Human Immunodeficiency Virus Type 1 Vpr Interacts with Antiapoptotic Mitochondrial Protein HAX-1

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Human immunodeficiency virus type 1 viral protein R (Vpr) is required for viral pathogenesis and has been implicated in T-cell apoptosis through its activation of caspase 3 and caspase 9 and perturbation of mitochondrial membrane potential. To understand better Vpr-mitochondria interaction, we report here the identification of antiapoptotic mitochondrial protein HAX-1 as a novel Vpr target. We show that Vpr and HAX-1 physically associate with each other. Overexpression of Vpr in cells dislocates HAX-1 from its normal residence in mitochondria and creates mitochondrial instability and cell death. Conversely, overexpression of HAX-1 suppressed the proapoptotic activity of Vpr.

Apoptosis or programmed cell death contributes to the elimination of damaged, aged, or virus-infected cells (13). Apoptosis can be initiated by an extrinsic pathway in which death receptors expressed at the cell surface trigger receptor-activation of caspases leading to mitochondrial membrane permeabilization (MMP) (6, 18, 42). Alternatively, a cell-intrinsic apoptotic pathway can act directly on mitochondria, leading first to mitochondrial membrane permeabilization and then activation of execution caspasases (6, 18, 30, 42). MMP is tightly regulated by Bcl2 family proteins which contain both pro- and antiapoptotic members (2, 5, 29). Once triggered, MMP marks the “point of no return” for the apoptotic process (29, 58), whether it be a caspase-dependent or caspase-independent death (26, 39, 50). Because apoptosis is used as a means by the host to defend against invading pathogens, viruses understandably have evolved strategies that target the intrinsic and extrinsic apoptotic pathways. Increasingly, examples illustrate that many viruses, including human immunodeficiency virus type 1 (HIV-1), hepatitis B virus, Sindbis virus, and baculovirus, encode proteins that modulate cell death (reviewed in reference 4).

Human immunodeficiency virus (HIV) principally infects T helper (T41) cells and cells of the monocyte-macrophage lineage, which express the CD4 cell surface protein. The gradual and selective loss of the CD4 subset of T-lymphocytes is a central feature of the pathogenesis of HIV which correlates with the progression from asymptomatic HIV infection to AIDS. Several mechanisms have been proposed to explain this decline, including the rapid turnover and death of infected host cells, as well as “bystander” cell death via indirect means (17). Moreover, several HIV-1 proteins, including Nef, Vif, Vpr, Vpu, Tat, and Rev, have been implicated in apoptosis induction.

HIV-1 Vpr, a 96-amino-acid, 14-kDa protein, is critically involved in HIV-1 pathogenesis in vivo (10, 15, 16). Several functions have been attributed to Vpr including (i) interaction with and translocation of the HIV-1 preintegration complex through the nuclear pore, (ii) induction of apoptosis, (iii) induction of host cell cycle arrest during G2-to-M transition, and (iv) stimulation of viral gene expression (1, 7, 9, 12, 14, 19–21, 25, 32, 40, 41, 43, 46, 48, 53, 55, 60, 61). Studies have documented Vpr-induced apoptosis in human fibroblasts, T-cell lines, and primary cells, including lymphocytes and monocytes (1, 23, 38, 44, 47). Indeed, death of uninfected bystander T cells has also been attributed to secreted Vpr protein. Among several explanations, a leading mechanistic model suggests that Vpr induces cellular apoptosis through dysregulation of MMP. Using isolated mitochondria, others have found that Vpr can target the mitochondrial permeability transition pore complex and promote permeabilization of mitochondrial membranes (23). Whether Vpr’s mitochondrion effect can be entirely explained through its binding of inner mitochondria membrane protein, adenine nucleotide translocator (ANT), remains to be clarified (52).

Apoptosis of infected cells may mute the host’s immune response to the virus (54). In this regard, we wanted to further understand the details of Vpr’s interaction with mitochondria and its apoptotic consequences. Here, we report the identification of HAX-1 (for HS1-associated protein X-1) as a new mitochondrial target for Vpr. HS-1 (for hematopoietic lineage cell-specific protein 1) is a B-cell signaling protein that is a substrate for intracellular protein tyrosine kinases involved in
the immune response to extracellular stimuli and in cell differentiation induced by cytokines. HAX-1 was initially reported as an HS-1-binding protein; HAX-1 is a 279-amino-acid (35-kDa) protein with homology to Bcl2. HAX-1 has been shown by others to be an antiapoptotic factor (51). We now document that (i) Vpr binds HAX-1 directly, (ii) overexpression of Vpr causes the egress of HAX-1 from the mitochondria into the cytoplasm, and (iii) overexpression of HAX-1 counters the proapoptotic effect of Vpr in cells.

