporter strain expressing pairs of hybrid proteins fused to GAL4BD and to GAL4AD was analyzed for histidine auxotrophy. Double transformants were patched on selective medium without histidine. GAL4AD alone was used as a negative control. ++, strong interaction; +, moderate interaction; + or −, weak interaction.
fected with HIV-1\textsubscript{WT}/dWF demonstrated significantly higher CAT activity compared with the virions from 293T cells co-transfected with HIV-1\textsubscript{D}VPR/dWF (28\% compared with 8\%, respectively, lanes 7 and 8 of Fig. 5B). This CAT activity can also be compared with CAT activity detected in virions from 293T cells co-transfected with HIV-1\textsubscript{WT}/Vpr-CAT and to the virions from 293T cells co-transfected with HIV-1\textsubscript{WT}/CAT (37\% compared with 8\%, respectively, lanes 5 and 6 of Fig. 5B) as positive controls. No difference was found in the CAT activity for fraction 11 (density of 1.19 g/ml), which was comparable to the background activity (data not shown). These results indicate that CAT activity was primarily associated specifically to the HIV-1 virions. The cell extracts from 293T cells co-transfected with these different plasmids demonstrated very similar CAT activity (Fig. 5B, lanes 1–4).

These data indicate that the W\textsubscript{XX}F motif is (i) sufficient to

![Image](http://www.jbc.org/Downloadedfrom)
deliver the CAT protein into HIV-1 virions by interaction with Vpr and (ii) allows CAT to retain its enzymatic activity upon fusion with WXXF.

DISCUSSION
The highly conserved HIV-1 Vpr protein expressed in the late stage of viral production (15) and incorporated into virions (16) was an ideal target for testing the docking protein model for intravirion protein delivery. HIV-1 Vpr functional studies also show that this protein has a diverse phenotype via interactions with multiple cellular or viral structural proteins (22–28, 30, 31, 34–37, 39, 40). These multiple interactions indicate the possibility of Vpr-protein binding through specific domains, which may be carried in the different target proteins.

To identify such potential Vpr-specific binding domains, random peptide libraries constructed in a phage-display system were used for initial screening. Subsequently, each of the identified Vpr-binding peptides were further confirmed by using the yeast two-hybrid system for intracellular interactions of Vpr and peptides. More than 32 different Vpr binding phages were identified after a third round of panning using both denatured His-Vpr or native GST-Vpr fusion proteins in different panning systems. Interestingly, the majority of the peptides binding to Vpr revealed by these assays contained a consensus motif, WXXF. Intracellular binding of Vpr and peptides was subsequently confirmed from 10 selected Vpr-binding phage-displayed peptides by using the yeast two-hybrid system. Computer searching with the WXXF motif against protein data bases revealed several cellular proteins containing the WXXF motif. One of these, UDG, the major uracil DNA glycosylase in human cells (47), has been reported in our recent work and has demonstrated that UDG specifically binds to Vpr (40). More importantly, we now demonstrate that the WXXF motif of UDG is implicated in the interaction of UDG with the Vpr protein, as mutants of the UDG motif bind differentially to Vpr. The mutant F225G lost its ability to interact with Vpr, and the mutant W222G lost more than 60% of the binding with Vpr. However, we could not exclude the participation of the first or second X of the WXXF motif in the interaction, since an appropriate conformation of the WXXF motif may be important. It is unclear at present why Vpr interacts with UDG, a DNA repair enzyme. A recent report excludes the involvement of UDG in contributing to G$_2$ arrest of cells. Mutational analysis of Vpr showed that binding to UDG is neither necessary nor sufficient for its effect on the cell cycle (52).

UDGs and deoxyuraciltriphosphate pyrophosphatases (dUTPs) are thought to prevent the misincorporation of deoxyuracil into DNA during DNA synthesis. Since UDG is not involved in the G$_2$ checkpoint, the hypothesis that association of Vpr and UDG may perform a role similar to that played by the dUTPases of non-primate lentiviruses is quite possible, i.e. the reduction of uracil misincorporation into proviral DNA. Recently, Turelli et al. (53) reported that dUTPase minus caprine arthritis encephalitis virus accumulates G-to-A substitutions in vivo (53). Mansky (54) reported that the vpr gene partially accounts for the lower than predicted in vivo mutation rate of HIV-1. A vpr-negative shuttle vector had an overall mutation rate as much as 4-fold higher than that of the parental vector (54).

We recently reported that TFIIB, a basal transcription factor, binds to Vpr (28). The portion of Vpr that interacts specifically with TFIIB ranges from amino acids 15 to 77. Also, it was indicated that the NH$_2$-terminal domain of TFIIB is required for this interaction. Interestingly, we located the WXXF motif in the NH$_2$-terminal domain of TFIIB. Preliminary data indicate that mutants of TFIIB, which have a point mutation in the WXXF motif, lose their ability to interact with Vpr (data not illustrated).

