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Viral Interferon Regulatory Factor 1 of Kaposi's Sarcoma-Associated Herpesvirus (Human Herpesvirus 8) Binds to, and Inhibits Transactivation of, CREB-Binding Protein

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Kaposi's sarcoma-associated herpesvirus (KSHV) contains many cellular homologue genes. The K9 open reading frame (ORF) of KSHV encodes a virus-encoded interferon regulatory factor (vIRF) which functions as a repressor for cellular interferon-mediated signal transduction, and as an oncogene to induce cell growth transformation. In addition, KSHV vIRF plays an important role in the regulation of gene expression. From genetic and biochemical analysis, we demonstrate that KSHV vIRF1 binds to a transcriptional coactivator CREB-binding protein (CBP) *in vivo* and *in vitro*. KSHV vIRF1 binds to the KIX domain and CH/3 region of CBP. The CH/3 region of CBP coincides with the binding region of adenovirus E1A. We also show that vIRF1 inhibits the transactivational activity of CBP in HeLa cells. These results demonstrate that vIRF1 can modulate gene expression by inhibiting the transactivation function of coactivator CBP. © 2000 Academic Press

Key Words: **KSHV; vIRF; CBP; transcription.**

Kaposi's sarcoma-associated herpesvirus (KSHV), also called human herpesvirus 8 (HHV8), is a novel human gammaherpesvirus that is related to Kaposi's sarcoma (KS) lesions (1, 2). Genomic sequencing of KSHV shows that KSHV has many genes which have homology to cellular proteins (3). Among these genes, KSHV encodes two analogues of interferon regulatory factors (IRF). One is K9 open reading frame (ORF) encoding the viral interferon regulatory factor (vIRF), which is named to vIRF1, and the other is vIRF2 (4). vIRF1 blocks interferon (IFN) and IRF1-mediated transcriptional activation. Moreover, vIRF1 transforms NIH3T3 cells. NIH3T3 cells, which express vIRF1 in a stable way, demonstrate characteristics of malignant fibrosarcoma (5–7). ORF K9, which encodes vIRF1, is transcribed without tetradecanoylphorbol acetate (TPA), but is induced to higher transcription levels by TPA treatment (8). The expression of antisense to vIRF1 in BCBL-1 cells reduced the expression of several KSHV-lytic genes, suggesting that vIRF1 may regulate the gene expression of KSHV (9).

The cAMP response element binding protein (CREB) binding protein (CBP) is a cellular coactivator that interacts with a variety of cellular and viral proteins, such as CREB (10), c-Jun (11), c-Fos (12), c-Myb (13), MyoD (14), YY1 (15), p300/CBP-associated factor (P/CAF) (16), NF-κB (17), adenovirus E1A (18–20), human T-cell leukemia virus type 1 (HTLV-1) Tax (21), and SV40 large T antigen (22). A recent report shows that human papillomavirus (HPV) E6 also interacts with CBP (23, 24). CBP possesses both intrinsic and associated histone acetyltransferase (HAT) activity. It plays a role in the chromatin remodeling of the target gene and is involved in different regulatory circuits, allowing cells to coordinate or to integrate signals from different pathways (25–27). P/CAF also has independent HAT activity. HAT activities of both CBP/p300 and P/CAF proteins were inhibited by E1A (16, 28). In a recent study, Burysek *et al.* (29) demonstrated that KSHV vIRF1 can interact with p300 *in vitro*. Therefore, it is very likely that KSHV vIRF1 interacts with CBP and is involved in IRF-mediated gene transcription. CBP/p300 also associates with cellular IRF3 following virus infection, and phosphorylated IRF3 binds to CBP/p300 (30–32). Here, we demonstrate that KSHV vIRF1 interacts with CBP *in vitro* and *in vivo*. vIRF1 interacts with the KIX domain and CH/3 region of CBP and inhibits the transcriptional activation activity of CBP.

