

HIV-1 VPR MODULATES CELL CYCLE G2/M TRANSITION THROUGH AN ALTERNATIVE CELLULAR MECHANISM OTHER THAN THE CLASSIC MITOTIC CHECKPOINTS

Robert T. Elder¹, Zsigmond Benko¹ and Yuqi Zhao¹⁻³

¹ Children's Memorial Institute for Education and Research, ² Department of Pediatrics, ³ Microbiology-Immunology, Northwestern University Medical School, 2430 N Halsted Street, MB218, Chicago, Illinois 60614

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1. ABSTRACT

HIV-1 Vpr induces cell cycle G2/M arrest in both human and fission yeast cells, suggesting a highly conserved activity of this viral protein. In this review, we summarize the current understanding of Vpr-induced G2 arrest based on studies from both mammalian cells and the fission yeast (*Schizosaccharomyces pombe*) model system. Fission yeast has proven to be an excellent model system to investigate cell cycle G2/M control of eukaryotic cells. Similarly, fission yeast has also been instrumental in defining the molecular mechanism underlying the G2 arrest induced by Vpr. We have compared the classic DNA-damage and DNA-replication checkpoint controls of the cell cycle G2/M transition to the G2 arrest conferred by Vpr. Based on the current findings, we hypothesize that Vpr induces cell cycle G2 arrest through an alternative novel cellular pathway(s) rather than through the classic mitotic checkpoint controls. A number of cellular proteins which may be involved in this new cellular pathway(s) have been identified and are discussed.

2. INTRODUCTION

Protein R (Vpr) of human immunodeficiency virus type 1 (HIV-1) is a virion-associated viral gene product with an average length of 96 amino acids, and a calculated molecular weight of 11.7 kD. Vpr is a highly conserved viral protein among HIV, simian immunodeficiency viruses (SIV) and other lentiviruses (1-3), indicating an important function or functions for this protein.

One of the highly conserved Vpr activities is its ability to blocks cell cycle progression by arresting cells in

the G2 phase of the cell cycle in both mammalian and fission yeast cells (4-8). The G2 arrest induced by Vpr is thought to suppress immune functions by preventing T cell clonal expansion (9, 10) and to provide an optimized cellular environment for increased transcription and maximal levels of viral replication (11, 12). However, the molecular mechanisms underlying the Vpr-induced G2 arrest are not fully elucidated. Much effort in the last six years has been devoted to determining how Vpr induces G2 arrest. Fission yeast has proven to be a reliable model system for studying the cell cycle G2/M controls of higher eukaryotic cells, and studies in fission yeast are also providing insight into Vpr-induced G2 arrest. In this review, we first compare Vpr-induced G2 arrest to the classic mitotic checkpoint controls where the models are based largely on studies in fission yeast and mammalian cells. We then describe developing ideas about how Vpr interacts with cellular processes and proteins during the induction of cell cycle G2 arrest.

3. CELL CYCLE G2/M CONTROL

3.1. The DNA damage and replication checkpoints act through inhibitory phosphorylation of Cdc2

The cell cycle is an orderly process that ensures faithful replication and distribution of all genes to the next generation, and there are controls on the cell cycle which help to protect the integrity of the genome. One of these important control systems is a cellular surveillance process known as the G2/M mitotic checkpoint. When DNA is damaged or DNA synthesis is inhibited, progression through the cell cycle is halted at the G2/M boundary by the DNA damage or DNA replication checkpoint. The G2 arrest induced by

Table 1. Homologues of fission yeast and human proteins that are involved in cell cycle G2/M control

