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Review

Human immunodeficiency virus type 1 (HIV-1) Vpr-regulated cell death: insights into mechanism

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Abstract

The destruction of CD4$^+$ T cells and eventual induction of immunodeficiency is a hallmark of the human immunodeficiency virus type 1 infection (HIV-1). However, the mechanism of this destruction remains unresolved. Several auxiliary proteins have been proposed to play a role in this aspect of HIV pathogenesis including a 14 kDa protein named viral protein R (Vpr). Vpr has been implicated in the regulation of various cellular functions including apoptosis, cell cycle arrest, differentiation, and immune suppression. However, the mechanism(s) involved in Vpr-mediated apoptosis remains unresolved, and several proposed mechanisms for these effects are under investigation. In this review, we discuss the possibility that some of these proposed pathways might converge to modulate Vpr's behavior. Further, we also discuss caveats and future directions for investigation of the interesting biology of this HIV accessory gene.
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Abbreviations: AIDS, Acquired Immunodeficiency syndrome; HIV-1, human immunodeficiency virus type 1; Vpr, viral protein R; NF-κB, nuclear factor kappa B; GR, glucocorticoid receptor; VIP-1, Vpr-interacting protein-1; CSN, COP9 signalosome; HSP, heat-shock protein

Introduction

A hallmark of human immunodeficiency virus type 1 (HIV-1) infection has been CD4$^+$ T-ell depletion and eventual immunodeficiency. Reports from different groups have suggested that various HIV proteins are involved in this process.[1] One of these proteins is viral protein R (Vpr), a 14 kDa accessory gene, implicated in various functions including cell cycle arrest, nuclear migration, and apoptosis.[2,3] Importantly, several key observations augment the importance of Vpr in engendering HIV-1 pathogenesis. First, mutations to key amino acids of Vpr have been associated with long-term nonprogressive HIV infections.[4] Second, patient-erived viral isolates with normal capability to replicate but without cytotoxicity have been described. These viruses contain Q3R mutations and premature stop codons in *vpr*.[5] Lastly, experiments involving Vpr-deleted viruses have suggested that Vpr is necessary for the infection of macrophages and nondividing CD4$^+$ T cells by facilitating nuclear entry.[6,7] Collectively, such evidence suggests that Vpr is an important cytotoxic component of HIV-1 infection (Table 1).

Although early experiments concentrated on the role of Vpr within a virus-intact infection setting, recent evidence suggests a physiological role for virus-free extracellular Vpr. For instance, a functional Vpr protein has been purified from serum and cerebrospinal fluid of infected patients.[8] The purified Vpr has been shown to have transactivational and cytotoxic properties in CD4$^+$ T cells and neurons, respectively.[8–10] Such results suggest that Vpr's cellular effects can manifest through various mechanisms including entry of free Vpr into uninfected, activated CD4$^+$ T cells for destruction. Accordingly, we review the questions surrounding the biochemical mechanism of Vpr-induced cell death. Other reviews have previously described the elucidated mechanism of Vpr-regulated cell death,[3,11,12] but this review will concentrate on the puzzles and enigmas associated with this death.

Role of the Glucocorticoid Pathway in Regulating Vpr-induced Cell Death

The functional interaction between the glucocorticoid signaling pathway and HIV-1 Vpr initially was uncovered with the finding of the physical interaction of these two molecules.[13] The protein complex was proposed to consist of glucocorticoid receptor (GR), Vpr, and a Vpr receptor-interacting protein (RIP-1), although other molecules may be part of the complex. This RIP-1 was later renamed the Vpr-interacting protein-1 (VIP-1) (also known as Mov34) when it was cloned via the yeast two-hybrid system.[14] Mutational analysis suggested that the carboxyl-terminus of VIP-1 was necessary for the physical interaction with the GR–Vpr complex.[15] However, the complete physiological role for VIP-1 remains speculative

Table 1 Cellular activities exerted by Vpr

Cellular activities	Vpr's effect
Viral replication	Acts in *trans* at the level of transcription to enhance virus replication Reactivation of viral replication from latency Influences nuclear transport Regulates nuclear import of the HIV-1 preintegration complex Micronuclei formation and aneuploidy induction Nuclear pore complex misassembly and nuclear envelope herneations
Cellular differentiation and growth arrest	Host cell differentiation Cell cycle arrest at G2/M stage Cell growth arrest in yeast Changes in host cell transcription
Host cell interaction	Translocates 34-kDa Mov34 homologue (hVIP) HHR23A phosphorylation Associates with uracil DNA glycosylase Interaction with adenine nucleotide translocator Interaction with cyclophilin A Binds to 14-3-3 proteins
Steroid pathways	SRC GR-II complex interaction Influences the transcription of GR-responsive genes
Apoptosis	*In vivo* tumor growth suppression Induction of apoptosis via a mitochondrial permeability transition pore Mitochondrial membrane potential depolarization Induces apoptosis through both caspase-dependent and -independent pathways
Suppression of immune activation	NF-κB suppression Negative regulation of β-chemokines and cytokines Inhibits dendritic cell maturation IκB upregulation
Transcription	Conformational changes in TFIIB Induces transcription of the HIV-1 and glucocorticoid-responsive promoters by binding directly to p300/CBP coactivators Cooperative actions by Vpr and p53 Promote transactivation by CREB
Kinase effect	Depletion of Wee1 kinase and Cdc25 Activates cell cycle inhibitor p21/Waf1/Cip1 Inhibition of p34CDC2 activity

because the experiments were conducted in an overexpression model and because a true *loss of function* experiment remains elusive. However, what information is available on this preserved member of the Vpr receptor–GR complex is interesting. For example, VIP-1 knockout mice have been shown to be embryonically lethal in mice shortly after transplantation into the blastocyst, suggesting a necessary role of this gene product in embryogenesis. In addition, antisense-mediated inhibition of VIP-1 expression in tumor cells lead to cell cycle arrest in a manner similar to Vpr treatment of cells, suggesting a role for VIP-1 in the cell cycle arrest function of Vpr (Figure 1a). However, whether the interaction between Vpr and VIP-1 is required for Vpr to manifest its cell cycle arrest remains undetermined.[14,16] Nonetheless, the activity of this complex also suggests a potential role for GR in the Vpr–VIP-1-mediated cellular effects. Specifically, it is believed that Vpr-mediated activation of GR regulates the nuclear migration of both VIP-1 and GR. Furthermore, this is further augmented by GR antagonists such as Mifepristone (Mif), which can reverse the Vpr-stimulated nuclear migration of VIP-1. However, an association with these results and Vpr's pleiotropic functions remain speculative as true *loss of function* experiments have yet to have been conducted.