MATERIALS AND METHODS

Plasmids. KSHV total DNA was purified from BCBL-1 tumor cells by the Hirt lysis method as previously described (33). To obtain vIRF1 expression plasmid, the KSHV ORF K9 gene was cloned by a polymerase chain reaction (PCR) from purified KSHV DNA using the

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oligonucleotides 5′-CCCGAATTCATGGACCCAGGCCAAAGA-3′ and 5′-GGGCTCGAGTTATTGCATGGCATCCCA-3′. The resulting 1.3 kb DNA product was inserted into *Eco*RI-*Xho*I sites of pcDNA3 plasmid (Invitrogen). This plasmid was designated as pcDNA3-vIRF1. To generate glutathione *S*-transferase (GST)-vIRF1 fusion protein, a 1.3 kb *Eco*RI-*Xho*I fragment from pcDNA3-vIRF1 was subcloned into *Eco*RI-*Xho*I sites of a pGEX4T-1 vector (Amersham Pharmacia Biotech). To generate GST-vIRF1 expression plasmid, pGEX4T-1-vIRF1 was digested with *Bam*HI-*Not*I, eluted, and inserted into the *Bam*HI-*Not*I sites of pEBG vector, a version of EF-1α promoter to express fusion protein with GST at the amino-terminus. pVP16-vIRF1 plasmid was generated by inserting a 1.3-kb *Eco*RI-*Xho*I fragment from pcDNA3-vIRF1 into *Eco*RI-*Sal*I sites of pVP16 vector (Clontech). pLexA-vIRF1 plasmid was generated by *Eco*RI-*Xho*I sites and pYEStrp2-CBP was generated by *Hin*dIII-*Not*I sites. pcDNA3-HA-mCBP plasmid was generated by inserting the CBP gene from pRc/RSVmCBP into *Hin*dIII-*Not*I sites of pcDNA3 vector. pRc/RSVmCBP plasmid was a generous gift from Dr. R. Goodman (Oregon Health Science Univ.). GST-CBP1 and GST-CBP3 were gifts from Dr. R. G. Roeder (Rockefeller Univ.). To generate the GST-CBP2 fusion protein, amino acids 1680 to 1891 of CBP gene was subcloned into *Nde*I-*Bam*HI sites of pGEX-2TL vector. Gal4-CBP (pM-CBP) plasmid was generated by inserting the CBP gene, respectively, into pM vector (Clontech). pRSETB-mmP/CAF plasmid which was used for *in vitro* translation was a generous gift from Dr. S. Y. Roth (Texas Univ.).

Cells, transfection, and reporter assay. HeLa or 293T cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). At 24 h before transfection, 3×10^5 cells were plated in 6-cm dishes. Transfections were performed by the calcium phosphate method (34). Total amount of transfected DNA was adjusted with the blank vector lacking the cDNA to be expressed. Equal amounts of cell lysates were employed for the luciferase assay (Promega). Each assay was normalized with total protein concentration.

GST pull-down assay. Wild-type GST protein and GST fusion proteins were expressed in *Escherichia coli*, bound to glutathione-Sepharose-4B beads (Amersham Pharmacia Biotech), and incubated with labeled proteins expressed by *in vitro* translation [using the TNT-coupled transcription-translation system as described by the manufacturer (Promega)]. Bound proteins were analyzed by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE) and autoradiography.

Yeast two-hybrid assay. The yeast strain EGY48 was transformed with pLexA-vIRF1 and pYEStrp2-CBP. β-Galactosidase expression in yeast was assayed as described (35).

In vivo binding assay. 293T cells in 10-cm dishes were transfected with the hemagglutinin (HA)-CBP expression vector (pcDNA3-HA-CBP, 7 μg) either in combination with the GST expression vector (pEBG, 3 μg) or in combination with the GST-vIRF1 expression vector (pEBG-vIRF1, 3 μg) as indicated using the calcium phosphate method (34). Forty-eight h post-transfection, cells were lysed for 1 h at 4°C in an EBC buffer [50 mM Tris-HCl (pH 7.5), 120 mM NaCl, 0.5% Nonidet P-40, 50 mM NaF, 200 μM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride (PMSF)]. The lysate was centrifuged at 14,000*g* for 10 min to pellet debris. The supernatant was incubated on a rocker for 2 h at 4°C with glutathione-Sepharose 4B beads. The precipitates were washed three times in EBC buffer. The presence of HA-CBP protein, GST protein, and GST-vIRF1 protein was assessed by immunoblot using monoclonal anti-HA and anti-GST antibodies.