**MATERIALS AND METHODS**

Plasmids, cell culture, and transfections. HeLa cells were maintained in Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum. Full-length and deletion mutants were generated by PCR amplification and cloning. Plasmids pCDNA-Vpr, pCDNA-FLAG Vpr, and pEYFP-Vpr were constructed by PCR amplification of pNL4-3 Vpr and cloning of PCR products into pCDNA3.1 and pEYFP vectors, respectively. pCDNA-HAX-1 and pCDNA-HA-HAX-1 were constructed by cloning HAX-1 ORF into pCDNA3.1 vector (Invitrogen). HeLa cells were transfected with Lipofectamine Plus and Lipofectamine according to the manufacturer’s instructions (Invitrogen).

Antibodies. Mouse monoclonal anti-HA and anti-FLAG M2 antibodies were commercially purchased (Sigma-Aldrich); rabbit polyclonal 46-amino-acid antibody was from the AIDS Reference and Reagent Program. Mouse monoclonal anti-HAX-1 antibody was from BD Pharmingen.

Western blotting, immunoprecipitation, and confocal imaging. Western blotting and immunoprecipitation were performed as previously described (28, 56). For confocal microscopy, HeLa cells were cultured on 25-mm coverslips (Thomas Scientific, Swedesboro, NJ) and transfected with plasmid DNA. One day later, cells were fixed with 3.7% formaldehyde, permeabilized with phosphate-buffered saline containing 0.1% Triton X-100 and 1 mg of bovine serum albumin/ml, and 1 mM EDTA) with RIPA (Tris-buffered saline [pH 8.0] containing 1% Triton X-100 or NP-40, 1 mg of bovine serum albumin/ml, and 1 mM EDTA) with Leica laser-scanning microscope. For staining of cells with Mitotracker (Invitrogen), cells were washed and stained with DAPI. We scored GFP-positive cells for 15 min in the incubator before washing and fixation for staining.

Coimmunoprecipitation. MT4/HeLa cells were lysed in radioimmunoprecipitation assay (RIPA) buffer (Tris-buffered saline [pH 8.0] containing 1% Triton X-100 or NP-40, 1 mg of bovine serum albumin/ml, and 1 mM EDTA) with protease inhibitor (phenylmethylsulfonyl fluoride and aproitinin [10 µg/ml]), 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate (SDS). Cell lysates were prepared by sonication and precleared first with anti-mouse/anti-rabbit immunoglobulin G-agarose before incubation with either anti-HAX-1 or Anti-Vpr antibody. Immune complexes were captured by using protein A- or G-agarose, followed by SDS-PAGE, followed by autoradiography. A portion of the reaction mixture was also analyzed by Coomassie blue staining to visualize GST fusion proteins.

**RESULTS**

Identification of HAX-1 as a Vpr-interacting protein. To search for Vpr-associated factor(s), we performed a yeast two-hybrid library screen using as bait a fusion protein comprised of full-length Vpr fused to the LexA protein (LexA-Vpr). HAX-1 was initially identified from an open-ended screening of the library. The specificity of the interactions between Vpr and HAX-1 was next verified by directly transforming yeast cells with isolated plasmids of LexA-Vpr and GalAD-HAX-1 (HAX-1 fused with the Gal4 activation domain); positive interaction between the two proteins was indicated by blue colonies on an X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) plate (Fig. 1A). Control transformation of yeast with LexA-lamin plus GalAD-HAX-1 produced no blue colonies, supporting the specificity of Vpr–HAX-1 interaction.

Vpr binds to HAX-1 in vitro and inside cells. To check further interaction between Vpr and HAX-1, GST pull-down assays were performed with recombinant GST-Vpr fusion protein and in vitro-synthesized 35S-labeled HAX-1 protein. Proteins captured by the beads were washed, solubilized, and resolved by SDS-PAGE, followed by autoradiography. As shown in Fig. 1B, GST-Vpr fusion protein but not GST alone captured HAX-1 (Fig. 1B, compare lanes 2 and 3); this result is fully consistent with a physical protein-protein interaction between Vpr and HAX-1.