The GR antagonist Mif was implemented in various studies to further probe these important molecular relationships. It was reported that Vpr functioned in a manner similar to steroids by inhibiting NF-kappa B (NF-κB) activation, perhaps through the upregulation of IκB-α.[17–19] Secondly, Mif was sufficient to reverse the cellular apoptosis phenotype of Vpr-treated cells, suggesting that at least the interaction between Vpr–GR was necessary for apoptosis (Figure 1b). A report from Kino *et al.*[20] also proposed that Vpr augments the transcriptional potential of the steroid–GR complex by functioning as a coactivator. These results, in conjunction with the ability of GR activation to stimulate cell death, provided clues into the claim that GR activation by Vpr is necessary for apoptosis.

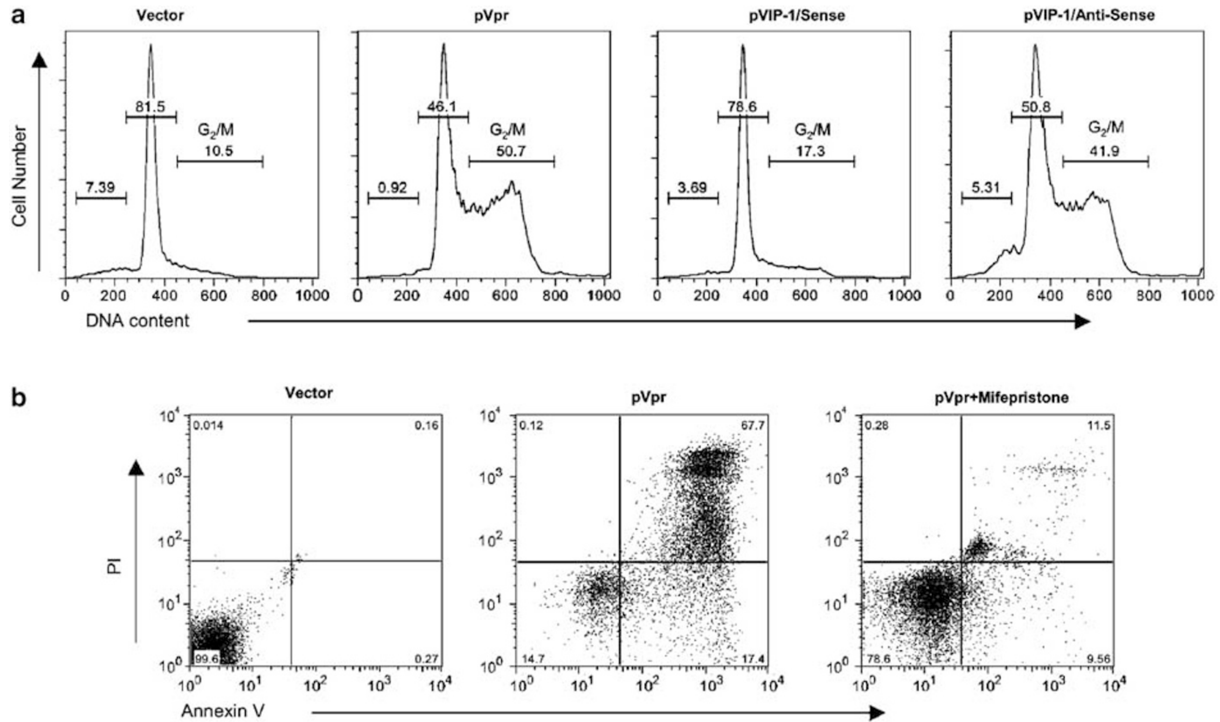

Figure 1 (**a**) VIP-1 is necessary for cell cycle progression through the G_2/M phase. HeLa cells were transfected with 5 μg of vector, pVpr, pVIP-1/sense, and pVIP-1/antisense expression vectors with pBabepurom (1 μg) (a vector that express the pruomycin gene), and the cells were maintained in DMEM medium containing puromycin (2 μg/ml).[14] Cells were harvested 36 h later, fixed in 1% formaldehyde–70% ethanol, and then incubated in phosphate-buffered saline (PBS) that contained PI (50 μg/ml), RNase A (50 μg/ml), and fetal calf serum (2% (vol/vol)) to analyze their DNA content using BD Cycletest™ Plus (BD PharMingen, USA). The fluorescence of 10 000 cells was analyzed directly on a Coulter EPICS® Flow Cytometer (Coulter, Hialeah, FL, USA) using FlowJo software (TreeStar, San Carlos, CA, USA).[21,46] (**b**) Vpr interaction with GR is required for Vpr-induced apoptosis. Flow cytometry analysis of Jurkat cells transfected with 5 μg of Vector, pVpr in the presence or absence of Mif (1 μM) as indicated. Cells were collected 2 days post-transfection and stained with Annexin V as recommended by the manufacturer (PharMingen, CA, USA). In brief, the transfected cells (1×10^6) were harvested and washed twice with PBS (pH 7.2). Cells were resuspended in binding buffer (0.1 M HEPES (pH 7.4); 1.4 M NaCl; 25 mM $CaCl_2$), stained with Annexin V and vital dye (PI), and incubated for 30 min at room temperature in the dark. Cells were washed once with binding buffer, resuspended in 400 μl of binding buffer and, subsequently, analyzed on a Coulter EPICS® Flow Cytometer (Coulter, Hialeah, FL, USA) using FlowJo software (TreeStar, San Carlos, CA, USA)[21,48]

However, several questions still remain unanswered. First, is the transcriptional potential of GR necessary for Vpr to induce apoptosis? Although inductive extrapolation suggests that this could be a required pathway, as Vpr could mimic a steroid-like effect and hence stimulate apoptosis, experiments proving this hypothesis remains elusive. For instance, it would be of interest to determine first if $GR^{-/-}$ cells are intractable to Vpr-stimulated apoptosis, and if so, can a transcriptionally defective GR recover the apoptotic phenotype? Secondly, what is the proteomic relationship of the Vpr–GR complex? Since Vpr can function as a coactivator with this complex, it is interesting to hypothesize the biochemical property of this complex. Does Vpr recruit yet to be determined factor(s) into the complex, which can yield GR-independent phenotypes? Lastly, is the Vpr–GR signal pathway a stimulator of apoptosis or a potentiator? Considering that NF-κB plays an essential role in protecting against various apoptotic factors, it remains undetermined if Vpr merely potentiates cell death by inhibiting antiapoptotic pathways, or if it can directly induce cell death. Interestingly, treatment of Vpr in Jurkat cells that endogenously overexpress Bcl-2 is sufficient to down-regulate its expression.[21] However, whether this phenotype is a direct consequence of GR–Vpr-dependent signaling remains undetermined.

