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Transcription factor TFIIH components enhance the GR coactivator activity but not the cell cycle-arresting activity of the human immunodeficiency virus type-1 protein Vpr

Tomoshige Kino,[a,*] Makoto Tsukamoto,[b] and George P. Chrousos[a]

[a] *Pediatric and Reproductive Endocrinology Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA*
[b] *Lymphocyte Biology Section, Laboratory of Immunology National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA*

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Abstract

The human immunodeficiency virus type-1 (HIV-1)-accessory protein Vpr interacts with and potentiates the activity of the glucocorticoid receptor (GR) and arrests the host cell cycle at the G2/M boundary. Here we report that three core components of the general transcription factor (TF) IIH, CDK7, Cyclin H, and MAT1, enhance Vpr's GR coactivator activity but inhibit its cell cycle-arresting function. A CDK7 mutant defective in kinase activity for the C-terminal tail of RNA polymerase II, which cannot form a functional TFIIH complex, did not enhance Vpr coactivator activity. Overexpression of all three TFIIH components and p300 cooperatively enhanced Vpr coactivator activity, whereas TFIIH overexpression did not potentiate the transcriptional activity of a Vpr mutant, which does not bind p300/CBP. These findings suggest that TFIIH participates in Vpr's GR coactivating activity, at a step beyond its interaction with p300/CBP.

Keywords: CDK7; Cyclin H; MAT1; Coactivator; Cell cycle arrest

The human immunodeficiency virus type-1 (HIV-1)-encoded protein, Vpr, a 15 kDa virion-associated auxiliary protein, is important for efficient viral replication in vivo, presumably due to its requirement for productive infection in nondividing cells, such as monocytes/macrophages, dendritic cells, and resting lymphocytes [1–3]. Since Vpr is part of the virion, it is thought to function at an early stage of the viral infection, possibly modulating host cell activities to achieve a host environment that is conducive to the replication/proliferation of HIV-1 [4,5]. Vpr induces several effects in vitro, including facilitation of nuclear translocation of the HIV-1 preintegration complex, coactivation of steroid hormone receptors, modulation of apoptosis, and induction of host cell cell cycle arrest at the G2/M transition [6–10].

We recently demonstrated that Vpr functions as a potent coactivator of nuclear receptors including the glucocorticoid receptor (GR) using an LXXLL motif, which is present in host cell nuclear receptor coactivators, such as the p160 family and p300/CBP molecules, and mediates the interaction of these proteins with nuclear receptors [11,12]. We further showed that Vpr efficiently attracts the host cell coactivator p300/CBP to both the glucocorticoid-responsive mouse mammary tumor virus (MMTV) promoter and the HIV-1 long terminal repeat (LTR) through direct binding to p300/CBP via another surface located between amino acids between 65 and 85 [13]. Vpr also arrests host cells at the G2/M phase of the cell cycle in mammalian cells and fission yeast [14–18]. Although the effects of this arrest on the virus life cycle are not fully understood, it has been proposed that it enhances HIV-1 replication [2]. Using a genetic approach in the fission yeast, Vpr cell cycle activity was associated with the gene products of

* Corresponding author. Fax: 1-301-480-2024.
E-mail address: kinot@mail.nih.gov (T. Kino).

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wee1, *ppa2*, and *rad24*, all of which function upstream of $p34^{cdc2}$, modulating its phosphorylation status [19].

By employing a yeast two-hybrid screening and using Vpr as bait, we and others found that Vpr interacts with HHR23A, a protein involved in the nucleotide excision repair system, which is important to correct damaged DNA sequences caused by agents such as ultraviolet and chemical compounds [20,21]. The nucleotide excision repair is carried out by concerted reactions catalysed by over 10 different proteins [22,23]. These proteins form a complex, containing molecules such as the xeroderma pigmentosum (XP) proteins and RAD3, which have ATPase and DNA helicase activities. Some of these proteins are also found within the complex of the general transcription factor (TF) IIH. Indeed, the nucleotide excision repair and the TFIIH complexes share the same three core proteins, cyclin-dependent kinase (CDK) 7, Cyclin H, and their associated protein MAT1 [23,24].

