Accessory protein Vpr of human immunodeficiency virus type 1 (HIV-1) arrests cell cycling at G2/M phase in human and simian cells. Recently, it has been shown that Vpr also causes cell cycle arrest in the fission yeast *Schizosaccharomyces pombe*, which shares the cell cycle regulatory mechanisms with higher eukaryotes including humans. In this study, in order to identify host cellular factors involved in Vpr-induced cell cycle arrest, the ability of Vpr to cause elongated cellular morphology (cdc phenotype) typical of G2/M cell cycle arrest in wild-type and various mutant strains of *S. pombe* was examined. Our results indicated that Vpr caused the cdc phenotype in wild-type *S. pombe* as well as in strains carrying mutations, such as the cdc2-3w, cdc25, rad1-1, &dhk1, &smk1, and &ppa1 strains. However, other mutants, such as the cdc2-1w, &wee1, &ppa2, and &rad24 strains, failed to show a distinct cdc phenotype in response to Vpr expression. Results of these genetic studies suggested that &wee1, &ppa2, and &rad24 might be required for induction of cell cycle arrest by HIV-1 Vpr. Cell proliferation was inhibited by Vpr expression in all of the strains examined including the ones that did not show the cdc phenotype. The results supported the previously suggested possibility that Vpr affects the cell cycle and cell proliferation through different pathways.

Human immunodeficiency virus type 1 (HIV-1) is a causative agent of AIDS. In addition to the viral genes, such as gag, pro, pol, and env, common to all of the replication-competent retroviruses, the HIV-1 genome has genes for accessory proteins that are thought to play important roles in viral replication and pathogenesis. One of the HIV-1 accessory proteins, Vpr, is a virion-associated protein of 14 kDa. Despite its small size, Vpr has been shown to have multiple functions including nuclear translocation of the preintegration complex (20, 53, 63), regulation of apoptosis (3, 61), inhibition of cell proliferation (30, 50), induction of cell differentiation (30), and host cell cycle arrest at G2/M phase (22, 55). The G2/M cell cycle arrest by Vpr is conserved among primate lentiviruses including HIV-2 and simian immunodeficiency viruses (14, 24, 51, 65), and a human homologue of mov34 (33), which have been implicated in cell cycle control. However, the functional significance of the interaction between Vpr and these cellular proteins is still unclear. In fact, it was shown that the ability of Vpr to bind UNG did not correlate with its ability to induce cell cycle arrest (60). Therefore, a different approach for identifying host factors functionally involved in Vpr-induced cell cycle arrest appeared necessary.

Previous studies have shown that Vpr-induced cell cycle arrest is associated with inactivation of p34\(^{\text{cdk2}}\) kinase, a key regulator of the G2/M transition (19, 54). The kinase activity of p34\(^{\text{cdk2}}\) is mainly regulated by proteins &wee1 and &cdc25 (28, 46). Specifically, p34\(^{\text{cdk2}}\) is inhibited by &wee1 via phosphorylation of its tyrosine residue at position 15 (Y15) when a cell is not ready for mitosis (37, 58). When a cell is prepared for mitosis, p34\(^{\text{cdk2}}\) is activated by &cdc25-mediated dephosphorylation of Y15, leading to G2/M transition (39). When DNA replication or repair of damaged DNA is incomplete, a checkpoint control mechanism is induced, which results in inhibition of p34\(^{\text{cdk2}}\) probably through activation of &wee1 or inactivation of &cdc25, causing cell cycle arrest at the G2/M boundary (28, 46). Therefore, it is possible that Vpr causes cell cycle arrest by affecting &wee1, &cdc25, or other checkpoint control molecules.

For elucidation of cell cycle regulatory mechanisms, the fission yeast *Schizosaccharomyces pombe* has been used as a good model system for a number of years, because (i) it shares the cell cycle regulatory mechanisms with higher eukaryotes including humans, (ii) a variety of well-defined mutant strains are available, facilitating genetic studies, and (iii) G2/M cell cycle arrest is manifested as an easily noticeable elongated morphology called the cdc phenotype (28, 40, 44). It has been studied...
described previously (35). Introduction of the mutation was verified by nucleo-
carrying C-to-T and T-to-C substitutions at the first and second nucleotides,
nmt1
stream of the thiamine-repressible
gene fragment was prepared by PCR using an infectious DNA clone of
HIV-1NL4-3 (1) given by Akio Adachi (Tokushima University, Tokushima, Ja-
The genetic background common to all of the strains (h− leu1-32) is omitted.