Fission Yeast (<i>S. pombe</i>)	Human (<i>H. sapiens</i>)	Putative activities	Reference
Mitotic regulators			
Cdc2	Cdk1	Cyclin B-dependent kinase	103
Cdc13	Cyclin B	B-type cyclin	104
Wee1	Wee1	Tyrosine kinase	105
Mik1	-----	Tyrosine kinase	
Cdc25	Cdc25B/C	Tyrosine phosphatase	106, 107
DNA damage and replication checkpoints			
Rad1	hRad1	Nuclease	108
Rad3	ATM/ATR	Protein kinase	57
Rad9	hRad9	3'-5' exonuclease	109
Rad17	hRad17	Unknown	110
Rad24/25	14-3-3	Binds to phosphorylated serine	41
Hus1	hHus1	A PCNA-related protein	111
Chk1	Chk1	Serine/Threonine Kinase	39
Cds1	Chk2	Serine/Threonine Kinase	45
Cellular proteins involved in Vpr-induced G2 arrest			
PP2A	PP2A	Protein phosphatase 2A	112
Wos2	P23	Inhibitor of Wee1	51
Rhp23	HHR23A/B	Excision DNA repair enzyme	82
-----	hVIP/Mov34	Regulation of transcription and proteolysis	84

Note: "-----", not found.

these checkpoint control systems is believed to allow sufficient time to repair DNA damage or completion of DNA synthesis prior to entering mitosis. Continued cell cycling without adequate DNA repair due to loss of the checkpoint control will result in subsequent mutations and genomic instability, processes contributing to carcinogenesis (13-16).

The G2/M transition in eukaryotes is normally regulated by the synergistic and opposing activities of a cascade of distinct protein kinases and phosphatases. This cascade converges on Cdc2, a cyclin-dependent kinase which determines onset of mitosis in all eukaryotic cells (The human homologue of Cdc2 is also called Cdk1. For reviews, see (17-20). For a list of fission yeast and human homologues that are involved in the G2/M checkpoint, see Table 1.) In fission yeast, entry into mitosis is regulated by the phosphorylation status of Tyr15 on Cdc2, which is phosphorylated by Wee1 and Mik1 kinases during G2 and rapidly dephosphorylated by the Cdc25 phosphatase to trigger entry into mitosis (21-24).

These mitotic regulators (Wee1, Mik1 and Cdc25) are the targets of two well-characterized DNA damage and DNA replication checkpoint pathways which induce cell cycle G2 arrest (Figure 1A. For recent reviews, see (17, 18, 25-28). The DNA damage checkpoint activated by radiation leads to inhibitory phosphorylation of Cdc2 by a pathway which is highly conserved between fission yeast and mammalian cells (29-31) (Table 1). In fission yeast the early genes in the pathway, which include Rad1, Rad3, Rad9, Rad17, Rad26 and Hus1 are thought to detect the DNA damage and lead to the phosphorylation of the Chk1 protein (32-38). The activated Chk1 kinase then directly phosphorylates the Cdc25 phosphatase (39). The phosphorylated Cdc25 binds the Rad24/25 proteins, and

this complex is transported out of the nucleus to render Cdc25 inactive (40). Both Rad24 and Rad25 are homologues of human 14-3-3 proteins which also inhibit Cdc25 activity (41, 42). Other reports have shown that upon DNA damage Chk1 also activates the Wee1 and Mik1 kinases to inhibit Cdc2 (31, 43, 44). DNA damage thus initiates a protein phosphorylation cascade ending in activation of the Chk1 kinase which then inactivates the Cdc25 phosphatase and activates the Wee1 and Mik1 kinases to increase inhibitory phosphorylation of Tyr15 on Cdc2.

Inhibition of DNA replication by chemical agent such as hydroxyurea in fission yeast also leads to cell cycle arrest through inhibitory phosphorylation of Cdc2 (29), and this DNA replication checkpoint pathway again appears to be highly conserved in mammalian cells (45) (Table 1). Parts of this DNA replication checkpoint are shared with the DNA damage checkpoint as Rad1, Rad3, Rad9, Rad17, Rad26 and Hus1 are required for both checkpoints in fission yeast (32-37). However, the DNA replication checkpoint acts primarily through phosphorylation of the Cds1 kinase with minor participation of the Chk1 kinase, and either kinase is sufficient by itself to give cell cycle arrest when DNA synthesis is inhibited (46). The activated Cds1 kinase inactivates Cdc25 through the same mechanism as Chk1 and may also activate the Wee1 and Mik1 kinases (46-48).