To examine Vpr-HAX-1 interaction inside cells, we next attempted to coimmunoprecipitate cell-endogenous HAX-1 with Vpr expressed from an infecting HIV-1 molecular clone, NL4-3. MT4 cells were infected with HIV-1 molecular clone NL4-3 and harvested 4 days postinfection. Cells lysed in RIPA buffer were sonicated and immunoprecipitated with anti-Vpr antibody. The immunoprecipitates were resolved by SDS-PAGE and analyzed by Western blotting with anti-HAX-1 antibody. As shown in Fig. 1C (lane 4), HAX-1 was found to coprecipitate with Vpr, a finding consistent with the results from yeast two-hybrid (Fig. 1A) and GST pull-down (Fig. 1B) assays. Taken together, the results suggest that the Vpr interacts directly with cell endogenous HAX-1 in HIV-1-infected cells.

Expression of DN HAX-1 mutant induces apoptosis. Previously, it was reported that HAX-1 contained limited homology with the BH1 and BH2 domains of Bcl-2 family members (45, 51). Elsewhere, in other experimental systems, HAX-1 was found to be a mitochondria protein (51), which when overexpressed blocked Bax-induced apoptosis (45). Formally, it has not been fully clarified whether HAX-1 serves a direct role in preventing cellular apoptosis or only plays an indirect role in influencing the expression, stability, and/or function of Bax. To address HAX-1 function in our experimental setting, we constructed several deletion mutants. We reasoned that some or all of the mutants might be dominant negative (DN) for HAX-1 function. One prediction is that if HAX-1 were to play a constitutive role in suppressing cellular apoptosis, then expression of DN HAX-1 should produce cell death. To check this prediction, we introduced wild-type HAX-1, HAX-1(142-173) mutant, or HAX-1(1-141) mutant separately into HeLa cells, along with a green fluorescent protein (GFP) plasmid that marked the transfected cells. After 36 to 48 h, cells were fixed and stained with DAPI. We scored GFP-positive cells for

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apoptosis based on the visualization of condensed nuclei with nuclear fragmentation. We found that neither full-length HAX-1 nor HAX-1(142-279) (Fig. 1, Fig. 2, Fig. 3, Fig. 4, Fig. 5 A1 through 6) induced apoptosis. However, overexpression of HAX-1 (1–141) produced frequent nuclear condensation and fragmentation consistent with apoptosis (Fig. 2A7 to A12). An interpretation of these results is that HAX-1(142-279) is a loss-of-function mutant, whereas HAX-1(1-141) is a DN mutant. The finding that DN HAX-1(1-141) induced apoptosis is consistent with an intrinsically protective role served by cell-endogenous HAX-1 against cell death.

**Vpr-induced apoptosis is suppressed by overexpression of HAX-1.** Several studies have shown that expression of Vpr causes apoptotic cell death (22, 23, 38, 44, 47). Because Vpr binds HAX-1 (Fig. 1) and because DN-HAX-1, in itself, promoted apoptosis (Fig. 2A), we wondered whether Vpr's effect on cell death might arise from its physical sequestration of limiting amounts of intracellular HAX-1. To explore this pos-
FIG. 2. Vpr-induced cell death is suppressed by HAX-1 overexpression. (A) HAX-1 influences cellular apoptosis. Expression of DN HAX-1 induces cell death in HeLa cells. pcDNA HAX-1 WT, pcDNA HAX-1(1-141), and pcDNA HAX-1(142-279) were cotransfected with pcCMV-GFP into HeLa cells. At 36 to 48 h posttransfection, cells were fixed and stained with the nuclear stain DAPI. The transfected GFP-expressing cells were examined by microscopy for differential interference contrast (DIC), DAPI, and green fluorescence. pcDNA HAX-1(1-141) expression in transfected cells resulted in significant cell death (compare subpanels 9 and 12 with subpanels 3 and 6). Apoptotic cells were detected as GFP-positive cells with nuclear condensation and fragmentation. (B) HAX-1 suppressed Vpr-induced cell death. HeLa cells were transfected with HAX-1 and Vpr as indicated along with cytomegalovirus (CMV)-GFP as a marker for transfected cells. Overexpression of HAX-1 suppressed
Vpr-induced cell death (compare subpanels 2 to 4 with subpanels 6 to 8). (C) Western blot analysis of the expression of Vpr and HAX-1 in transfected HeLa cells. Cell lysates prepared from transfections above (Fig. 2B) were analyzed by blotting for expression of the indicated proteins from transiently transfected plasmids. The top row shows detection of transfected HAX-1 with a light exposure of film. The middle row shows detection of Flag-tagged transfected Vpr. The bottom row shows β-actin signals as loading controls. (D) Graphic representation of suppression cell death induced by HIV-1 Vpr. Cell viability of transfected cells was observed 36 to 48 h posttransfection by counting the number of live green (total cells) and apoptotic green (apoptotic cells) fluorescent cells under the microscope. Values are representative of three independent assays.