TFIIH plays an important role in transcription, forming a large complex consisting of nine subunits that is crucial for the initiation of transcription and promoter clearance [24]. CDK7 has kinase activity, which is stabilized through association with Cyclin H and MAT1. CDK7 phosphorylates the C-terminal tail of the RNA polymerase II (CTD activity) and plays a critical role in the transcriptional regulation exerted by TFIIH. Phosphorylation of a serine residue at amino acid 170 of CDK7 is required for stable complex formation with Cyclin H and MAT1, greatly stimulating the activity of the complex towards the C-terminal domain of RNA polymerase II [25]. TFIIH also phosphorylates several nuclear receptors and participates in their transcriptional activity [26,27]. The same protein complex also phosphorylates molecules involved in cell cycle regulation, such as CDK 2 in vitro (CAK activity), further suggesting that it may also contribute to cell cycle control [24,28–30].

We previously demonstrated that overexpression of HHR23A modulates both the coactivator and the cell cycle-arresting activities of Vpr [20]. Therefore, we hypothesised that core components of TFIIH, such as CDK7, Cyclin H, and MAT1, might play a role in these Vpr activities. Here we examined the effects of TFIIH on the coactivator and cell cycle-arresting activities of Vpr.

Materials and methods

Plasmids. pCDNA3-VPR, which expresses the wild-type Vpr protein under the control of the Cytomegalovirus (CMV) promoter, was described previously [11]. pCDNA3-CDK7, pCDNA3-Cyclin H, and pCDNA3-MAT1 were constructed by inserting cDNAs of each protein amplified from MO15-3M, CyclH-HA, and pHT7-MAT1, which are gifts from Dr. R. Fisher (University of California, San Francisco, CA) and Dr. R. Roeder (The Rockefeller University, New York, NY), respectively, into pCDNA3 (Invitrogen, Palo Alto, CA). pCDNA3-CDK7 S170A expressing a mutant CDK7, in which a serine residue at amino acid 170 is replaced with alanine, was constructed by PCR-assisted mutagenesis reaction using pCDNA3-CDK7 as a template. This mutant CDK7 is defective in kinase activity directed towards the C-terminal tail of RNA polymerase II but still active in the phosphorylation of CDK2. pCMV-β-p300-CHA, which expresses the human p300 coactivator under the control of the CMV promoter, was a gift from Dr. D.M. Livingston (Dana Farber Cancer Institute, Boston, MA). pMMTV-Luc contains the luciferase gene under the control of the mouse mammary tumor virus promoter that has four functional glucocorticoid-responsive elements is a gift from Dr. G.L. Hager (National Institutes of Health, Bethesda, MD). pSV40-β-Gal and pHook-1 were purchased from Promega (Piscataway, WI) and Invitrogen (Carlsbad, CA), respectively.

Cell cultures, *reporter assays*, *and cell cycle analyses*. Human cervical carcinoma, HeLa, and human rhabdomyosarcoma A204 cells were maintained and transfected with a $CaPO_4$ method as described previously [11]. For the reporter assay using pMMTV-Luc, the cells were transfected with 1.0 μg/well pCDNA3-VPR, 1.0 μg/well pCDNA3-CDK7, -CDK7 S170A, -Cyclin H, and/or -MAT1 together with 1.5 μg/well pMMTV-Luc and 0.5 μg/well pSV40-β-Gal. Empty plasmids were used to keep the same amount of DNA. Twenty-four hours after transfection, cells were treated with 10^{-6} M dexamethasone and cell lysates were collected after additional incubation for 24 h. Luciferase and β-galactosidase activities were determined as described previously [11].

For the analysis of the cell cycle effect of Vpr, HeLa cells were transfected with 1.0 μg/well pCDNA3-VPR in the absence or presence of 1.0 μg/well pCDNA3-CDK7, -Cyclin H, and/or -MAT1 together with 2.0 μg/well pHook-1. After transfection, cells were cultured for 48 h and transfection-positive cells were enriched using the single-chain antibody, expressed by pHook-1 on the cell surface, following the company's protocol. DNA of transfected cells was stained with propidyl iodide and the DNA content was determined by FACS.