Role of the Vpr/VIP-1 Interaction in the Context of the COP9 Signalosome (CSN)

It is thought that Vpr interacts intracellularly with its ligand VIP-1 (Mov34) and this may have effects on GR-mediated apoptosis. In recent years, interesting insight has been acquired into the function of this interacting protein VIP-1. VIP-1 (a CSN6 protein) is a member of a family of proteins that make up the COP9 signalosome (CSN) complex. The CSN is a highly conserved, multifunctional protein complex that is comprised of eight different subunits, CSN1–CSN8.[22] Initially discovered in the mustard weed *Arabidopsis thaliana*, most or all of the subunits of this complex are encoded in the genomes of humans, *Caenorhabditis elegans*, *Drosophila melanogaster*, *Schizosaccharomyces pombe*, and *Schizosaccharomyces cerevisiae*. The characteristic feature of these proteins is the PCI/PINT and the MPN/Mov34 signature domains; six contain the PCI domain and two the MPN domain.[22,23] Interestingly, these two domains are found in two

other large protein complexes: the 26S proteasome lid complex and the eukaryotic translation initiation factor 3 (eIF3). In fact, CSN often copurifies with components of the proteasome and eIF3 in protein assays.[24]

Initially in *A. thaliana*, CSN was found to play a role in the ubiquination of the light-inducible HY5 transcription factor, preferentially targeting it for proteolysis during periods of darkness.[25] It has now been revealed that the CSN complex participates in a wide variety of cellular processes ranging from ubiquination to cell cycle regulation. A major target of CSN is the SCF ubiquitin ligase complex, a key E3 enzyme that catalyzes a key step in ubiquitin conjugation to proteins destined for destruction. CSN binds to a key component of the SCF, CUL1, and regulates its activity through the cleavage of a regulatory molecule Nedd8, a compound that neddlyates CUL1. Neddylation of CUL1 enhances the ability of SCF to ubiquinate proteins.[26–28] Thus, CSN is a negative regulator of ubiquination in this setting. Paradoxically, in genetic analysis, a role for CSN as a positive regulator of ubiquination has been observed. Interestingly, neddylation is required for ubiquitin-dependent proteolysis of $p27^{kip1}$, IκB, and HIF-1α. Through the regulation of p27 by the CSN complex, it is thought that CSN may play a role in regulating the cell cycle. In fact, microinjection of purified CSN temporarily blocks the S-phase entry in a deneddylation-dependent manner when injected into synchronized G1 cells. In addition, CSN1 and CSN2 also play a role in S-phase progression in *S. pombe*.[28–30]

CSN can also act as a protein kinase, phosphorylating *c*-Jun, IκBα, p105, and tumor suppressor p53. In this setting, it is thought that CSN targets p53 for degradation, meanwhile, stabilizing the *c*-Jun molecule and contributing to the JNK activation of AP-1. It is thought that the members of the CSN complex, namely CSN2 and CSN5, also play a role in nuclear hormone-mediated gene expression through the direct interaction with nuclear receptors such as the thyroid hormone receptor, the progesterone receptor, and with coactivators such as steroid receptor coactivator-1 (SRC-1).[22,25,31]

It is clear that the CSN complex plays an important role in the regulation of diverse cellular processes, but more work is necessary to further examine the role of the Vpr/VIP-1 interaction in the context of this signalosome (Figure 2). For instance, only one subunit (VIP-1) of CSN has been biochemically and genetically purified with Vpr thus far, suggesting that any mechanisms involving CSN in Vpr-mediated effects are currently speculative. It has been shown that eIF3/INT-6 interacts strongly with CSN6 and that *A. thaliana* deficient in CSN6 exhibit diverse developmental defects, including homeotic organ transformation, symmetric body organization, and organ boundary definition, and have a high level of ubiquinated proteins.[32,33] In addition, VIP-1 has been shown to be embryonically lethal in mice shortly after transplantation into the blastocyst, and antisense-mediated inhibition leads to cell cycle arrest. However, any conclusions drawn from Vpr–VIP-1 interaction as a causation of apoptosis or cell cycle arrest remains premature because *loss of function* experiments with antisense VIP-1 could be a result of various bystander effects including alterations in proteolysis, ubiquitination, translation, etc. Therefore, further work needs to be conducted on the effect of Vpr on mediating these and other CSN activities and the potential role of these interactions in regulating apoptosis.

Direct Effect on the Mitochondria Membrane Potential (MMP) by Vpr

Some of the earliest reports of Vpr's effects on apoptosis suggests that inhibiting caspase activation by overexpression of antiapoptotic genes or chemical treatment was sufficient to repress Vpr-induced apoptosis.[34,35] Pathways involving activation of both caspases 8 and 9 have been reported,