Vpr's capacity to induce cell death. In our cultured cells, we observed that nuclear fragmentation rapidly ensued after Vpr expression (Fig. 2B2-4). Provocatively, when HAX-1 was coexpressed with Vpr in cells, mortality of recipient cells normally expected from receiving Vpr-alone disappeared (Fig. 2B, compare panels 6 to 8 to 2 to 4). These results are compatible with Vpr’s proapoptotic effect being manifest through its sequestration of cell-endogenous HAX-1 and with...
such sequestration being competitively muted by overexpression of exogenous HAX-1. In Fig. 2C, we performed control Western blotting to monitor the degree of expression of transfected HAX-1 and Vpr. Indeed, based on Western blotting, it was evident that the level of expression of transfected HAX-1 exceeds the amount detected for cell endogenous HAX-1 (Fig. 2C, compare lanes 1 and 3 to lanes 2 and 4).

We next quantified the ability of HAX-1 to suppress Vpr-
induced apoptosis. In Fig. 2D, we counted the number of GFP-positive cells (total cells) and the number of green cells that were apoptotic (apoptotic cells). We found that Vpr-induced apoptosis was reduced by threefold when HAX-1 was coexpressed in trans. Expression of HAX-1-alone, Vpr1-70 mutant alone or of Vpr1-70 mutant plus HAX-1 produced apoptosis similar to the controls cells transfected with pCDNA3 vector.

Mutually altered cellular localizations of HAX-1 and Vpr upon simultaneous overexpression. Although Vpr is generally located at the nucleus and nuclear membrane, some investigators have found its additional presence in mitochondria (22). On the other hand, HAX-1 is largely mitochondrial and is found rarely in the endoplasmic reticulum (51). To verify the notion that Vpr might sequester HAX-1 and perturb the latter’s activity, we investigated whether the two proteins show some level of colocalization inside cells. We transfected HeLa cells with either Flag-Vpr (Fig. 3A1) or GFP-HAX-1 (Fig. 3A2) or both (Fig. 3A3 and 4). Cells were fixed 24 h later and stained with anti-Flag antibody, as well as visualized for GFP. We found that GFP-HAX-1 (Fig. 3A2) showed a speckled pattern that mirrored Mitotracker staining of mitochondria (Fig. 3B3); on the other hand, Flag-Vpr alone was diffusely nuclear (Fig. 3A1). Intriguingly, coexpression of GFP-HAX-1 with Flag-Vpr resulted in a remarkable redistribution of both molecules (Fig. 3A3 and 4). Under such conditions, Flag-Vpr relocated from the nucleus into the cytoplasm, whereas GFP-HAX-1 departed the mitochondria to appear with the cytoplasmic Flag-Vpr. The redistributed Vpr-HAX-1 bodies do not colocalize with Golgi, endoplasmic reticulum, or mitochondria (data not shown). Currently, it is not clear whether these bodies localize with bona fide cytoplasmic organelles.

As controls, we also visualized mitochondrion-resident GFP-Bcl2 (Fig. 3A5) and GFP-Bcl2 plus Flag-Vpr (Fig. 3A6). Here, simultaneous expression of Bcl2 and Vpr had neither a significantly perturbing nor a colocalizing effect on each other (compare Fig. 3A3, 4). A minor “blurring” by confocal visualization of Bcl2 in the mitochondria may be due to perturbation of mitochondrial membrane potential by Vpr. Overall, our findings suggest that Vpr has a specific interaction with HAX-1 but not Bcl2. Next, to make sure that our results are not idiosyncratic to GFP-tagged HAX-1, we also examined the behavior of an hemagglutinin (HA)-tagged HAX-1 (Fig. 3B). In agreement with GFP-HAX-1, HA-HAX-1 was also dislocated from the mitochondria by overexpressed Flag-Vpr (compare Fig. 3B4). Altogether, our results are compatible with a scenario in which the mitochondrion-intrinsic cell-protective function of HAX-1 is disturbed by Vpr expression.