RESULTS

vIRF1 binds to coactivator CBP in vivo. In order to find whether KSHV vIRF1 can binds to CBP, we adopted the yeast two-hybrid assay. vIRF1 was fused to LexA DNA binding domain (pLexA-vIRF1) and CBP was fused to B42 transactivation domain (pYEStrp2-CBP). β-Galactosidase activities of yeast cells containing pLexA + pYEStrp2 or pLexA + pYEStrp2 were almost negligible (Fig. 1A). Since vIRF1 is an intrinsic activator, the yeast cells harboring pLexA-vIRF1 show relatively high β-galactosidase activity. Therefore, it is natural that the yeast cells containing pLexA-vIRF1 and pYEStrp2 also showed high β-galactosidase activ-

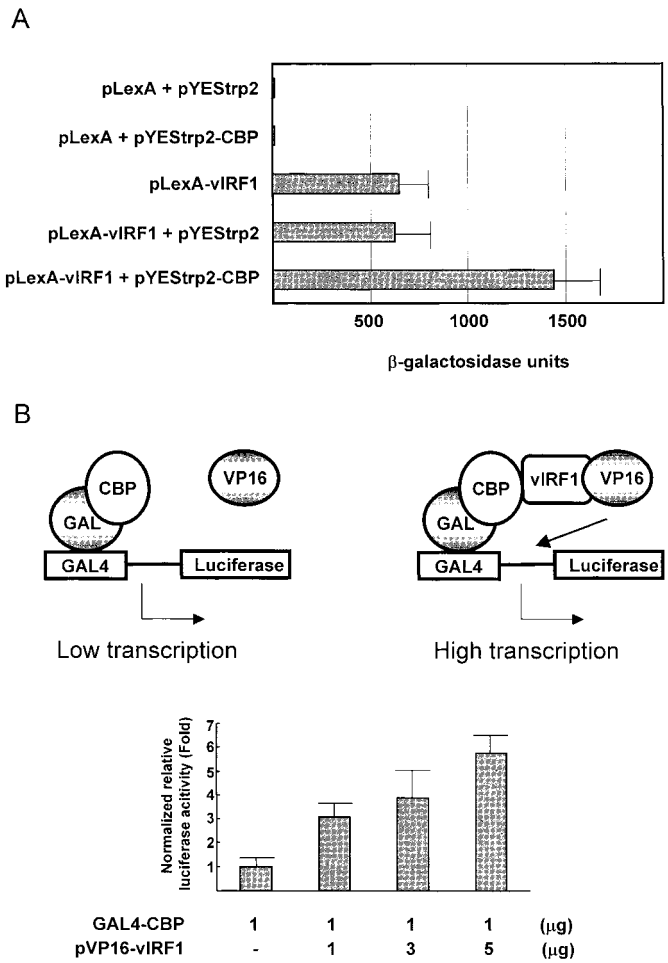

FIG. 1. Yeast and mammalian two-hybrid assay for the detection of vIRF1 and CBP interaction. (A) Yeast two-hybrid assay. Two independent transformants harboring the plasmids indicated on the left were isolated and grown, and their β-galactosidase activity was measured. The values are averages of duplicate assays of two independent transformants with standard deviation indicated. (B) Mammalian two-hybrid assay. Full-length CBP fused to the GAL4 DNA binding domain (GAL4-CBP) interacted with full-length vIRF1 fused to the VP16 transcriptional activation domain in HeLa cells. The HeLa cells were cotransfected with 1 μg of pFR-Luc, 1 μg of GAL4-CBP, and increasing amounts of the VP16-vIRF1. The total DNA amounts were equally adjusted with blank vector. Cells were transfected as described (34), and the luciferase activity was measured 48 h later using extract quantities normalized to the total protein concentration. Each result is an average expressed as fold activation and obtained from three independent experiments.