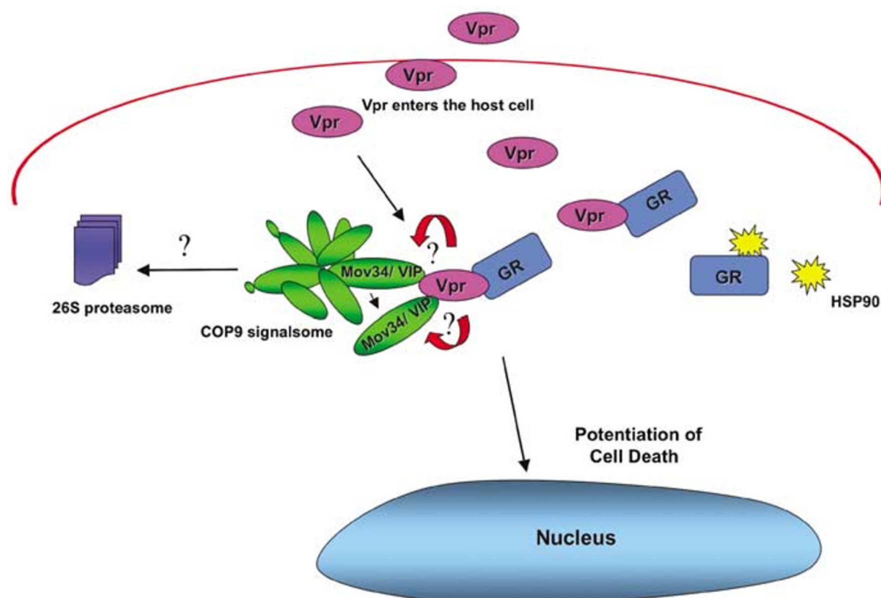

Figure 2 Possible interaction of Vpr and CSN through Mov34/VIP-1

although the former was in differentiated neuronal cells with gp120 cotreatment.[21,36] Recently, the hypothesis that mitochondria disruption is involved in Vpr-induced cell death has been fortified by various reports (Figure 3). A potential mechanism was postulated by Jacotot *et al.*,[37] who studied the effect of Vpr directly on isolated mitochondria, whereby it was reported to interact with the mitochondria transition pore complex. Specifically, the carboxyl-terminus of Vpr interacts with the adenine nucleotide translocator (ANT) in the nM range and forms large conductance channels and, consequently, decouples the respiratory chain and induces inner mitochondria depolarization.[38] The effect can be attenuated with the overexpression of Bcl-2, which prevents the interaction of Vpr with the complex. However, the role of the mitochondria gateway factors Bak/Bax in Vpr-mediated disruption of MMP remains undetermined,[39] although the fact that Vpr can directly interact with ANT suggests that Vpr may be an aberrant apoptotic molecule that can bypass this requirement.

Recently, an interesting report by Roumier *et al.*[40] applied *loss of function* experiments to determine the physiological role for the mitochondrial pathway for Vpr-induced cell death. The authors used cells deficient in caspase activators (APAF-1 and caspase 9) or AIF-1 and caspase 9 inhibitors to suggest that a caspase-independent mitochondrial death pathway was involved. However, considering that overexpression of Bcl-2 and the cytomegalovirus-encoded ANT-targeting protein vMIA is sufficient to prevent both Vpr-induced MMP and cell death, it indicates that another factor not yet determined is likely required for cell death to manifest. The purification of this factor(s) may answer several interesting questions regarding the role of Vpr and the mitochondria in cell death. First, if this is the necessary factor, it suggests that any observations of caspase action may just be bystander activation and not necessarily a causation factor. Second, if caspase activation is not necessary, then perhaps an ATP-independent form of cell death-including necrosis may also be physiologically important as observed in primary neuronal cells.[41] Lastly, the

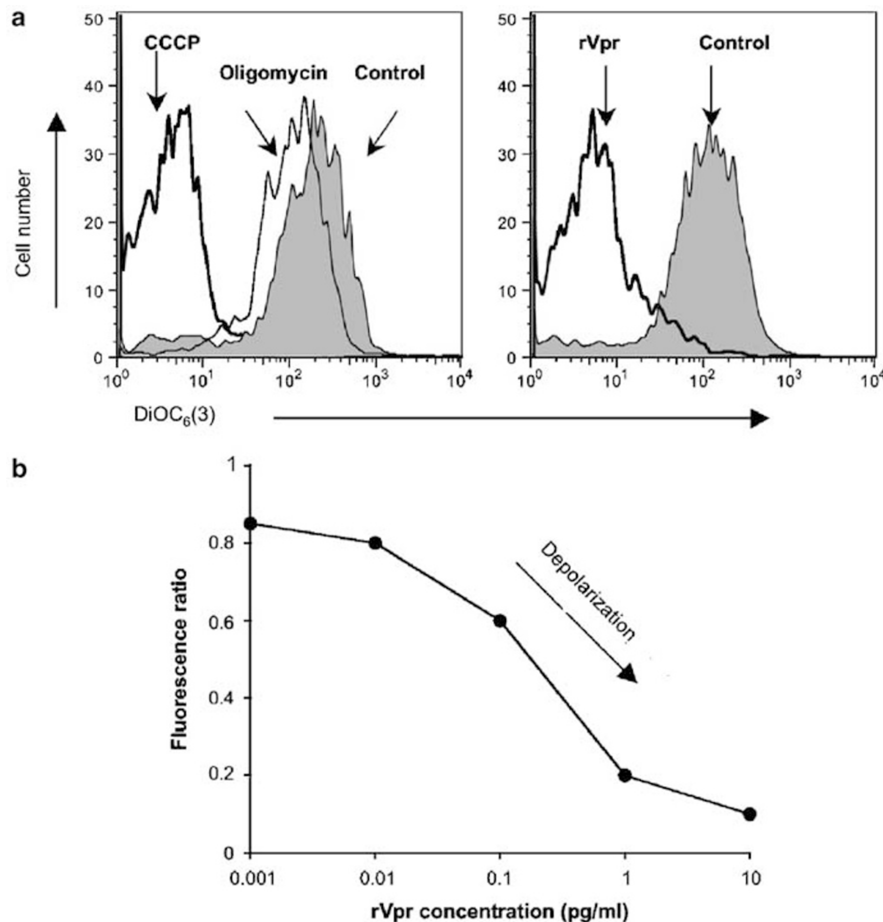

Figure 3 (**a**) Vpr disrupts the MMP. Representative FACS analysis of 3,3-dihexyloxacarbocyanine iodide (DiOc$_6$(3)) (Molecular Probes, USA)-labeled Jurkat T cells either were treated with untreated (control) or CCCP (50 μM, 37°C, 10 min; Molecular Probes, USA), a protonophore that abolishes $\Delta\Psi_{mt}$, or oligomycin (2.5 μg/ml, 37°C, 10 min; Sigma, USA), an uncoupling agent known to hyperpolarize the mitochondrial membranes or treated with recombinant Vpr protein (10 pg/ml). The cells were treated with the drug or rVpr for 24 h prior to the labeling with 25 nM DiOc$_6$(3).[37,38] DiOc$_6$(3) was excited at 488 nm, and fluorescence analyzed at 525 nm (FL-1). (**b**) Variations of the red/green (FL-2/FL-1) fluorescence ratio as a function of the Vpr concentration. Jurkat cells were treated with different concentrations of rVpr as indicated for 24 h prior to staining with the probe JC-1 (1 μM) (Molecular Probes, USA) and both green (FL-1) and red (FL-2) fluorescences were recorded. The green fluorescence refers to the JC-1 monomers and the red fluorescence corresponds to the formation of J-aggregates

possibility that the mitochondria is not necessary for apoptosis *per se*, but rather this unknown factor, raises intriguing teleological questions about the involvement of the mitochondria to potentiate this form of caspase-independent cell death. If the paradigm of mitochondria disruption is not required, then what is the benefit for HIV, and specifically Vpr, to target a critical organelle involved in various cellular homeostasis including ATP generation, metabolism, etc.? Indeed, it will be interesting to ascertain answers to these perplexing questions, which will provide important insight into the enigmatic behavior of HIV on host cells as influenced by Vpr.