3.2. Vpr-induced G2 arrest acts through inhibitory phosphorylation of Cdc2 but does not use the checkpoint pathways

Vpr induces G2 arrest through inhibitory phosphorylation of Cdc2/Cdk1 both in fission yeast and human cells. Three main lines of evidence support this conclusion. 1) Vpr induces hyperphosphorylation of

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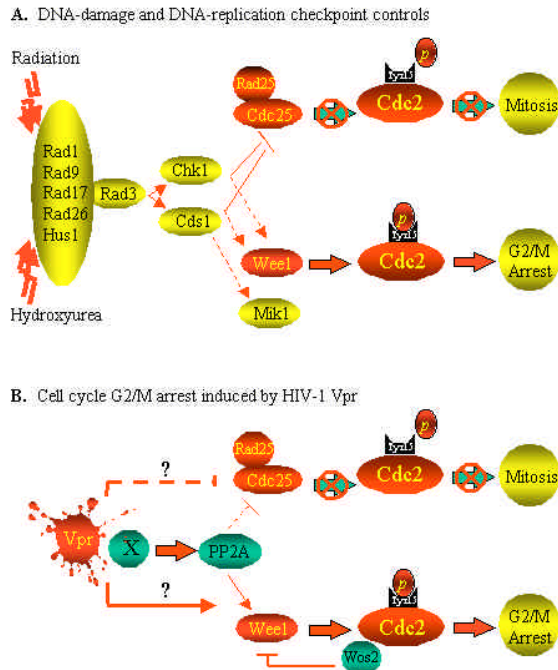


Figure 1. Cell cycle G2/M controls. (A) DNA damage and DNA replication checkpoint control pathways. (B) Working model for Vpr-induced G2 arrest in fission yeast.

Cdc2/Cdk1 (5, 7, 49). 2) Expression of nonphosphorylatable Cdc2 mutants, T14A Y15F of Cdk1 and Y15F of Cdc2, prevents Vpr-induced G2 arrest (49, 50). 3) Overexpression of the Cdc25 phosphatase reduces Vpr-induced G2 arrest in both human and fission yeast cells (51, 52). Consistent with the essential role of inhibitory phosphorylation of Cdc2 in Vpr-induced G2 arrest, Vpr does not induce G2 arrest in budding yeast where inhibitory phosphorylation of the Cdc2 homologue Cdc28 does not control the G2 to M transition (53-55). Since Vpr induces G2 arrest through inhibitory phosphorylation of Cdc2 both in human and fission yeast cells, fission yeast is an excellent model system to elucidate the underlying mechanisms for Vpr-induced G2 arrest.

Given that the DNA checkpoints and Vpr both induce G2 arrest through inhibitory phosphorylation of Cdc2, Vpr might induce G2 arrest through a mitotic checkpoint pathway. This possibility has been thoroughly evaluated in fission yeast by expressing *vpr* in mutant fission yeast strains defective in early and late steps of the mitotic checkpoint pathways. None of the early checkpoint-specific mutants (*rad1*, *rad3*, *rad9* and *rad17*) have a significant effect on the induction of G2 arrest by Vpr (50, 51). Furthermore, mutations in both *chk1* and *cds1*, which are thought to be the last steps specific for the mitotic checkpoint (39, 46, 47), also do not block Vpr-induced G2 arrest (51, 56). Therefore, Vpr does not use the DNA-damage or DNA-replication checkpoint pathways to induce G2 arrest in fission yeast.