MATERIALS AND METHODS
Molecular cloning of the HIV-1 Vpr expression vector.
The Vpr gene fragment was prepared by PCR using an infectious DNA clone of
HIV-1NL4-3 (1) given by Akio Adachi (Tokushima University, Tokushima, Ja-
pan) as a template and a pair of oligonucleotide primers (5'-CGGGATCCCG
AGGACAGATGGAAACAGGCC-3' and 5'-CAATTACGAAAAGGTACCAAG
CAGTCTTAAACC-3'). The amplified product was digested with BamHI and
MfeI and subcloned between the BamHI and EcoRI sites of pBluescript SK II (+)
(Stratagene), and its nucleotide sequence was verified. Then, the BamHI-EcoRV
fragment containing the Vpr-coding region was prepared from the plasmid and
inserted between the BamHI and Smal sites of the pREP-1 vector (36) down-
stream of the thiamine-repressible nmt1 promoter. The constructed vector was
named pREP1-Vpr. With pREP1-Vpr as a template, a mutant vpr gene fragment
containing C-to-T and T-to-C substitutions at the first and second nucleotides,
respectively, of codon 67 was generated by primer-directed PCR mutagenesis as
described previously (35). Introduction of the mutation was verified by nucleo-
tide sequencing so that the mutant gene encodes Vpr whose Leu67 is replaced by
Ser. The fragment was cloned in the pREP-1 vector to construct pREP1-L67S.
Culture and transformation of S. pombe. S. pombe strains used in this study are
listed in Table 1. Mutant strains were originally obtained from Paul Nurse
(Imperial Cancer Research Fund, London, United Kingdom), Antony M. Carr
(University of Sussex, Brighton, United Kingdom), and Mitsuo Yanagida
(Kyoto University, Kyoto, Japan). Strains designated as “our stock” in Table 1
were generated in our laboratory by modifying their nutrition requirement prop-
cesses through mating and tetrad analysis. Fission yeast cells were grown at 30°C
in minimal medium (MM) supplemented or not supplemented with leucine (250
µg/ml), using standard culture techniques (2). As for temperature-sensitive (ts)
mutants, different conditions were used as specified below. For repression of the
nmt1 promoter, 10 µM thiamine was added to the medium. To induce transcription
from the nmt1 promoter, cells were washed twice with MM without thiamine
and then reincubated into MM lacking thiamine. Transformation of S. pombe
with plasmid DNA was carried out by the lithium acetate method as described
previously (47).

Examination of proliferation and morphology of yeast cells. Yeast cells were
grown in MM supplemented or not supplemented with thiamine, and an aliquot
was taken at various time points. The number of cells in the sample was mea-
sured by using a particle counter (Z1; Beckman Coulter, Inc.). Morphology of
the cells was visualized by using a charge-coupled device camera (KV-26B; Hitachi Densi,
Ltd.) and printed by a video copy processor (SCT-P67; Mitsubishi Electric Corp.). Staining of the nucleus with 4'-dia-
midino-2-phenylindole (DAPI) was carried out by the standard method (2).