Results

Effect of CDK7, Cyclin H, and MAT1 on the GR coactivator activity of Vpr

To examine whether TFIIH contributes to Vpr's GR coactivator activity, we examined overexpression of the three core components of TFIIH, CDK7, Cyclin H, and MAT1, separately or altogether, with and without Vpr, on the glucocorticoid-responsive MMTV promoter in HeLa and A204 cells (Fig. 1). CDK7, Cyclin H, and MAT1, when examined individually, did not alter the activity of the dexamethasone-stimulated MMTV promoter, while coexpression of all three proteins enhanced the activity of this promoter by up to 2–3-fold in both cell lines. Vpr enhanced the dexamethasone-stimulated MMTV promoter activity by 4- or 10-fold in HeLa and A204 cells, respectively. CDK7 or MAT1 alone enhanced the potentiation of Vpr on this promoter by 2.5- and 4-fold in HeLa cells and 2.3- and 2.8-fold in A204 cells, respectively. Cyclin H did not change the effect of Vpr in HeLa cells but suppressed it by 60% in A204 cells. When all these proteins were expressed at the same time, they enhanced the Vpr activity by 5- and 4-fold in HeLa and A204 cells, respectively. These results suggest that TFIIH may participate in the coactivator activity of Vpr on GR-induced transactivation.

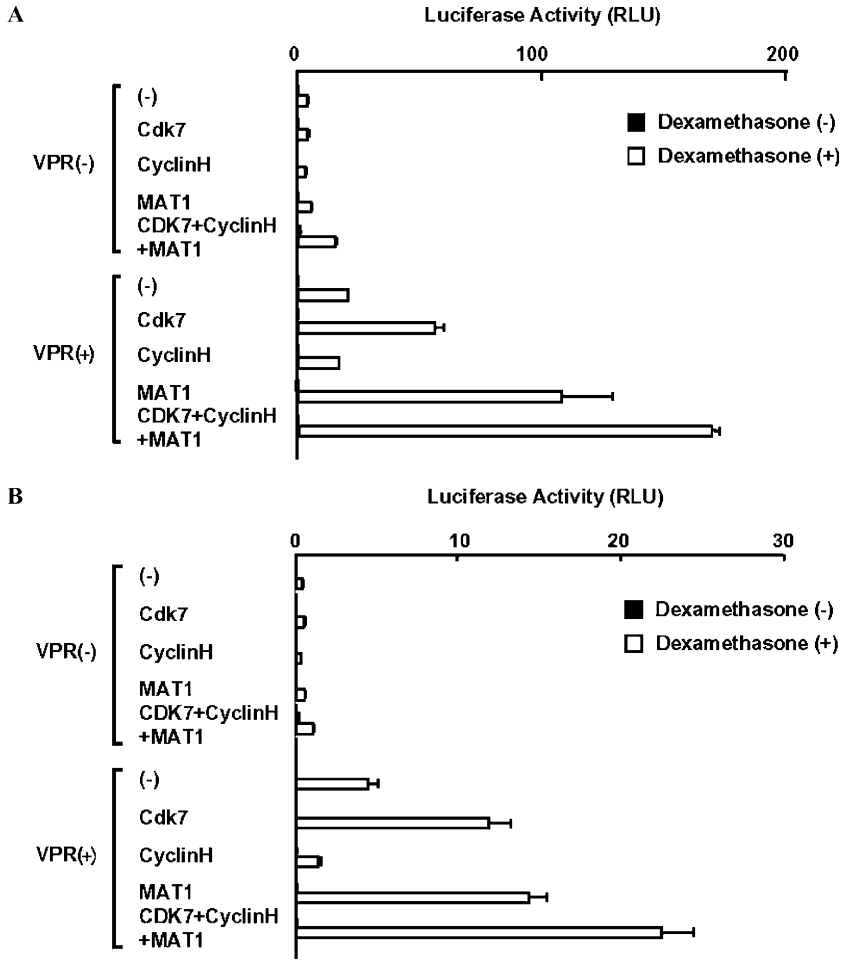

Fig. 1. TFIIH core component enhances GR coactivator activity of Vpr in HeLa and A204 cells. HeLa (A) and A204 (B) cells were transfected with plasmid-expressing indicated proteins together with pMMTV-Luc and pSV40-β-Gal. Bars show mean ± SE values of luciferase activity normalized for β-galactosidase activity.