More limited data in human cells tends to support the conclusion that Vpr does not induce G2 arrest through

the mitotic checkpoints pathways. Vpr still induced G2 arrest in cells from patients with ataxia telangiectasia (AT) (52). These AT cells are mutant for the ATM gene, which is the human homologue of fission yeast Rad3, and do not arrest in G2 in response to DNA damage (45, 57, 58). Thus, Vpr does not use this early part of the DNA damage checkpoint to induce G2 arrest in human cells. However, Poon *et al.* (59) have suggested that Vpr does induce G2 arrest through the DNA damage checkpoint in human cells based primarily on studies with pentoxifylline (PTX), a methyl xanthine. PTX prevents G2 arrest after DNA damage (60), and Poon *et al.* (59) found that PTX also inhibits Vpr-induced G2 arrest. PTX similarly inhibits Vpr-induced G2 arrest in fission yeast (51, 61). However, since PTX inhibits Vpr-induced G2 arrest in fission yeast where the DNA damage checkpoint apparently plays no role, PTX inhibition alone is not strong evidence that the DNA damage checkpoint has a role in Vpr-induced G2 arrest.

4. POSSIBLE REGULATORY STEPS BETWEEN VPR AND THE CELL CYCLE REGULATORS WEE1 AND CDC25

4.1. Protein phosphatase 2A (PP2A)

Since Vpr does not appear to use the mitotic checkpoint pathways in fission yeast or human cells but does act through Wee1 and Cdc25, what is the regulatory pathway that operates between Vpr and the Wee1 kinase and Cdc25 phosphatase? A clue comes from studies with okadaic acid (OA), a potent inhibitor of protein phosphatase 2A (PP2A) (62, 63). Both in fission yeast and human cells, OA inhibits the induction of G2 arrest by Vpr (5, 7). This further similarity between Vpr-induced G2 arrest in fission yeast and human cells suggests that Vpr induces G2 arrest by activating PP2A. This possibility is supported by the observation that PP2A is known to play a role in cell cycle control both in fission yeast and higher eukaryotes (63, 64). Further evidence that PP2A has a role in Vpr-induced G2 arrest comes from the finding that mutations in genes (*ppa2* and *pab1*) coding for the catalytic and regulatory subunits of the fission yeast PP2A partially blocked Vpr-induced G2 arrest (51, 56). Furthermore, an elevated PP2A protein level was observed in cell extracts isolated from the *vpr*-expressing cells, confirming that Vpr up-regulates PP2A in fission yeast cells (51). [A paper proposing a specific role of PP2A in Vpr-induced G2 arrest in mammalian cells (65) has been withdrawn by the corresponding author (pers. comm.)]. Thus, activation of PP2A plays a major role in Vpr-induced G2 arrest in fission yeast and possibly in human cells.

PP2A is thought to affect the cell cycle by acting on the Wee1 and Mik1 kinases and the Cdc25 phosphatase which control phosphorylation of Tyr15 (63, 64). In fission yeast, expression of *vpr* in strains with mutations in these genes suggests that Vpr inhibits Cdc2 predominantly by activation of Wee1 with inhibition of Cdc25 playing a minor role. A working model for the novel regulatory pathway(s) that Vpr uses to induce G2 arrest in fission yeast is shown in Figure 1B. Expression of *vpr* cells

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activates PP2A activity either by direct association with the PP2A enzyme complex or by association with an intermediate protein(s) X. A protein phosphorylation cascade (depicted as “?”) including PP2A is probably ultimately responsible for activation of the mitotic repressor Wee1 by altering its phosphorylation levels, which in turn inhibits Cdc2 by Tyr15 phosphorylation. While Wee1 plays the major role in the induction of G2 arrest by Vpr, Cdc25 appears to play a minor role and is inhibited by this proposed regulatory pathway. Although PP2A is a major part of this proposed regulatory pathway, it is not yet known whether PP2A is completely or only partially responsible for Vpr-induced G2 arrest. Other protein kinases and phosphatase may also be regulated by Vpr to induce G2 arrest in fission yeast.