Is there a Functional Link between Vpr-Induced Cell Cycle Arrest and Apoptosis?

Early observations from different cell lines suggested that Vpr possesses potent differentiating and antiproliferative properties.[42] The growth arrest was induced in the G_2/M phase with a concomitant hyperphosphorylation of p34cdc2.[43–45] Early observations suggested that cell cycle arrest and apoptosis were coupled as a direct correlation of the two were observed.[46,47] Further, cells grown with serum had a greater propensity for Vpr-induced toxicity, indicating that growth arrest was necessary for apoptosis.[48] It is also interesting to note that certain factors that regulate the cell cycle have been invariably associated with Vpr-induced death. First, RNAi experiments suggest that the depletion of the cell cycle inhibitory kinase Wee1 is necessary for Vpr to prompt apoptosis, as Wee1 overexpression attenuates cell death.[49] Secondly, Vpr was shown to mimic the DNA-alkylating agent nitrogen mustard for its effects on cell cycle arrest.[50] Furthermore, other DNA damage checkpoint factors, including ATR (ataxia-telangiectasia and Rad3-related), were shown to be required for Vpr to stimulate cell cycle arrest.[51] Lastly, both cell viability and viral gene expression regulated by Vpr correlated with its ability to regulate cell cycle arrest.[52] However, a caveat to these findings is that Vpr does not require the tumor suppressor protein p53 to manifest cell death.[21] Therefore, a complete linear pathway involving the activation of p53-dependent genes under DNA damage does not seem to be necessary for the Vpr-related effects.[53] Nonetheless, when taken together, these several results suggest that an intricate relationship might exist between these two processes.

However, an analysis of the differing motifs of Vpr suggests otherwise. For instance, Nishizawa *et al*.[54] have suggested that cell cycle arrest may not be necessary for apoptosis to transpire. Further, mere treatment of purified mitochondria with Vpr induces membrane potential dissipation, suggesting that cell cycle effects may not be directly required or may merely be an ancillary function to augment or potentiate cell death.[37] Similar dissection experiments have determined that these various functions including nuclear localization, apoptosis, cell cycle arrest, and transactivation do not require the same regional motifs of Vpr.[55–57] A caveat here is that *in vitro* experiments with isolated organelles provide important information but may not mimic the entire *in vivo* functions of Vpr's and interactions with the host cells. This is potentially due to lack of interaction or potentiation with other relevant host pathways. Although such mutagenesis studies have delineated the differing regions of Vpr necessary to manifest its pleiotropic functions, a complete repudiation of their dependence on one another is premature. For instance, many of these experiments were predicated on highly correlative patterns of phenotype, which fails to provide an unequivocal *loss of function* conclusion. This is important because there is a possibility that Vpr's interaction via a certain motif may potentiate another motif to functionally carry out its phenotype. Therefore, mutagenesis studies may suggest the dichotomy of these functions, even though they may be inexplicably linked. Therefore, a more comprehensive investigation involving the mix matching of differing mutants and subsequent recovery assays is likely warranted.

Heat-shock Protein 70 (HSP70) is an Antagonists of Vpr

The initial observation of any relationship between Vpr and HSP70 involved the compensation of HSP70 for Vpr in regulating the nuclear migration of HIV's preintegration complex.[58] Interestingly, mild heat shock or recombinant HSP70 was sufficient to impede viral replication, but failed to do so in viruses lacking Vpr. Furthermore, such heat-shock conditions actually augmented infection in Vpr-deleted viruses.[59] In addition, HSP70 overexpression was also sufficient to attenuate both cell cycle arrest and apoptosis, and RNAi of HSP70 increased the sensitivity of host cells for apoptosis, cell cycle arrest, and viral replication.[60] The authors also purified Vpr in complex with HSP70 with the mild detergent CHAPS, suggesting that a direct or indirect interaction is involved.[59,60] A fascinating question that arises is whether this interaction is a direct or a complex involving certain adaptors form. If a direct interaction is responsible, then it suggests that HSP70 perturbs Vpr's various functions by binding and nullifying it in a manner similar to a neutralizing antibody. However, if a complex is involved, HSP70 could prevent Vpr from binding to its target host receptor or could perturb the function of this receptor. If the latter of the possibilities is true, then it suggests that Vpr's interaction with this receptor is responsible for commencing its various pleiotropic phenotypes.

Levels of Vpr can Influence Apoptotic Sensitivity

Despite the many proapoptotic reports of Vpr activity discussed above, a potential caveat is that they were conducted predominantly in an overexpression setting. Reports by several groups have suggested that the quantity of *Vpr* expression may have different biological phenotypes. For instance, $CD4^+$ Jurkat or HEP-2 cells expressing low and constitutive levels of Vpr become more resistant to apoptosis rather than promoting or potentiating it.[61,62] Further, Jurkat $CD4^+$ T cells exposed to Vpr also exhibit an upregulation of Bcl-2 and a concomitant downregulation of Bax, suggesting that post-transcriptional regulation is involved in manifesting an antiapoptotic phenotype. These findings raise important questions regarding Vpr's pernicious *versus* benevolent

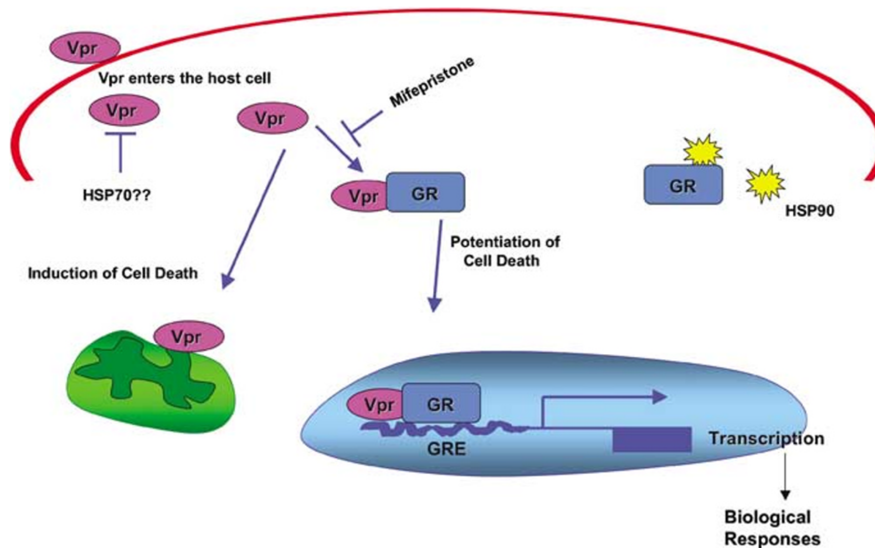

Figure 4 Vpr includes the convergence of two different pathways to induce apoptosis. Upon entry into cells, Vpr binds to the ANT to induce MMP and targets the GR and potentiates the cell death. Mif reverse the cellular apoptosis phenotype of Vpr-treated cells

functions. It is interesting to speculate as others have, if early entry and infection of the host cell by HIV-1 is associated with low expression to prevent the host cell from dying prior to high levels of viral particles being produced. These studies also raise the possibility that Vpr's cytotoxic effects may be important even as a shed protein.