RESULTS

Wild-type Vpr, but not the L67S mutant, induced G2/M cell
Cyte arrest in S. pombe associated with the cdc phenotype. It
has previously been shown that expression of HIV-1 Vpr in S.
pombe causes cell cycle arrest (67, 68). To confirm the obser-
vation, the vpr gene was cloned in thiamine-repressible expres-
sion vector pREP-1 and introduced into wild-type S. pombe
cells. When the cells were grown in the absence of thiamine,
FIG. 1. Effects of wild-type Vpr and Vpr<sup>L67S</sup> expression on <i>S. pombe</i> cell growth properties. Wild-type <i>S. pombe</i> cells carrying pREP-1 (Ctrl.), pREP1-vpr (Vpr), and pREP1-L67S (L67S) were compared. (A) Immunoblot analysis of Vpr expression. Cells were grown in the presence (+) or absence (-) of thiamine for 15 h, and cell extracts were prepared, fractionated by SDS–10% PAGE, and transferred to a PVDF membrane. Vpr was detected by a rabbit antiserum to HIV-1<sub>NL4-3</sub> Vpr (NIH AIDS Research and Reference Reagent Program) and a peroxidase-conjugated mouse anti-rabbit Ig antibody. Binding of the secondary antibody was visualized by using a BM chemiluminescence blotting kit (Roche Diagnostics). (B) Cells were grown under Vpr-inducing (n) or noninducing (h) conditions and were counted at the indicated time points. (C) Cells were grown in the low-nitrogen medium supplemented (2) or not supplemented (1) with thiamine for 36 h, fixed with ethanol, treated with RNase A, and stained with propidium iodide. Then, cellular DNA content was measured by flow cytometry. The numbers in each graph indicate the percentages of the cells in G<sub>1</sub> and G<sub>2</sub> phases. (D) Photomicrographs of cells grown under Vpr-inducing (+) and noninducing (-) conditions for 36 h. (E) DAPI staining of wild-type <i>S. pombe</i> manifesting the Vpr-induced cdc phenotype. Original magnification, ×400. (F) DAPI staining of wild-type <i>S. pombe</i> manifesting the Vpr-induced cdc phenotype. Original magnification, ×630.
Vpr expression was induced (Fig. 1A, lane 4) and cell proliferation was markedly inhibited (Fig. 1B). Normally, nitrogen-starved S. pombe cells are arrested in G1 phase of the cell cycle, as shown by flow cytometric analysis of the control cells carrying the pREP-1 vector (Fig. 1C). Although S. pombe cells carrying pREP1-vpr showed a similar cell cycle profile under Vpr-repressing conditions, a large proportion of Vpr-expressing cells were arrested at G2/M phase (Fig. 1C). Microscopic observation demonstrated that the Vpr-expressing cells manifested an elongated morphology typical of the cdc phenotype representing G2/M cell cycle arrest (Fig. 1D). In addition, DAPI staining of the Vpr-expressing cells revealed that the elongated cells carried a single nucleus (Fig. 1E), further confirming that the cells were arrested at G2/M phase. The Leu57-to-Ser (L67S) substitution in Vpr has been shown to decrease the ability of the protein to induce G2/M cell cycle arrest in human cells (34). When the Vpr with the L67S substitution (VprL67S) was expressed in S. pombe (Fig. 1A, lane 6), cell proliferation was affected only slightly and the level of G2/M arrest was reduced compared with the arrest induced by wild-type Vpr (Fig. 1B and C). Most of the VprL67S-expressing cells failed to manifest the cdc phenotype, consistent with the reduced effects on the cell cycle (Fig. 1D). These results indicated that the effects of wild-type and mutant Vpr on the cell cycle of S. pombe cells were similar to those on the human cell cycle, suggesting that a common mechanism is involved in Vpr-induced cell cycle arrest in these different species. It was also shown that microscopic detection of the elongated morphology (cdc phenotype) was useful for evaluating the level of Vpr-induced G2/M arrest.