PP2A appears to be a common cellular target for viruses (66). In addition to HIV-1 Vpr, PP2A is affected by the SV40 small t antigen, the polyoma virus small and middle t antigens and the adenovirus E4orf4. The SV40 t antigen inhibits PP2A in its role of promoting cellular proliferation (67, 68). Activation of PP2A towards certain substrates by polyoma t antigens is thought to be necessary for the t antigens to promote DNA replication and cellular proliferation (69). The adenovirus E4orf4 interaction with PP2A may be the closest analogy to Vpr. Expression of E4orf4 induces G2/M arrest and cell death in both mammalian and budding yeast cells, and the interaction of E4orf4 with PP2A is required for the G2/M arrest and cell death (70, 71).

4.2. Other cellular proteins which may be involved in Vpr-induced G2 arrest

One way to identify other regulatory steps in the pathway for Vpr-induced G2 arrest is to screen for multicopy suppressors and enhancers of Vpr-induced G2 arrest. Multicopy suppressors of Vpr-induced G2 arrest might represent genes which are inhibited by Vpr to cause G2 arrest, and enhancers might be genes which are activated by Vpr to cause G2 arrest. In the fission yeast system where Vpr-induced G2 arrest leads to longer cells, multicopy Vpr suppressors and enhancers have been isolated by screening for shorter or even longer cells after *vpr* expression (51). One suppressor giving shorter cells is *wos2*, a homologue of the human p23 protein which is a Hsp90-associated co-chaperone (72). In fission yeast, *wos2* is an inhibitor of Wee1 which is additional evidence for the importance of Wee1 in Vpr-induced G2 arrest. One enhancer isolated in this screen is *rad25*, an inhibitor of Cdc25 (40). As discussed below, isolation of *rad25* as an enhancer suggests that Vpr inhibits Cdc25 by moving it to the cytoplasm. *Wos2* and *Rad25* may be regulatory steps in the pathway for Vpr-induced G2 arrest, and characterization of additional suppressors and enhancers will help to define the regulatory pathway for Vpr-induced G2 arrest.

A number of Vpr binding proteins have been identified, and some of these have been proposed to have a role in Vpr-induced G2 arrest. One such Vpr-binding protein is HHR23A, the human homologue of budding yeast Rad23 protein. Two reports (73, 74) initially showed

that HHR23A bound to Vpr and that overexpression of HHR23A inhibited Vpr-induced G2 arrest. Rad23 homologues are known to be involved in nucleotide excision repair of UV damaged DNA (75), but have also been shown to have roles in 26S proteasome function and the cell cycle (76-79). These cell cycle-related functions of Rad23 homologues and the inhibition of Vpr-induced G2 arrest supported the proposal that HHR23A plays an important role in the induction of G2 arrest by Vpr.

Structural studies of the Vpr binding site on HHR23A raise interesting possibilities about the role of this binding to Vpr activities. Vpr was found to bind to HHR23A at the UBA (UBiquitin-Associated) domain which was originally identified in proteins involved in the ubiquitination pathway (80). Recently it has been shown that ubiquitin also binds to the UBA domain (81). Since Vpr and ubiquitin both bind to the UBA domain, one implication from these results is that Vpr may have a direct effect on ubiquitination and proteasome pathways. This idea is certainly supported by a number of reports showing that Rad23 homologues are involved in proteasome activity (76, 77, 79). Since the UBA domain is found in a number of proteins, a second implication from these binding studies is that Vpr may interact with more than one protein through the UBA domain. Perhaps it is this interaction with the UBA on more than one protein is important for Vpr-induced G2 arrest. Support for this possibility comes from budding yeast where a single mutation in *rad23* has no effect on the cell cycle, but when combined with a *dsk2* mutation, a gene which also has an UBA domain and does not affect the cell cycle as a single mutation, the cell cycle is arrested (78). We have recently isolated the fission yeast homologue of Rad23, Rhp23, and found that Rhp23 interacts directly with the fission yeast homologue of budding yeast Dsk2 and that deletion of *rhp23* by itself gives rise to a G2 delay in a fraction of the cell population (82). Thus Vpr may induce G2 arrest by interacting with the UBA domain on multiple proteins.