Concluding Thoughts

Despite the influx of reports on the mechanism of Vpr-induced apoptosis, a universal model remains nebulous. Several reports have isolated proteins or interactions that are necessary for this death process to manifest (Figure 4). An early report suggested that the interaction of Vpr and GR was necessary for apoptosis to be initiated.[17] Studies also suggested that Vpr directly targets the mitochondria for membrane potential depolarization, suggesting that Vpr–ANT interaction is required.[37] Chen and colleagues, via RNAi-mediated *loss of function* experiments, concluded that the depletion of Wee1 is also required for Vpr to prompt apoptosis.[49] Lastly, investigations into the role of heat-shock proteins have determined that HSP70 is likely both necessary and sufficient to inhibit many of Vpr's destructive effects, which highlights its importance.[60] Despite these differences in complexes and targets, these studies do not preclude the potential of an inclusive model that requires all of these factors. It is also a possibility that each necessary component functions to initiate inductively apoptosis or potentiate the host to allow apoptosis to transpire. However, several remaining gaps need to be filled. First, the mechanism of HSP70-mediated attenuation of Vpr-induced cell death needs to be ascertained. Since HSP70 is sufficient to inhibit several aspects of Vpr's pleiotropic properties, it is likely that HSP70's effects are manifested early on during Vpr's cellular entry. Therefore, elucidation of the mechanism of HSP70's inhibition (i.e. direct interaction *versus* inhibition of Vpr's receptor function) will resolve some of these ambiguities and may clarify its functions compared to pathway inhibition. Second, the role of GR (i.e. transcriptional or mere interaction) and Vpr needs to be further resolved. Although the GR–Vpr interaction is necessary, whether Vpr functions exclusively as a steroid imitator or if other *gain of function* properties are involved in this activity remains undetermined. Lastly, it is clear that Vpr can directly target the mitochondria for depolarization, but whether this in itself is sufficient for driving apoptosis *in vivo* remains undetermined. For instance, Vpr's signaling through the GR pathway has been shown to inhibit the survival NF-κB pathway,[17] which can ideally repress antiapoptotic factors such as Bcl-2. Therefore, the elucidated factors and the pathways discussed may not be mutually exclusive.

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References

1. Emerman M and Malim MH (1998) HIV-1 regulatory/accessory genes: keys to unraveling viral and host cell biology. Science 280: 1880–1884
2. Sherman MP, De Noronha MC, Williams SA and Greene WC (2002) Insights into the biology of HIV-1 viral protein R. DNA Cell Biol. 21: 679–688
3. Muthumani K, Choo AY, Hwang DS, Chattergoon MA, Dayes NN, Zhang D, Lee MD, Duvvuri U and Weiner DB (2003) Mechanism of HIV-1 viral protein R-induced apoptosis. Biochem. Biophys. Res. Commun. 304: 583–592
4. Lum JJ, Cohen OJ, Nie Z, Weaver JG, Gomez TS, Yao XJ, Lynch D, Pilon AA, Hawley N, Kim JE, Chen Z, Montpetit M, Sanchez-Dardon J, Cohen EA and Badley AD (2003) Vpr R77Q is associated with long-term nonprogressive HIV infection and impaired induction of apoptosis. J. Clin. Invest. 111: 1547–1554