wee1*, but not cdc25*, was required for induction of the cdc phenotype by Vpr. In order to examine whether Vpr affects the cell cycle through the Wee1 or the cdc25 pathways (1), cdc2 mutant cdc2-3w and cdc2-1w strains (Table 1). cdc2-3w encodes p34cdc2, whose Y15 is dephosphorylated in a Cdc25-independent manner. On the other hand, cdc2-1w encodes p34cdc2, which is refractory to Wee1-mediated Y15 phosphorylation. Although both of them are defined as constitutively active mutants, cdc2-3w responds to the negative regulation by overexpression of Wee1, whereas cdc2-1w does not (58). When Vpr was expressed in the cdc2-3w strain, the cdc phenotype was clearly observed and cell proliferation was inhibited (Fig. 2A and B). On the other hand, Vpr expression in the cdc2-1w strain failed to show the cdc phenotype, and only inhibition of cell proliferation was observed (Oeg 2C and D). These results suggested that Wee1-mediated phosphorylation of p34cdc2 might be necessary for Vpr-induced cell cycle arrest. To further examine this possibility, Vpr was expressed in a wee1-50 mutant (Table 1). As shown in Fig. 3A and B, the cdc phenotype was not manifested by Vpr in the wee1 strain, whereas cell proliferation was inhibited. The level of Vpr expression in the wee1 strain in the absence of thiamine was comparable to that in wild-type S. pombe (Fig. 4, lanes 2 and 4). When the effects of Vpr expression on the viability of wild-type and wee1 strains of S. pombe were compared, no significant difference was observed (data not shown). Therefore, failure of the wee1 strain to manifest a Vpr-induced cdc phenotype was not due to lack of Vpr expression or a higher susceptibility to Vpr-mediated cell killing. The requirement of Wee1 activity for the Vpr-induced cdc phenotype was also confirmed with ts mutant wee1-50 strain (Table 1). The wee1-50 cells grown at a permissive temperature (23°C) clearly showed the cdc phenotype in response to Vpr expression (Fig. 3C). In contrast, manifestation of the distinct cdc phenotype was no longer observed when the temperature was shifted to 32.5°C (Fig. 3E), demonstrating that Wee1 activity was required for Vpr-induced cell cycle arrest. Prolinelation of the wee1-50 strain was inhibited by Vpr at both temperatures (Fig. 3D and F). A temperature shift from 23 to 32.5°C did not affect the susceptibility of wild-type S. pombe to the effects of Vpr (Fig. 3G and H). As a “twins kinase” of Wee1, Mik1 plays a supplementary role in regulating p34cdc2 phosphorylation of Y15 (29, 31). Vpr expression in a double mutant wee1-50 mik1 strain (Table 1) at 23°C caused both the cdc phenotype and inhibition of proliferation (Fig. 3I and J), indicating that Mik1 was not required for Vpr-induced cell cycle arrest. At 32.5°C, the wee1-50 mik1 strain manifested a lethal phenotype both in the presence and absence of induction of Vpr expression (data not shown). The effects of Vpr in the absence of cdc25 on a cdc25 cdc2-3w double mutant were examined (Table 1) since S. pombe carrying the cdc25 null mutation alone is not viable (55). Induction of Vpr expression in this strain caused the cdc phenotype as well as inhibition of proliferation (Fig. 3K and L), indicating that Cdc25 was dispensable for Vpr-induced cell cycle arrest. In a mutant mik1 strain (Table 1) deficient in the kinase which negatively regulates Wee1 (11, 48, 66), Vpr expression induced the cdc phenotype as well as inhibition of proliferation (data not shown).

Requirement of rad24* for the Vpr-induced cdc phenotype. It has previously been suggested that Vpr may cause cell cycle arrest through a pathway similar to the DNA damage checkpoint pathway (52). To examine this possibility, the effects of Vpr on various cell cycle checkpoint mutants were examined. Rad1 and Chk1 are signal transducers required for both DNA damage and replication checkpoints (46). Strains carrying both rad1-1, which encodes nonfunctional Rad1, and chk1, which expresses no Chk1, manifested the cdc phenotype in response to Vpr expression (Table 1; Fig. 5A and C). DAPI staining of the Vpr-expressing rad1-1 and chk1 cells revealed that most of the elongated cells carried one nucleus (data not shown). Although proliferation of the rad1-1 and chk1 cells was clearly inhibited under Vpr-inducing conditions at earlier time points up to 36 h postinduction, their growth curves appeared to catch up with that of the controls at later time points (Fig. 5B and D). These results indicated that both of rad1 and chk1 were dispensable for Vpr-induced cell cycle arrest, while they may play some role in sustaining the arrest, rad24* was identified as a multicopy suppressor of a radiation-sensitive mutation of S. pombe and is thought to be involved in regulation of the cell cycle timing and DNA damage checkpoint control through negative effects on Cdc25 (12, 15, 16, 49, 59). Unlike rad1-1 and chk1 mutants, the rad24 strain (Table 1) failed to reveal the cdc phenotype in response to Vpr expression, although its proliferation was markedly inhibited (Fig. 5E and F). The level of Vpr expression in the rad24 strain was comparable to that in wild-type S. pombe (Fig. 4, lane 8). Another mutant, the delt1 strain, deficient in the DNA replication checkpoint (Table 1) was susceptible to both induction of the cdc phenotype and inhibition of proliferation by Vpr (Fig. 5G and H).