Physical interaction between Vpr and HAX-1 correlates with apoptosis. To characterize how HAX-1 is targeted by Vpr, we sought to better understand the domain in Vpr responsible for this function. We tested one point (VprS79A) and two deletion [Vpr(1-70) and Vpr(1-51)] mutants of Vpr (Fig. 4A and B) for their association with HAX-1. As shown in Fig. 4A, two distinct intracellular profiles were observed. VprS79A was entirely nuclear (Fig. 4A1), whereas Vpr(1-70) (Fig. 4A4) and Vpr(1-50) (data not shown) were located in extranuclear speckles. Interestingly, when the Vpr mutants were coexpressed with HAX-1, two different patterns were seen. VprS79A exited the nucleus and colocalized with HAX-1 (Fig. 4A3). On the other hand, neither Vpr1-70 (Fig. 4A4) nor Vpr1-51 (data not shown) appeared to colocalize with HAX-1, nor did these two mutants perturb HAX-1’s normal mitochondrial location (Fig. 4A6).

We also checked the region of interaction between HAX-1 and Vpr by coimmunoprecipitations. We transfected HA-tagged HAX-1 with YFP alone, YFP-Vpr, YFP-Vpr(1-70) and YFP-Vpr(1-51) (Fig. 4B) into cells. Cell lysates were immunoprecipitated with anti-HA, and immunoprecipitates were Western blotted with anti-YFP. As shown in Fig. 4B, YFP-Vpr coppedipitated with HAX-1, a finding consistent with the results from yeast two-hybrid (Fig. 1A) and GST pull down (Fig. 1B) and earlier coimmunoprecipitation (Fig. 1C) assays. In contrast, Ypr(1-70) and Vpr(1-51) failed to associate with HAX-1 (Fig. 4B, lanes 3 and 4), suggesting that the C terminus of Vpr spanning amino acids 71 to 96 is required for interaction with HAX-1.

We also examined our transfected cells for signs of apoptosis (Fig. 4C). We found agreement with previous data on Vpr-induced apoptosis. Hence, Jacotot et al. (23) had reported that full-length Vpr and a carboxy-Vpr form (VprS2-95), but not an amino-Vpr form [Vpr(1-51) and Vpr(1-70)], triggered cellular apoptosis. Our data are consistent with those earlier results and further suggest a correlation between Vpr-induced apoptosis and Vpr’s ability to associate with HAX-1. Thus, we observed that Vpr proteins competent or incompetent for HAX-1 binding are, respectively, active or inactive for inducing apoptosis (Fig. 4C).

Sequestration of Vpr and suppression of apoptosis maps to amino acids 118 to 141 of HAX-1. Our results to this point are consistent with a model in which Vpr sequesters and dislocates mitochondrion-protective HAX-1, leading to cellular apoptosis. This model predicts that there should be a HAX-1 mutant which, when overexpressed, would bind Vpr competitively and prevent Vpr’s disturbance of wild-type HAX-1 function. To check this prediction, we constructed several overlapping HAX-1 deletion mutants (Fig. 5B). In Fig. 5A, we observed that some HAX-1 deletion mutants showed minor differences from wild-type HAX-1 in intracellular localization (Fig. 3). Interestingly, all mutants, with the exception of HAX-1(141-279), were competent for sequestering Vpr (Fig. 5A and B).

When we examined the functional consequences of HAX-1 mutant overexpression, a one-to-one correlation emerged between HAX-1 mutants capable of binding Vpr and their ability to suppress Vpr-induced apoptosis in cells (Fig. 5B). These results are complementary to the above findings (Fig. 4) and demonstrate that overexpressed HAX-1 mutants can competitively capture Vpr and abrogate Vpr’s ability to perturb the limiting apoptosis-protective function of cell-endogenous mitochondria-located HAX-1. We quantified that overexpression of HAX-1 mutants [HAX-1(81-279), HAX-1(91-279), HAX-1(118-279), and HAX-1(1-129)] suppressed Vpr induced apoptosis by ~2-3-fold (data not shown). On the other hand, HAX-1(141-279) failed entirely to suppress Vpr-induced apoptosis. One interpretation of these results is that HAX-1 is required to stabilize mitochondria and that Vpr can physically bind and dislocate HAX-1 from mitochondria leading to destabilized MMP and apoptosis.
Mitochondrial physiology is intimately tied to cellular apoptosis (18, 31, 57). One hypothesis for mitochondrial cell death involves changes in MMP culminating in the release of apoptogenic factors such as cytochrome c and apoptosis inhibiting factor from the mitochondrial intermembrane space (34, 35, 49, 59). Whether cells live or die is tied to a balance between the actions of antiapoptotic (e.g., Bcl-2/Bcl-XL) and proapoptotic (e.g., Bax, ANT-1, and VDAC) factors. Within this context, HAX-1 is an antiapoptotic factor thought most likely to be located at the outer mitochondrial membrane (8). Overexpression of HAX-1 has been shown previously to prevent Bax-induced MMP and the subsequent apoptosis of cells (45).