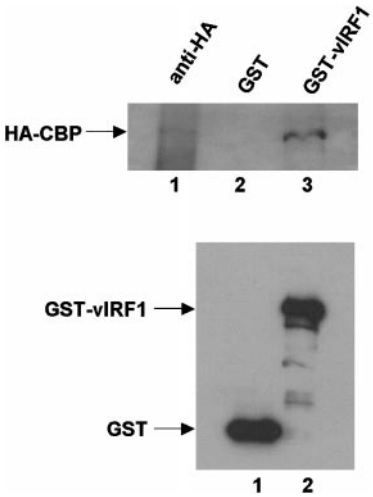

FIG. 2. Association of vIRF1 and CBP proteins *in vivo*. 293T cells were transfected with either pEBG or pEBG-vIRF1 plasmid that contained pcDNA3-HA-CBP plasmid. Whole-cell extracts were prepared 48 h after transfection and they were pulled down with glutathione-Sepharose 4B beads. The pulled down proteins were separated on 7% gel and 12% gel. Each was immunoblotted with anti-HA (upper panel) or anti-GST (lower panel).

ity. The yeast cells containing pLexA-vIRF1 and pYEStrp2-CBP showed about 2.5-fold higher β-galactosidase activity than the yeast cells containing pLexA-vIRF1 and pYEStrp2. These results show that vIRF1 interacts with CBP in yeast.

Next, we carried out the mammalian two-hybrid assay to find vIRF1 binds to CBP in mammalian cells. Transient cotransfection experiments were performed with HeLa cells in which a luciferase reporter plasmid (pFR-Luc), driven by five GAL4 binding sites, was introduced along with an expression vector for full-length CBP fused to the DNA binding domain of GAL4 (GAL4-CBP). Activation of transcription was determined for those cells containing GAL4-CBP in conjunction with the expression plasmid (VP16-vIRF1) for the VP16 fused to the full-length vIRF1. As shown in Fig. 1B, VP16-vIRF1 activates transcription via GAL4-CBP chimeric protein in a dose-dependent manner. We confirmed that vIRF1 does not activate luciferase reporter (pFR-Luc) containing multiple GAL4 binding site (data not shown). These results showed that KSHV vIRF1 binds to CBP in mammalian cells.

To confirm that vIRF1 interacts with CBP *in vivo*, we also carried out the co-immunoprecipitation experiment between vIRF1 and CBP in 293T cells. The plasmids encoding GST fusion proteins (GST alone or GST-vIRF1) and HA–tagged CBP were cotransfected in 293T cells. In 293T cells containing GST-vIRF1 and HA-tagged CBP proteins, the HA-tagged CBP protein was coprecipitated with GST-vIRF1 (Fig. 2, upper panel, lane 3). However, the HA-tagged CBP protein was not coprecipitated with GST in cells containing GST and HA-tagged CBP proteins. (Fig. 2, upper panel, lane 2). To confirm that GST and GST-vIRF1 proteins were expressed in 293T cell, the precipitated proteins were immunoblotted with anti-GST (Fig. 2, lower panel). GST and GST-vIRF1 proteins were clearly shown. These results indicate that vIRF1 and CBP are physically associated in mammalian cells.

vIRF1 specifically binds to the KIX domain and CH/3 region of CBP. In order to determine the binding domain of CBP to KSHV vIRF1, we carried out the GST-pull-down assay. Figure 3A showed that CBP and vIRF1 interacted with each other *in vitro*. *In vitro* binding using GST fusion proteins encoding various domains of CBP were performed to characterize the interaction domains between CBP and vIRF1 (Fig. 1B). KSHV vIRF1 specifically bound to GST-CBP1 (KIX domain) and GST-CBP2 (CH/3 region) with a relatively high affinity; however, vIRF1 was not detected in the eluants from the GST-CBP3 (Q-rich) binding reaction.