Vpr failed to induce the cdc phenotype in the Δppa2 strain. It has been shown that okadac acid, a potent inhibitor of protein phosphatase 2A (PP2A), can abrogate Vpr-induced cell cycle arrest in mammalian and fission yeast cells, suggesting that PP2A may be required for manifestation of the effects of Vpr (54, 68). Fission yeast PP2A consists of a catalytic subunit, either Ppa1 or Ppa2, and two regulatory subunits, Paa1 and Pab2 (25, 27). To investigate the possibility that PP2A might be involved in Vpr-induced cell cycle arrest, the effects of Vpr expression on cellular morphology and proliferation in mutant Δppa1 and Δppa2 strains were examined (Ta-
ble 1). Vpr expression in the Δppa1 strain caused clearly elongated morphology and inhibition of cell proliferation (Fig. 6A and B). On the other hand, Vpr affected the morphology of the Δppa2 strain only marginally (Fig. 6C). Vpr was expressed in the Δppa2 strain as efficiently as in wild-type S. pombe (Fig. 4, lane 6) and inhibited cell proliferation (Fig. 6D). These results suggested that ppa2\textsuperscript{+}, but not ppa1\textsuperscript{+}, was necessary for the inhibitory effects of Vpr on the cell cycle.

Susceptibility of several mutants to Vpr-induced cell cycle arrest was discordant with their responsiveness to DNA damage. In order to compare the mechanisms of Vpr-induced cell cycle arrest and the DNA damage checkpoint, several mutant strains of S. pombe carrying pREP1-vpr were grown under Vpr-repressing conditions and treated with bleomycin, which induces DNA double-strand breaks. As expected from previous studies (16), wild-type S. pombe and the wee1 wee1-50 Δmik1 strains incubated at the permissive temperature responded to bleomycin-induced DNA damage, revealing the cdc phenotype, whereas the cdc2-3w Δcdc25 strain did not (Table 2). The size of cdc2-3w cells was somewhat increased by bleomycin treatment (Table 2), probably because the mutant remains responsive to DNA damage-induced Cdc25 inhibition (16). However, the change in the cell size of the cdc2-3w strain was marginal compared with the cdc phenotype induced by
FIG. 3. Requirement of wee1 for manifestation of the Vpr-induced cdc phenotype. 

S. pombe strains transformed with pREP1-vpr (Vpr; squares) or pREP-1 (Ctrl.; circles) were grown in the presence (2; open symbols) or absence (1; solid symbols) of thiamine at 30°C (A, B, K, and L) or 23°C (C, D, I, and J) throughout the experiment or at 23°C until 12 h (arrowhead) and 32.5°C thereafter (E, F, G, and H). Photomicrographs (A, ... the cells at 36 h. Graphs (B, D, F, H, J, and L) indicate the numbers of the cells counted at the indicated time points.
than inhibiting Cdc25. Like Wee1, Mik1 has been shown to affect the host cell cycle by increasing the Wee1 activity rather than inhibiting Cdc25. The kinase activity of p34*cdcc* is mainly regulated by the relative activities of Wee1 and Cdc25, although other mechanisms also appear to contribute to the regulation (28, 46). Our data demonstrated that Wee1, but not Cdc25, was required for induction of the cdc phenotype by Vpr in S. pombe, suggesting that Vpr may affect the host cell cycle by increasing the Wee1 activity rather than inhibiting Cdc25. Like Wee1, Mik1 has been shown to negatively regulate p34*cdc2* through Y15 phosphorylation. However, our data indicated that Mik1 was dispensable for the Vpr-induced cdc phenotype.

What might be the mechanism by which Vpr affects the Wee1 activity? Although Vpr may activate Wee1 directly (Fig. 7), Vpr has no known domain or activity that mediates immediate interaction with Wee1. Therefore, it is possible that Vpr affects Wee1 through a mechanism which involves additional cellular factors. In S. pombe, kinase Nim1 negatively affects Wee1 activity (11, 48, 66). However, Vpr caused the cdc phenotype in a nim1 mutant, making it unlikely that Nim1 or the pathway upstream of Nim1 is involved in Vpr-induced cell cycle arrest. A Nim1-related kinase, Cdr2, which may also negatively regulate Wee1, has recently been identified (8, 23). Additional experiments using a cdr2 mutant may reveal whether Cdr2 plays any role in the effects of Vpr on the cell cycle. Alternatively, it is possible that Vpr blocks the G2/M transition by inhibiting Wee1 degradation, since a recent study suggested that degradation of Wee1 might be a prerequisite for entry into mitosis (38). These possibilities are currently being investigated in our laboratories.