While there is some evidence that HHR23A plays a role in the induction of G2 arrest by Vpr, a recent report (83) showed by the yeast two-hybrid assay that binding of the Vpr mutants to HHR23A did not correlate with the induction of G2 arrest. It is difficult at the moment to interpret the evidence for and against a role for HHR23A in Vpr-induced G2 arrest. If the Vpr-HHR23A interaction were not important for G2 arrest, one interpretation of the suppression by overexpression of HHR23A (73, 74) is that the strong interaction between Vpr and HHR23A may have precluded Vpr from interacting with its actual targets for G2 arrest. On the other hand, if the Vpr-HHR23A interaction were important in G2 arrest, Vpr may interact with a protein complex containing HHR23A to induce G2 arrest. In this case, assaying the single interaction between Vpr and HHR23A by the yeast two-hybrid system may not accurately indicate the interaction of Vpr with this protein complex.

4.3. Alterations of cellular localization may be important for Vpr-induced G2 arrest.

Another Vpr-binding protein (hVIP/MOV34) which might have a role in G2 arrest is the MOV34

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homologue (84), which belongs to a family of mammalian proteins involved in regulation of transcription, proteasome function and cell cycle. Anti-sense inhibition of the *hVIP/MOV34* gene leads to G2 arrest indicating a role for this gene in the cell cycle. Interestingly, the expression of *vpr* moves hVIP/MOV34 from the nucleus to a perinuclear localization indicating that Vpr may affect localization of cellular proteins. These results suggest that Vpr may induce G2 arrest by moving hVIP/MOV34 from a nuclear location where it may be important for G2 arrest to a location where it is no longer active.

The connections between *rad24/rad25* and Vpr-induced G2 arrest also suggest that altered protein localizations may be important for G2 arrest. Multicopy *rad25* is an enhancer of Vpr-induced G2 arrest (51), and deletion of *rad24* suppresses Vpr-induced G2 arrest (56). Fission yeast *rad24* and *rad25* are homologues of mammalian 14-3-3 proteins, and these proteins are known to affect the localization of Cdc25 and to play a role in the DNA damage and replication checkpoints (40, 85). In the mitotic checkpoint pathways, Rad24/25 bind to phosphorylated serines on Cdc25 generated by the checkpoint kinase Chk1 and Cds1 leading to export of Cdc25 from the nucleus (40, 42). Although Vpr does not induce G2 arrest through the Chk1 and Cds1 kinases, it will be interesting to see if another kinase phosphorylates serines on Cdc25 so that Vpr inhibits Cdc25 through binding of Rad24/25.

de Noronha *et al.* (86) have recently demonstrated in human cells a novel mechanism through which Vpr alters the nuclear-cytoplasmic localization of proteins. They showed that Vpr induces transient nuclear herniations and that proteins such as Wee1 and Cdc25 leak across these disruptions in the nuclear envelope. Two Vpr mutants which did not induce G2 arrest were tested, and they did not induce nuclear herniations suggesting a correlation between G2 arrest and the nuclear herniations. We have also observed nuclear herniation in fission yeast after expression of Vpr indicating that this is a highly conserved activity of Vpr (87). While the mislocalization of cell cycle regulators might have some effect on the cell cycle, de Noronha *et al.* (86) suggested that the herniations do not directly affect the cell cycle but rather that this nuclear damage may induce a known or unknown checkpoint leading to G2 arrest.