5. Somasundaran M, Sharkey M, Brichacek B, Luzuriaga K, Emerman M, Sullican JL and Stevenson M (2002) Evidence for a cytopahtogenicity determinant in HIV-1 Vpr. Proc. Natl. Acad. Sci. USA 99: 9503–9508
6. Subbramanian RA, Kessous-Elbaz A, Lodge R, Forget J, Yao XJ, Bergeron D and Cohen EA (1998) Human immunodeficiency virus type 1 Vpr is a positive regulator of viral transcription and infectivity in primary human macrophages. J. Exp. Med. 187: 1103–1111
7. Eckstein DA, Sherman MP, Penn ML, Chin PS, De Noronha CM, Greene WC and Goldsmith MA (2001) HIV-1 Vpr enhances viral burden by facilitating infection of tissue macrophages but not nondividing $CD4^+$ T cells. J. Exp. Med. 194: 1407–1419
8. Levy DN, Refaeli Y, MacGregor RR and Weiner DB (1994) Serum Vpr regulates productive infection and latency of human immunodeficiency virus type 1. Proc. Natl. Acad. Sci. USA 91: 10873–10877
9. Piller SC, Jans P, Gage PW and Jans DA (1998) Extracellular HIV-1 virus protein R causes a large inward current and cell death in cultured hippocampal neurons: implications for AIDS pathology. Proc. Natl. Acad. Sci. USA 95: 4595–4600
10. Tungaturthi PK, Sawaya BE, Singh SP, Tomkowicz B, Ayyavoo V, Khalili K, Collman RG, Amini S and Srinivasan A (2003) Role of HIV-1 Vpr in AIDS pathogenesis: relevance and implications of intravirion, intracellular, and free Vpr. Biomed. Pharmacother. 57: 20–24
11. Bukrinsky M and Adzhubei A (1999) Viral protein R of HIV-1. Rev. Med. Virol. 9: 39–49
12. Ferri KF, Jacotot E, Blanco J, Este JA and Kroemer G (2000) Mitochondrial control of cell death induced by HIV-1-encoding proteins. Ann. NY Acad. Sci. 926: 149–164
13. Refaeli Y, Levy DN and Weiner DB (1995) The glucocorticoid receptor type II complex is a target of the HIV-1 vpr gene product. Proc. Natl. Acad. Sci. USA 92: 3621–3625
14. Mahalingham S, Ayyavoo V, Patel M, Kieber-Emmons T, Kao GD, Muschel RJ and Weiner DB (1997) HIV-1 Vpr interacts with a human 34-kDa move34 homologue, a cellular factor linked to the G2/M phase transition of the mammalian cell cycle. Proc. Natl. Acad. Sci. USA 95: 3419–3424
15. Ramanathan MP, Curley III E, Su M, Chambers JA and Weiner DB (2002) Carboxyl terminus of hVIP/mov34 is critical for HIV-1-Vpr interaction and glucocorticoid-mediated signaling. J. Biol. Chem. 277: 47854–47860
16. Gridley T, Jaenisch R and Gendron-Maguire M (1991) The murine Mov-34 gene: full length cDNA and genomic organization. Genomics 11: 501–507
17. Ayyavoo V, Mahboubi A, Mahalingam S, Ramalingam R, Kudchodkar S, Williams WV, Green DR and Weiner DB (1997) HIV-1 Vpr suppresses immune activation and apoptosis through the regulation of nuclear factor kappa B. Nat. Med. 3: 1117–1123
18. Scheinman RI, Cogswell PC, Lofquist AK and Baldwin Jr AS (1995) Role of transcriptional activation of I kappa B alpha in mediation of immunosuppression by glucocorticoids. Science 270: 283–286
19. Auphan N, DiDonato JA, Rosette C, Helmberg A and Karin M (1995) Immunosuppression by gluocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. Science 270: 286–290
20. Kino T, Gragerov A, Kopp JB, Stauber RH, Pavlakis GN and Chrousos GP (1999) The HIV-1 virion-associated protein vpr is a coactivator of the human glucocorticoid receptor. J. Exp. Med. 189: 51–62
21. Muthumani K, Hwang DS, Desai BM, Zhang D, Dayes N, Green DR and Weiner DB (2002) HIV-1 Vpr induces apoptosis through caspase 9 in T cells and peripheral blood mononuclear cells. J. Biol. Chem. 277: 37820–37831
22. Wei N and Deng XW (2003) The COP9 signalosome. Annu. Rev. Cell Dev. Biol. 19: 261–286
23. Ponting CP, Aravind L, Schultz J, Bork P and Koonin EV (1999) Eukaryotic signalling domain homologues in archaea and bacteria. Ancient ancestry and horizontal gene transfer. J. Mol. Biol. 289: 729–734
24. Glickman MH, Rubin DM, Coux O, Wefes I, Pfeifer G, Cjeka Z, Baumeister W, Fried VA and Finley D (1998) A subcomplex of the proteasome regulatory particle required for ubiquitin-conjugate degradation and related to the COP9-signalosome and eIF3. Cell 94: 615–623
25. Wei N, Chamovitz DA and Deng XW (1994) *Arabidopsis* COP9 is a component of a novel signaling complex mediating light control of development. Cell 78: 117–124
26. Deshaies RJ (1999) SCF and cullin/ring H2-based ubiquitin ligases. Annu. Rev. Cell Dev. Biol. 15: 435–467
27. Cope GA and Deshaies RJ (2003) COP9 signalosome: a multifunctional regulator of SCF and other cullin-based ubiquitin ligases. Cell 114: 663–671
28. Read MA, Brownell JE, Gladysheva TB, Hottelet M, Parent LA, Coggins MB, Pierce JW, Podust VN, Luo RS, Chau V and Palombella VJ (2000) Nedd8 modification of cul-1 activates SCF(beta(TrCP))-dependent. Mol. Cell. Biol. 20: 2326–2333
29. Ohh M, Kim WY, Moslehi JJ, Chen Y, Chau V, Read MA and Kaelin Jr WG (2002) An intact NEDD8 pathway is required for cullin-dependent ubiquitylation in mammalian cells. EMBO Rep. 3: 177–182
30. Mundt KE, Liu C and Carr AM (2002) Deletion mutants in COP9/signalosome subunits in fission yeast *Schizosaccharomyces pombe* display distinct phenotypes. Mol. Cell Biol. 13: 493–502
31. Bech-Otschir D, Kraft R, Huang X, Henklein P, Kapelari B, Pollmann C and Dubiel W (2001) COP9 signalosome-specific phosphorylation targets p53 to degradation by the ubiquitin system. EMBO J. 20: 1630–1639
32. Dressel U, Thormeyer D, Altincicek B, Paululat A, Eggert M, Schneider S, Tenbaum SP, Renkawitz R and Baniahmad A (1999) Alien, a highly conserved protein with characteristics of a corepressor for members of the nuclear hormone receptor superfamily. Mol. Cell. Biol. 19: 3383–3394
33. Peng Z, Serino G and Deng XW (2001) Molecular characterization of subunit 6 of the COP9 signalosome and its role in multifaceted developmental processes in Arabidopsis. Plant Cell. 13: 2393–2407
34. Stewart SA, Poon B, Song JY and Chen IS (2000) Human immunodeficiency virus type 1 vpr induces apoptosis through caspase activation. J. Virol. 74: 3105–3111
35. Shostak LD, Ludlow J, Fisk J, Pursell S, Rimel BJ, Nguyen D, Rosenblatt JD and Planelles V (1999) Roles of p53 and caspases in the induction of cell cycle arrest and apoptosis by HIV-1 vpr. Exp. Cell Res. 251: 156–165
36. Patel CA, Mukhtar M and Pomerantz RJ (2000) Human immunodeficiency virus type 1 Vpr induces apoptosis in human neuronal cells. J. Virol. 74: 9717–9726
37. Jacotot E, Ravagnan L, Loeffler M, Ferri KF, Vieira HL, Zamzami N, Costantini P, Druillennec S, Hoebeke J, Briand JP, Irinopoulou T, Daugas E, Susin SA, Cointe D, Xie ZH, Reed JC, Roques BP and Kroemer G (2000) The HIV-1 viral protein R induces apoptosis via a direct effect on the mitochondria permeability transition pore. J. Exp. Med. 191: 33–46
38. Jacotot E, Ferri KF, El Hamel C, Brenner C, Druillennec S, Hoebeke J, Rustin P, Metivier D, Lenoir C, Geuskens M, Vieira HL, Loeffler M, Belzacq AS, Briand JP, Zamzami N, Edelman L, Xie ZH, Reed JC, Roques BP and Kroemer G (2001) Control of mitochondria membrane permeabilization by adenine nucleotide translocator interacting with HIV-1 viral protein R and Bcl-2. J. Exp. Med. 193: 509–519
39. Wei MC, Zong WX, Cheng EH, Lindsten T, Panoutsakopoulou V, Ross AJ, Roth KA, MacGregor GR, Thompson CB and Korsmeyer SJ (2001) Proapoptotic Bax and Bak: a requisite gateway to mitochondrial dysfunction and death. Science 292: 727–730
40. Roumier T, Vieira HL, Castedo M, Ferri KF, Boya P, Andreau K, Druillennec S, Joza N, Penninger JM, Roques B and Kroemer G (2002) The C-terminal moiety of HIV-1 Vpr induces cell death via a caspase-independent mitochondrial pathway. Cell Death Differ. 9: 1212–1219
41. Huang MB, Weeks O, Zhao LJ, Saltarelli M and Bond VC (2000) Effects of extracellular human immunodeficiency virus type 1 vpr protein in primary rat cortical cell cultures. J. Neurovirol. 6: 202–220
42. Levy DN, Fernandes LS, Williams WV and Weiner DB (1993) Induction of cell differentiation by human immunodeficiency virus 1 Vpr. Cell 72: 541–550
43. Jowett JB, Planelles V, Poon B, Shah NP, Chen ML and Chen IS (1995) The human immunodeficiency virus type 1 vpr gene arrests infected T cells in the G2+M phase of the cell cycle. J. Virol. 69: 6304–6313
44. He J, Choe S, Walker R, Di Marzio P, Morgan DO and Landau NR (1995) Human immunodeficiency virus type 1 viral protein R (Vpr) arrests cells in the G2 phase of the cell cycle by inhibiting p34cdc2 activity. J. Virol. 69: 6705–6711
45. Re F, Braaten D, Franke EK and Luban J (1995) Human immunodeficiency virus type 1 Vpr arrests the cell cycle in G2 by inhibiting the activation of p34cdc2–cyclin B. J. Virol. 69: 6859–6864
46. Stewart SA, Poon B, Jowett JB and Chen IS (1997) Human immunodeficiency virus type 1 Vpr induces apoptosis following cell cycle arrest. J. Virol. 71: 5579–5592
47. Zhu Y, Gelbard HA, Roshal M, Pursell S, Jamieson BD and Planelles V (2001) Comparison of cell cycle arrest, transactivation, and apoptosis induced by the