**DISCUSSION**

In this study, we exploited the fission yeast S. pombe for identifying the cellular factors involved in Vpr-induced cell cycle arrest. The fission yeast S. pombe, serving as a useful model organism for elucidating the mechanism for cell cycle regulation, has been shown to be susceptible to Vpr-induced cell cycle arrest (67, 68). Although it has previously been suggested that the effects of Vpr on the cell cycle were species or cell type specific (43, 62), a recent study demonstrated that the effects of mutations in Vpr on its functions in S. pombe and human cells were similar (10). Our data on Vpr expression (Fig. 4), which was not examined in S. pombe in the previous study (10), also indicated that the effects of the Vpr mutation in S. pombe were similar to those in human cells (34). These observations suggest that Vpr may induce cell cycle arrest in S. pombe and human cells through a common mechanism despite the large phylogenetic distance between these species.

It has previously been shown that G2/M cell cycle arrest by HIV-1 Vpr is associated with inactivation of p34*cdcc* kinase, a key regulator of the G2/M transition (19, 54). The kinase activity of p34*cdcc* is mainly regulated by the relative activities of Wee1 and Cdc25, although other mechanisms also appear to contribute to the regulation (28, 46). Our data demonstrated that Wee1, but not Cdc25, was required for induction of the cdc phenotype by Vpr in S. pombe, suggesting that Vpr may affect the host cell cycle by increasing the Wee1 activity rather than inhibiting Cdc25. Like Wee1, Mik1 has been shown to negatively regulate p34*cdcc* through Y15 phosphorylation. However, our data indicated that Mik1 was dispensable for the Vpr-induced cdc phenotype.

**TABLE 2. Effects of genetic background of S. pombe on induction of cell cycle arrest in response to Vpr and DNA damage**

| Brief genotype | Vpr phenotype induction in response to: | DNA damage c
| Wild type | + | + |
| cdc2-3w | + | +/- |
| cdc2-3w Δcdc25 | + | - |
| Δwee1 | - | - |
| wee1-50 Δmik1Δ | - | |
| rad1-1 | - | - |
| Δrad24 | - | |
| Δppa1 | + | + |
| Δppa2 | - | - |

* Data shown in Fig. 2, 3, 5, and 6 are summarized.

**FIG. 4. Immunoblot analysis of Vpr expression.** Wild-type (WT), Δwee1, Δppa2, and Δrad24 S. pombe cells carrying the pREP1-vpr vector were grown under noninducing (−) or inducing (+) conditions for 15 h, and cell extracts were prepared, fractionated by SDS-10% PAGE, and transferred to a PVDF membrane. Vpr was detected by a rabbit antiserum to HIV-1NL4-3 Vpr (NIH AIDS Research and Reference Reagent Program) and peroxidase-conjugated mouse anti-rabbit Ig antibody. Binding of the secondary antibody was visualized by using a BM chemiluminescence blotting kit (Roche Diagnostics).

**FIG. 5. Induction of DNA damage response by Vpr in S. pombe.** The response of Δwee1, Δppa1, Δppa2, and Δrad24 S. pombe cells carrying the pREP1-vpr vector was examined by scoring the percentage of cells with a chromosome bridge (B) or with a chromosome bridge and a micronucleus (M). The results are presented as the percentage of cells with DNA damage in response to: A. DNA damage, B. nuclear division, C. cell cycle arrest, D. micronucleus formation, and E. chromosome bridge formation. The data shown are representative of two independent experiments with similar results.