To confirm the contribution of TFIIH on Vpr's GR coactivator activity, we next employed a CDK7 mutant, in which a serine residue at amino acid 170 was replaced with alanine, rendering this molecule defective in its transcriptional activity. The wild-type CDK7 minimally affected Vpr's GR coactivator activity in HeLa cells. Coexpression of this molecule with Cyclin H and MAT1, however, significantly enhanced the Vpr effect on this promoter. In contrast, the expression of CDK7 S170A alone or with Cyclin H and MAT1 did not affect GR coactivator activity of Vpr, suggesting that TFIIH contributes to Vpr's GR coactivator activity through the kinase activity of CDK7 directed to the C-terminal tail of RNA polymerase II (Fig. 2).

TFIIH cooperates with p300 and affects the GR coactivator activity of Vpr downstream of its binding to p300/CBP

Vpr stimulates both the glucocorticoid-responsive and HIV-1-LTR promoters by efficiently attracting p300/CBP to the promoter region through direct binding to p300/CBP coactivator [13]. Thus, we examined how TFIIH components cooperate with p300/CBP. p300 enhanced Vpr coactivator activity on the dexamethasone-stimulated MMTV promoter by about 3-fold in HeLa cells, as previously reported [13]. Added coexpression of TFIIH components further enhanced the synergistic activity of Vpr and p300 (Fig. 3A). We next tested TFIIH on a Vpr mutant I74,G75A, which is defective in binding to p300/CBP but active in binding to the GR [13] (Fig. 3B). Overexpression of CDK7, Cyclin H, and MAT1 enhanced the wild-type Vpr activity on the MMTV promoter, while it did not change the transcriptional activity of this mutant. These results suggest that TFIIH and p300 cooperatively enhance Vpr effect on GR-induced transactivation, and TFIIH may function at a step downstream from Vpr's binding to p300/CBP.

Effect of CDK7, Cyclin H, and MAT1 on the cell cycle-arresting activity of Vpr

We examined the effect of the same proteins on a second important activity of Vpr, the arrest of the host cell cycle in HeLa cells. Table 1 and Fig. 4 demonstrate

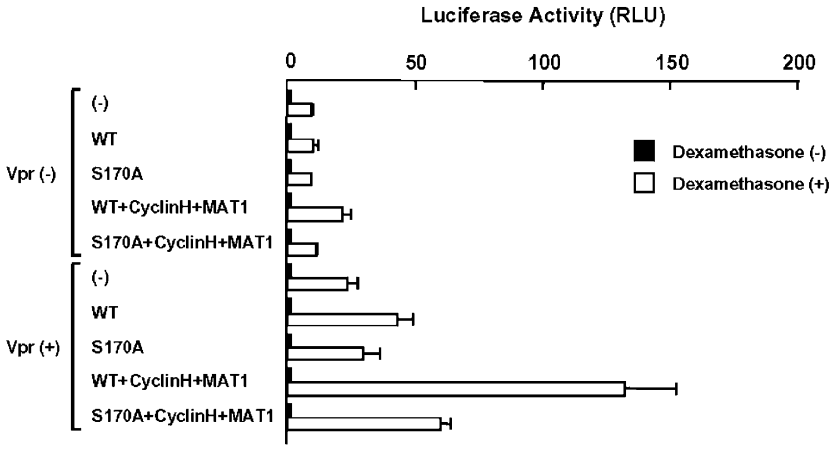

Fig. 2. CDK7 S170A failed to enhance Vpr coactivator activity in HeLa cells. HeLa cells were transfected with plasmid-expressing indicated proteins together with pMMTV-Luc and pSV40-β-Gal. Bars show mean ± SE values of luciferase activity normalized for β-galactosidase activity.