simian immunodeficiency virus SIVagm and human immunodeficiency virus type 1 vpr genes. J. Virol. 75: 3791–3801
48. Stewart SA, Poon B, Jowett JB, Xie Y and Chen IS (1999) Lentiviral delivery of HIV-1 Vpr protein induces apoptosis in transformed cells. Proc. Natl. Acad. Sci. USA 96: 12039–12043
49. Yuan H, Xie YM and Chen IS (2003) Depletion of Wee-1 kinase is necessary for both human immunodeficiency virus type 1 Vpr- and gamma irradiation-induced apoptosis. J. Virol. 77: 2063–2070
50. Poon B, Jowett JB, Stewart SA, Armstrong RW, Rishton GM and Chen IS (1997) Human immunodeficiency virus type 1 vpr gene induces phenotypic effects similar to those of the DNA alkylating agent, nitrogen mustard. J. Virol. 71: 3961–3971
51. Roshal M, Kim B, Zhu Y, Nghiem P and Planelles V (2003) Activation of the ATR-mediated DNA damage response by the HIV-1 viral protein R. J. Biol. Chem. 278: 25879–25886
52. Yao XJ, Mouland AJ, Subbramanian RA, Forget J, Rougeau N, Bergeron D and Coehn EA (1998) Vpr stimulates viral gene expression and indueces cell killing in human immunodeficiency virus type 1-infected dividing Jurkat T cells. J. Virol. 72: 4686–4693
53. Muthumani K, Zhang D, Hwang DS, Kudchodkar S, Dayes NS, Desai BM, Malik AS, Yang JS, Chattergoon MA, Maguire Jr HC and Weiner DB (2002) Adenovirus encoding HIV-1 Vpr activates caspase 9 and induces apoptotic cell death in both p53 positive and negative human tumor cell lines. Oncogene 21: 4613–4625
54. Nishizawa M, Kamata M, Mojin T, Nakai Y and Aida Y (2000) Induction of apoptosis by the Vpr protein of human immunodeficiency virus type 1 occurs independently of G(2) arrest of the cell cycle. J. Biol. Chem. 276: 16–26
55. Mahalingam S, Ayyavoo V, Patel M, Kieber-Emmons T and Weiner DB (1997) Nuclear import, virion incorporation, and cell cycle arrest/differentiation are mediated by distinct functional domains of human immunodeficiency virus type 1 Vpr. J. Virol. 71: 6339–6347
56. Sherman MP, de Noronha CM, Pearce D and Greene WC (2000) Human immunodeficiency virus type 1 Vpr contains two leucine-rich helices that mediate glucocorticoid receptor coactivation independently of its effects on G(2) cell cycle arrest. J. Virol. 74: 8159–8165
57. Subbramanian RA, Yao XJ, Dilhuydy H, Rougeau N, Bergeron D, Robitaille Y and Cohen EA (1998) Human immunodeficiency virus type 1 Vpr localization: nuclear transport of a viral protein modulated by a putative amphipathic helical structure and its relevance to biological activity. J. Mol. Biol. 278: 13–30
58. Agostini I, Popov S, Li J, Dubrovsky L, Hao T and Bukrinsky M (2000) Heat-shock protein 70 can replace viral protein R of HIV-1 during nuclear import of the viral preintegration complex. Esp. Cell Res. 259: 398–403
59. Iordanskiy S, Zhao Y, DiMarzio P, Agostini I, Dubrovsky L and Bukrinsky M (2004) Heat-shock protein 70 exerts opposing effects on Vpr-dependent and Vpr-independent HIV-1 replication in macrophages. Blood 104: 1867–1872
60. Iordanskiy S, Zhao Y, Dubrovsky L, Iordanskaya T, Chen M, Liang D and Bukrinsky M (2004) Heat shock protein 70 protects cells from cell cycle arrest and apoptosis induced by human immunodeficiency virus type 1 viral protein R. J. Virol. 78: 9697–9704
61. Conti L, Rainaldi G, Matarrese P, Varano B, Rivabene R, Columba S, Sato A, Belardelli F, Malorni W and Gessani S (1998) The HIV-1 vpr protein acts as a negative regulator of apoptosis in a human lymphoblastoid T cell line: possible implications for the pathogenesis of AIDS. J. Exp. Med. 187: 403–413
62. Fukumori T, Akari H, Iida S, Hata S, Kagawa S, Aida Y, Koyama AH and Adachi A (1998) The HIV-1 Vpr displays strong anti-apoptotic activity. FEBS Lett. 432: 17–20