**FIG. 6. Cell cycle arrest by Vpr in S. pombe.** The effect of Vpr expression on cell cycle progression was examined by scoring the percentage of cells in each phase of the cell cycle. The results are presented as the percentage of cells in each phase of the cell cycle in response to: A. DNA damage, B. nuclear division, C. cell cycle arrest, D. micronucleus formation, and E. chromosome bridge formation. The data shown are representative of two independent experiments with similar results.
FIG. 5. Influence of cell cycle checkpoint molecules on the susceptibility to the effects of Vpr. *rad1-1* (A and B), *Δchk1* (C and D), *Δrad24* (E and F), and *Δcds1* (G and H) *S. pombe* cells transformed with pREP1-vpr (Vpr; squares) or pREP-1 (Ctrl.; circles) were grown in the presence (−; open symbols) or absence (+; solid symbols) of thiamine. Photomicrographs (A, C, E, and G) show representative morphology of the cells at 36 h. Graphs (B, D, F, and H) indicate the numbers of the cells counted at the indicated time points.
phenotype by Vpr. These results suggest that Vpr does not utilize Rad1, Chk1, or the DNA damage checkpoint control pathway upstream of these molecules for inducing cell cycle arrest. It should be mentioned that the \textit{rad1-1} and \textit{Dchk1} strains appeared to recover from the effects of Vpr after prolonged induction of Vpr expression (Fig. 5B and D), suggesting that Rad1 and Chk1 might play some role in the maintenance, if not the induction, of cell cycle arrest by Vpr. Unlike \textit{rad1}^{+} and \textit{chk1}^{+}, \textit{rad24}^{+} was shown to be necessary for manifestation of the Vpr-induced \textit{cdc} phenotype. Rad24, an \textit{S. pombe} homolog of the mammalian 14-3-3 protein, was identified as a DNA damage checkpoint molecule which determines the timing of mitosis (15). Recent studies have suggested that 14-3-3 binds to Cdc25 phosphorylated by Chk1, inhibits its nuclear translocation, and thereby prevents it from dephosphorylating \textit{p34}^{+}\textit{cdc2} (12, 16, 49, 59). Our data indicated that Cdc25 does not play a major role in induction of the \textit{cdc} phenotype by Vpr. Therefore, it is possible that Rad24 negatively regulates \textit{p34}^{+}\textit{cdc2} through an alternative pathway, which may involve \textit{Wee1} (Fig. 7). Taken together, these findings suggest that Vpr does not mimic the entire DNA damage checkpoint control, but rather may partially utilize it for inducing \textit{G2/M} arrest.
Also supporting this possibility are the data in this study indicating that the susceptibility of *S. pombe* mutants to Vpr-induced cell cycle arrest is not always correlated with their responsiveness to DNA damage (Table 2). Vpr inhibited proliferation of all of the strains used in this study including the ones that did not show the cdc phenotype in response to Vpr expression. It has been suggested in a previous study (67) that Vpr-induced cell cycle arrest may be Wee1 independent, based on an observation that Vpr inhibited the proliferation of the cdc2-1w strain. However, the morphology of cdc2-1w *S. pombe* cells expressing Vpr was not described in the study. Our present study reveals that Vpr could inhibit proliferation of cdc2-1w cells without causing the cdc phenotype, indicating that Wee1 is required for the Vpr-induced cdc phenotype but not inhibition of cell proliferation. Therefore, Vpr appears to affect the cell cycle and proliferation through distinct pathways (Fig. 7) as suggested by previous studies (43, 69). Since the toxicity of Vpr has been documented, not only for mammalian cells but also for other systems including bacteria (6) and the budding yeast *Saccharomyces cerevisiae* (32), Vpr may affect the viability of *S. pombe* by deteriorating a basic biological function common to various species.

Although it is still unknown whether the human homologs of Wee1, Ppa2, and Rad24 are involved in Vpr-induced G2/M arrest, our preliminary data suggested that expression of human *WEEl* in the *wee1Δ* strain of *S. pombe* restored susceptibility to the Vpr-induced cdc phenotype (Y. Nagai and M. Masuda, unpublished data). Further studies using *S. pombe* on the mechanism by which Vpr affects human cellular functions may provide useful insights into the molecular basis for AIDS pathogenesis and development of a novel therapeutic intervention.

**ACKNOWLEDGMENTS**

We thank A. Adachi for the molecular clone of HIV-1NL4-3 and P. Nurse, A. M. Carr, and M. Yanagida for the mutant strains of *S. pombe*. Y.N. and N.O. are students of the Faculty of Medicine, University of Tokyo, participating in the Free Quarter internship program.

This study was supported in part by research grants from the Ministry of Human Health and Welfare and the Ministry of Education, Science, Sports and Culture of Japan.

**REFERENCES**

2A (PP2A) affect cell morphogenesis, cell wall synthesis and cytkinesis. Genes Cells 1:29–45.