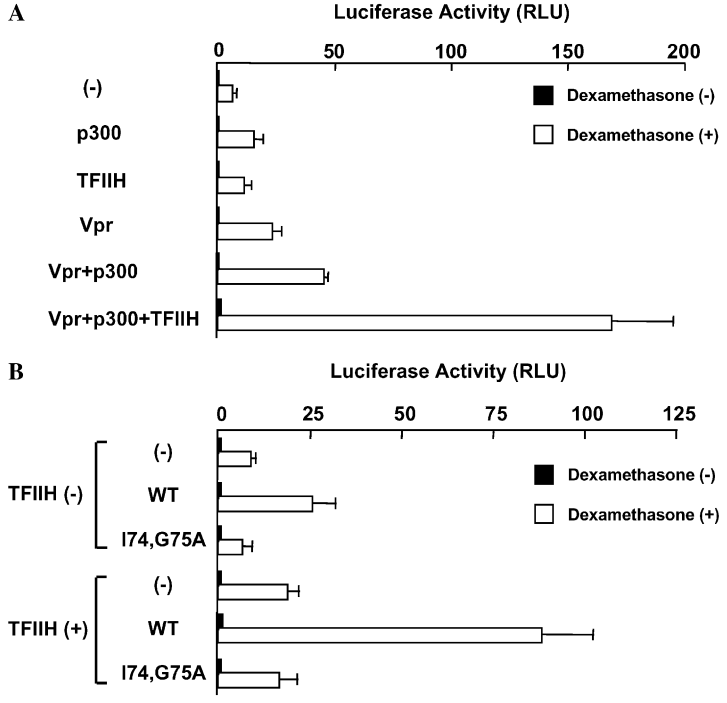

Fig. 3. TFIIH and p300 cooperatively enhances Vpr coactivator activity (A) and TFIIH did not reverse the defect of coactivator activity seen in Vpr I74,G75A mutant (B) in HeLa cells. HeLa cells were transfected with plasmid-expressing indicated proteins together with pMMTV-Luc and pSV40-β-Gal. Bars show means ± SE values of luciferase activity normalized for β-galactosidase activity.

Table 1
Effect of TFIIH core components on the Vpr cell cycle-arresting activity in HeLa cells

		G1 (%)	G2/M (%)	G2/G1 ratio
Vpr(−)	(−)	63.91	16.28	0.26
	CDK7	59.04	18.66	0.32
	CyclinH	64.03	15.88	0.25
	MAT1	61.02	20.07	0.33
	CDK7 + Cyclin H + MAT1	62.41	17.79	0.29
Vpr(+)	(−)	49.72	30.64	0.62
	CDK7	54.95	24.74	0.45
	Cyclin H	57.89	22.89	0.40
	MAT1	61.55	19.51	0.32
	CDK7 + Cyclin H + MAT1	63.18	17.44	0.28

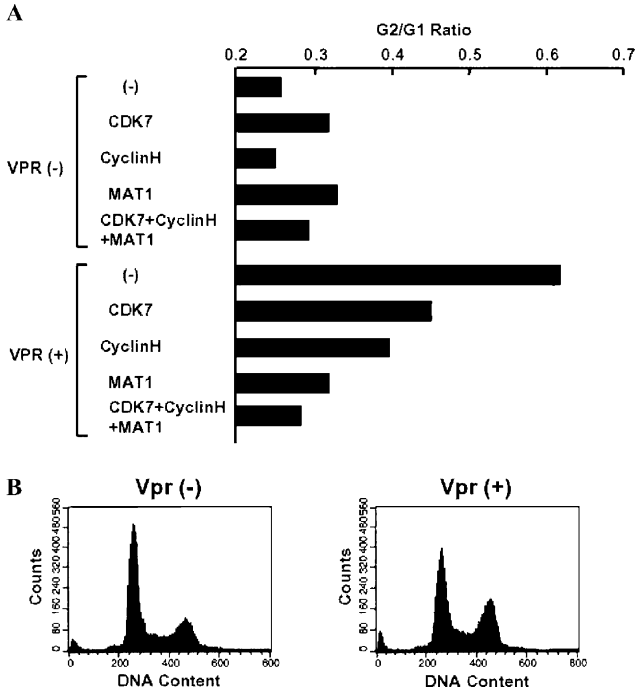

Fig. 4. TFIIH components, CDK7, Cyclin H, and MAT1 antagonise with Vpr's cell cycle-arresting activity in HeLa cells. (A) HeLa cells were transfected with plasmids-expressing indicated proteins. Bars show ratios of cell populations in G2/M phase vs. in G1 phase of the cell cycle. (B) Representative cell cycle profiles in the absence or presence of Vpr.

the percentage of cells in G1 and G2/M phases and their ratios. CDK7 and MAT1 weakly increased cell population in G2/M phase, while Cyclin H showed no effect. Vpr increased the percentage of cells in the G2/M, as previously reported. CDK7, Cyclin H, and MAT1 all antagonized with the cell cycle-arresting activity of Vpr. Among them, MAT1 showed the strongest activity. Expression of all three components also suppressed Vpr's cell cycle-arresting effect. These results suggest that overexpression of the TFIIH core complex is inhibitory to the cell cycle-arresting activity of Vpr, in contrast to its transcriptional activity, further supporting a link between TFIIH and the host GR machinery that is modulated by Vpr.