4.4. Structural requirements for G2 arrest and relationship to other Vpr activities

Studies of Vpr mutants have allowed correlations of G2 arrest activity with Vpr structure and have suggested that induction of G2 arrest is independent of several other Vpr activities. A tertiary structure of Vpr proposed on the basis of NMR consists of an alpha-helix-turn-alpha helix domain in the amino half from amino acids 17 to 46 and a long alpha helix from aa 53 to 78 in the carboxy half (88, 89). These two main structural features of Vpr in the amino and carboxyl half are likely to interact with each other (89, 90). Mutagenesis studies in both mammalian and fission yeast cells indicate that the C-terminal end of Vpr is largely responsible for the G2 arrest

induced by Vpr (91-95). Positively charged amino acids in carboxy-end seem to be particularly important for the induction of G2 arrest. For example, changing a single arginine at position 73 eliminated G2 arrest, and this mutant Vpr was transdominant over the activity of wild-type Vpr (96). Zhou and Ratner (97, 98) have proposed that phosphorylation of Ser79 of Vpr is also essential for G2 arrest but an earlier report indicated that Ser79 could be changed to Ala without affecting G2 arrest in both human and fission yeast cells (91, 94).

Vpr has activities such as cell killing and nuclear localization in addition to G2 arrest [For a recent review, see (99)]. Studies of mutant Vpr indicate that Vpr-induced G2 arrest is independent of the cell killing and nuclear localization activities of Vpr, studies in fission yeast clearly indicate that G2 arrest is independent of cell killing based on studies with mutations in Vpr and Cdc2. Mutations in Vpr often had opposite effects on G2 arrest and cell killing in wild type fission yeast cells (91). One example is the F34I mutation which nearly eliminates cell killing but has no significant effect on the levels of G2 arrest. Further support for the independence of G2 and cell death comes from studies of the Y15F Cdc2 mutation in fission yeast (50). In the Y15F strain, where Vpr-induced G2 arrest is completely abolished, Vpr still induces cell killing. Studies in mammalian cells with Vpr mutants also indicate that G2 arrest is independent of cell killing (100).

Similarly nuclear localization and G2 arrest are separate functions of Vpr. One example is again the F34I mutation of Vpr which no longer localizes to the nucleus but still induces G2 arrest in both mammalian and fission yeast cells (51, 91, 101). The independence of G2 arrest and nuclear localization activities is further indicated by the separation of these functions in the *vpr* homologues in HIV-2. Two genes in HIV-2, *vpr* and *vpx*, are homologous to the *vpr* gene of HIV-1. One of these homologues, *vpr*, has the G2 activity of HIV-1 *vpr* while the other homologue, *vpx*, has the nuclear localization function (102).

5. SUMMARY AND FUTURE PROSPECTS

It is firmly established that Vpr induces G2 arrest through inhibitory phosphorylation of Cdc2. Strong experimental evidence also supports the view that Vpr regulates the Wee1 kinase and Cdc25 phosphatase to inhibit Cdc2. There are two major goals for future work about the mechanism(s) by which Vpr induces G2 arrest. First, to define the regulatory pathways between Vpr and the cell cycle regulators, Wee1 and Cdc25. The best case can be made at the moment for PP2A being an important part of this pathway, but its exact molecular role remains to be established. For instance, it is not presently known whether it is the overall activity of PP2A that is important or whether specific substrates are crucial for Vpr-induced G2 arrest. For other candidates for the pathway such as HHR23A and hVIP/MOV34, the extent and exactly how they might contribute to Vpr-induced G2 arrest remains to be determined. The importance of altered cellular localization to Vpr-induced G2 arrest also needs to be

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defined. A second major goal is to determine if Vpr uses some normal cellular mechanism of G2 control. The observation that Vpr induces G2 arrest in organisms as distant as human and fission yeast cells indicates that Vpr induces G2 arrest through a highly conserved, and presumably, important pathway. Vpr does not apparently use any part of the DNA damage or replication checkpoint pathways, but it may use some other normal cellular mechanism of cell cycle control and could possibly use some other checkpoint pathway(s). Thus, studies of Vpr-induced G2 arrest may help define a new system for G2/M control in all eukaryotic cells.

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Send correspondence to: Dr Yuqi Zhao, 2430 N Halsted Street, MB218, Chicago, Illinois, USA. Tel: 773-880-6608, Fax: 773-880-6609, E-mail: yzhao@northwestern.edu