Vpr has many different potential roles in HIV-1 expression. Vpr is able to transactivate several heterologous viral promoters lacking a common DNA sequence element (29). It has been shown that the addition of exogenous Vpr can reactivate HIV-1 replication in latently infected cell lines, indicating that Vpr could play a role in increasing HIV-1 expression through transcriptional or translational events (32, 33). Also, Vpr has been reported to influence the regulation of some cellular functions. Vpr induces terminal differentiation of rhabdomyosarcoma cells (31) and causes arrest in the G$_2$/M phase of the cell cycle (34–37), and recently this viral protein has been shown to be capable of inducing apoptosis after cell cycle arrest (38). All of these effects by Vpr are probably mediated by interactions with cellular proteins. Our findings could assist in the screening of proteins that are implicated in these functions and have the motif WXXF. We have already demonstrated the potential implications of this motif (WXXF) for UDG and TFIIB in their interactions with Vpr. Because of the strict conformation of UDG, it is formally possible that other regions are important for the interaction with Vpr. UDG is an enzyme that is extraordinarily specific in its function and so must have a very strict and defined three-dimensional conformation (48).

Recently, Chen and co-workers (55) demonstrated a weak
interaction between Vpr and the cellular DNA repair protein, HH23A. The authors show that the carboxyl-terminal 45-amino acid region of HH23A interacts with Vpr. Interestingly, this carboxyl-terminal region has 2 Phe residues that are conserved between different species. This may explain the weak interaction of this protein with Vpr (55). Of interest, we show in Fig. 3 that the UDG mutant, W222G, lost 60% of its interaction with Vpr.

Since the Vpr protein can be packaged into virions in quantities similar to those of the major structural proteins (16, 17), this protein has been used to target fusion proteins to progeny virus. Serio et al. (56) have generated a chimeric protein based on Vpr utilizing the conserved protease cleavage site sequences from Gag and Gag-Pol precursor polyproteins as fusion partners. Kappes and co-workers (8, 9, 13, 57) have generated chimeric proteins based on HIV-1 Vpr and HIV-2 Vpx, utilizing

**Fig. 5. Virion association of enzymatically active CAT fusion proteins.** A, HIV-1 virions collected from the culture supernatants of 293T cells co-transfected with pSLXCMV-CAT, pSLXCMV-VPR-CAT, or pSLXCMV-dWF-CAT were sedimented in gradients of 20–65% sucrose. Fractions of 0.7 ml were collected and analyzed for HIV-1 p24 antigen. B, CAT enzyme activity was determined for either the fraction corresponding to the peak (Fraction 9, lanes 5–8) or the cell extract (lanes 1–4) by standard methods. Lanes 1 and 5, NL4-3WT/pSLXCMV-CAT (WT/CAT); Lanes 2 and 6, NL4-3WT/pSLXCMV-VPR-CAT (WT/VPR-CAT); Lanes 3 and 7, NL4-3WT/pSLXCMV-dWF-CAT (WT/dWF-CAT); Lanes 4 and 8, NL4-3VPR/pSLXCMV-dWF-CAT (VPR/dWF-CAT). CAT activity was detected by thin-layer chromatographic separation of [14C]chloramphenicol from its acetylated derivatives and was quantitated by radioactivity counting in liquid scintillation. The experiment was repeated three times with similar results in each replicate.
CAT, staphylococcal nuclease, wild-type and mutated HIV-1 protease, integrate, and reverse transcriptase. All of these reports use Vpr or Vpx as partners for chimeric proteins. As HIV-1 Vpr may functionally block the cell cycle, possibly involving the cellular apoptosis pathways, fusion proteins with Vpr for therapeutic purposes will be difficult to utilize, as this retroviral protein may (i) perturb viral replication, (ii) lead to viral escape, and (iii) interfere in cellular functions. In this study, a new strategy is proposed. HIV-1 Vpr was targeted as a docking protein, by which one could target anti-viral agents or any foreign proteins fused to the WXXF motif into HIV-1 virions.

We base on our demonstration that the WXXF motif in fusion with CAT, staphylococcal nuclease, wild-type and mutated HIV-1 Vpr and WXXF motif docking protein, by which one could target anti-viral agents or any foreign proteins fused to the WXXF motif into HIV-1 virions. This study illustrates a potential new strategy in the targeting of these approaches.

The data presented here reconstitute in vivo and with interaction by this motif, one can deliver a fusion protein into the HIV-1 virion through a new docking strategy. The interesting features of the chimeric proteins generated here are the minimal addition of residues (22 amino acids) and the use of Vpr not as a partner for the fusion protein but as a receptor for docking to deliver this fusion protein into virions. This study illustrates a potential new strategy in the targeting of anti-viral agents into virions that may permit a feasible avenue to interfere with HIV-1 replication in vivo.

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