Discussion

We demonstrated that overexpression of all three major TFIIH core components enhanced Vpr's GR coactivator activity. When only each protein was overexpressed, however, CDK7 and MAT1 enhanced this activity, while Cyclin H suppressed it. Also, CDK7 S170A, which is defective in CTD phosphorylating activity, failed to enhance Vpr's GR coactivator activity. These results indicate that the TFIIH core complex may participate in Vpr's GR coactivator function. On the other hand, the TFIIH core proteins overexpressed alone or altogether suppressed the cell cycle-arresting activity of Vpr, supporting our hypothesis that the Vpr coactivator and cell cycle-arresting activities are two distinct functions. It appears as if overexpression of the TFIIH components shifts the distribution of Vpr from the fraction mediating its cell cycle-arresting activity to that mediating its GR coactivator activity. We previously demonstrated that HHR23A had a similar effect on these two Vpr activities [20]. It is possible that Vpr might directly interact with yet unknown protein(s), which is (are) associated with HHR23A and/or the TFIIH core complex to help it exert its GR coactivation effect.

TFIIH has several important roles in the transcriptional regulation organized by RNA polymerase II [23,24,31]. It is part of the holo-enzyme complex of the RNA polymerase II and participates in the unwinding of the DNA duplex through its helicase and ATPase activities [32–34]. Through the kinase activity of CDK7, it phosphorylates the C-terminal tail of RNA polymerase, contributing to promoter clearance and transcriptional elongation [35]. TFIIH also plays a multifaceted role in the transcriptional activity of nuclear receptors. CDK7 and Cyclin H, but not MAT1, bind to the N-terminal portion of the androgen receptor (AR) and overexpression of each or all of CDK7, Cyclin H, and MAT1 enhances AR-induced transactivation in a ligand-dependent fashion [26]. TFIIH also potentiates the transcription induced by the estrogen receptor (ER) [27]. Thus, CDK7 phosphorylates a serine residue at amino acid 118 of the ER and induces ligand-dependent binding of TFIIH to activation function 2 domain of the ER. CDK7 regulates the transcriptional activity of other nuclear receptors with similar mechanisms [36,37].

Since Vpr binds to GR, it is possible that TFIIH enhances the GR coactivator activity of Vpr through their mutual interaction with the GR molecule. Nuclear receptors interact with many proteins and protein complexes, including coactivators, the SWI/SNF and TRAP/DRIP complexes, as well as components of general transcription machineries in a time-sequential fashion [38,39]. We previously demonstrated that Vpr binds directly to p300/CBP in addition to the GR and activates GR-induced transcription by acting as an adaptor, linking promoter-bound components and coactivators [13]. Since TFIIH core components and p300 cooperatively enhanced the GR coactivator activity of Vpr and the expression of the former failed to generate the coactivator effect of VprI74,G75A, which is defective in binding to p300/CBP, TFIIH may function at a step downstream from Vpr binding to p300/CBP and may play a supportive role in Vpr's transcriptional activity. Although it is not demonstrated as yet, it is highly possible that p300/CBP also interacts with TFIIH. If it

were so, physical and functional interaction of Vpr and TFIIH may be dependent on the presence of p300/CBP.

CDK7, Cyclin H, and MAT1 alone or altogether inhibited the Vpr's cell cycle-arresting effect in HeLa cells (Fig. 2 and Table 1). It is likely that TFIIH components squelched Vpr from different complexes through which Vpr affects the cell cycle. Since TFIIH contains CAK activity, it is also possible that Vpr functions antagonistically at the step where TFIIH is operative in cell cycle progression, although the contribution of TFIIH to the control of the cell cycle has not been unequivocally demonstrated as yet [23].

Since several compartments of TFIIH, including CDK7, Cyclin H, MAT1, and HHR23A, are also present in the nucleotide excision repair complex, Vpr might also affect the host's DNA repair system. It is known that Vpr participates in viral integration by facilitating the translocation of the HIV-1 preintegration complex into the nucleus [40–42]. Since the first step of the viral integration is detected as DNA damage [43], it is also possible that modulation of the nucleotide excision repair system by Vpr might directly affect the integration of the HIV-1 into the host genome.

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