Vpr has been found to perturb MMP (23). Previously, Jacotot et al. (23) used a synthetic HIV-1 Vpr peptide to study its interaction with isolated mitochondria in vitro. At micromolar concentrations of Vpr, these authors observed rapid dissipation of MMP; this change in potential correlated with a leakage from purified mitochondria of apoptogenic proteins such as cytochrome c. Similarly, in Jurkat T cells, Vpr expression also produced aberrant MMP with accompanying apoptosis (23, 37). Currently, Vpr’s apoptogenic MMP effect has been largely attributed to its interaction with an inner mitochondrial membrane protein, ANT-1 (22, 23). Although Vpr-ANT-1 interaction is consistent with many extant findings, it remains perplexing how an interaction or disturbance at the inner mitochondrial membrane mechanistically provokes perforation of the outer membrane leading to the release of apoptogenic factors normally resident in the intermembranous space.

Our new finding that an outer membrane factor, HAX-1, is also targeted by Vpr clarifies how HIV-1 might fully perforate both layers of mitochondria membranes leading to apoptosis. Our Vpr-HAX-1 finding also validates a prediction made by Jacotot et al. based on their Vpr52-96 results that, separate from ANT-1, Vpr must have a second target located in the outer mitochondrial membrane (22). The current finding that overexpression of HAX-1 is sufficient to abolish Vpr-induced apoptosis in cells (Fig. 2) is further consistent with our interpretation and suggests that for purposes of apoptosis, stabilization of the outer mitochondrial membrane is dominant over destabilization of the inner membrane (i.e., via ANT-1).

HAX-1 was originally identified by yeast two-hybrid screen as a protein that associates with HS-1 (for hematopoietic lineage cell-specific protein 1). HS-1 is a B-cell signaling protein and is a substrate for intracellular protein tyrosine kinases involved in the immune response to extracellular stimuli and in cell differentiation induced by cytokines (51). HAX-1 possesses two Bcl2 homologous domains BH1 and BH2 which are conserved among Bcl-2 family proteins. Additional amino acid sequences in HAX-1 retain similarities with apoptosis regulating protein Nip3, which has been found to bind antiapoptotic factor Bcl2 (51). Indeed, recent findings have also shown that HAX-1 can directly bind to Bcl2, which is also predominantly located in the outer mitochondrial membrane (36). It is interesting that two other human viruses, Epstein-Barr virus and Kaposi’s sarcoma herpevirus, have also been found to encode proteins that target HAX-1 (11, 27, 45). Currently, it is not fully understood why disparate viruses would commonly converge on this mitochondrial protein. Potentially, if apoptosis of
FIG. 5. Mapping of amino acids 118 to 141 of HAX-1 as a region of interaction with Vpr. (A) Confocal examination of expression and distribution of HAX-1 in HeLa cells. Fluorescent images indicate that the various HAX-1 deletion mutants are either capable or not capable of sequestering Vpr. (B) Diagrammatic summary of HAX-1 deletion mutant correlating the ability to sequester Vpr with ability to halt Vpr-induced apoptosis. Bcl2 homology domains 1 and 2 (BH1 and -2, green), PEST sequence (PEST, red), and transmembrane domain (TMD, brown) are indicated.
infected cells is one way for viruses to evade the elicitation of a vigorous host immune response, then it could be reasonable why Epstein-Barr virus, Kaposi’s sarcoma herpesvirus, and HIV-1 would share this interaction. If this reasoning is correct, then the discovery of small molecules which might interdict Vpr-HAX-1 interaction could contribute as an adjunct to the better development of an immunity-eliciting AIDS vaccine.

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