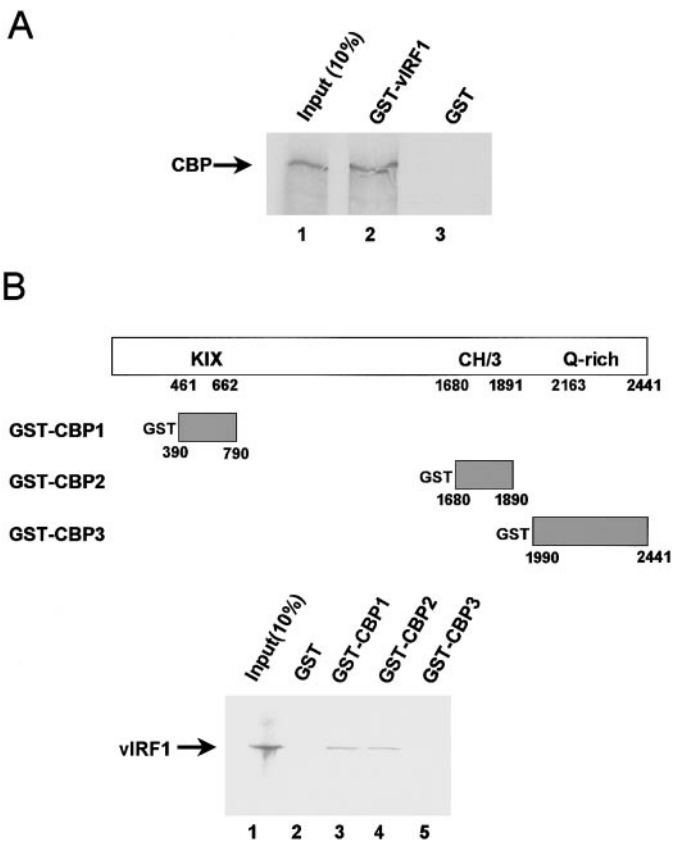

FIG. 3. vIRF1 interacts with CBP *in vitro*. (A) The interaction of an *in vitro* ^{35}S-labeled full-length CBP with immobilized GST-vIRF1 protein is shown in lane 2, and ^{35}S-labeled CBP input (10%) and its binding to GST protein are shown in lanes 1 and 3, respectively. (B) A schematic representation of GST-CBP fusion proteins used in GST pull-down assays is shown. KIX, Kinase induced domain interacting domain; C/H, cysteine/histidine-rich domain; Q-rich, glutamine-rich domain. (upper diagram) Interactions of *in vitro* ^{35}S-labeled full-length vIRF1 with GST, GST-CBP1, GST-CBP2, and GST-CBP3 are shown in lanes 2, 3, 4, and 5, respectively. Lane 1 shows ^{35}S-labeled vIRF1 input (10%).

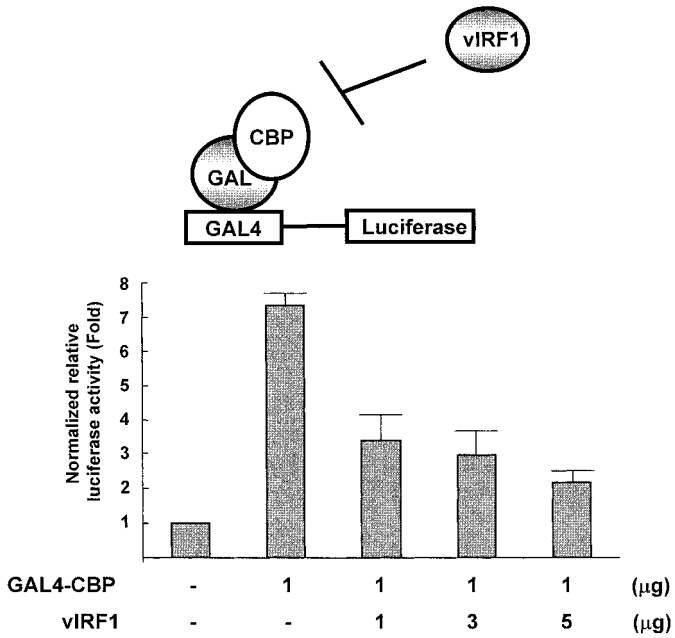

FIG. 4. GAL4-CBP stimulates transcription and its activation is repressed by vIRF1. HeLa cells were cotransfected with 1 μg of pFR-Luc, 1 μg of GAL4-CBP, and with increasing amounts of the expression vector encoding full-length vIRF1. Total DNA amounts were equally adjusted with blank vector. Cells were transfected as described (34), and luciferase activity was measured 48 h later using extract quantities normalized to total protein concentration. Each result is an average obtained from three independent experiments, with the standard deviation indicated.

These results showed that vIRF1 specifically binds to the KIX domain and CH/3 region of CBP.

vIRF inhibits transactivation of CBP. Patel *et al.* (24) reported that HPV-16 E6 binds to CBP and p300 and inhibits the intrinsic transcriptional activity of CBP/p300. Since vIRF1 interacts with the CH/3 domain of CBP like the other DNA virus transforming proteins (18–20, 23), we examined whether the KSHV vIRF1 modulates the transcriptional activation of CBP. Transient cotransfection was performed to investigate the influence of vIRF1 on the transcriptional activity of CBP. The transactivation function of the fusion protein was measured with a luciferase reporter plasmid (pFR-Luc) containing five GAL4 binding sites. The level of luciferase activity obtained with cells transfected with the reporter plasmid (pFR-Luc) alone was set to 1, and the luciferase activities of other cells were compared with this. Before examining the ability of vIRF1 to modulate transactivation of CBP, we checked that vIRF1 does not activate the luciferase reporter plasmid (pFR-Luc) (data not shown). As shown Fig. 4, vIRF1 repressed the transcriptional activity of GAL4-CBP in dose dependent manner. These data demonstrate that vIRF1 can inhibit the transactivation activity of CBP in HeLa cells.

DISCUSSION

CBP is a coactivator for a number of genes that are important for cell growth and differentiation. CBP functions as coactivator of several transcription factors, such as MyoD (14), c-Jun (11), c-Fos (12), and c-Myb (13). CBP is also harnessed by viral proteins, including adenovirus E1A (18, 19, 20), HTLV-1 Tax (21), SV40 large T (22), HPV E6 (23, 24), and EBV BZLF1 (36). Thus, the harboring of CBP by viral protein can cause the dysregulation of gene expression which is a general mechanism for viral regulation of host environment. KSHV vIRF1 is capable of interfering response to IFN-α, IFN-β and IFN-γ at the transcriptional level. It was reported that KSHV vIRF1 represses IFN- and cellular IRF-mediated transcription without binding to interferon-stimulated response element (ISRE) (6, 7). They suggested that the mechanism of vIRF1 action is distinct from DNA binding and might involve in other factor. vIRF1 transforms NIH3T3 cells, and NIH3T3 cells stably expressing vIRF1 demonstrate features of a malignant fibrosarcoma in nude mice, but its molecular mechanism is not fully understood (5, 6). vIRF1 also inhibits tumor necrosis factor (TNF) α-mediated apoptosis NIH3T3 cells and binds to the C-terminal region of p300 *in vitro* (29).

Because CBP is a known coactivator of various transcription factors, CBP might be involved in action of vIRF1. In this report, we have shown that vIRF1 binds to CBP and inhibits transactivation activity of CBP. Biochemical approaches were employed to show that vIRF1 species could really interact with CBP. These approaches include yeast two-hybrid assay, mammalian two-hybrid assay, *in vitro* pull-down assay, and *in vivo* co-immunoprecipitation of GST-vIRF1 with CBP. We also demonstrate that vIRF1 specifically binds to the KIX domain and CH/3 region of CBP. CBP is target for several viral proteins, such as adenovirus E1A (18–20), SV40 large T (22), and HPV E6 (23, 24), and cellular proteins such as c-jun (11), c-fos (12), MyoD (14), and P/CAF (16). Because CBP plays an important role in cell differentiation and in the regulation of transcriptional factors, the dysregulation of CBP is beneficial for virus to regulate host environment. AP1 families such as c-Jun and c-Fos interact with CBP, and E1A sequesters CBP from c-Fos, resulting in silencing of AP1-dependent promoters (11, 12). It is possible that vIRF1 may sequester AP1 using similar mechanism. It is also known that CBP/p300 participates in causing cell cycle arrest by activating certain enhancer and stimulating differentiation pathway (37). Inhibition of transactivating function of CBP could cause certain gene expression, which is important for cell cycle arrest. Because vIRF1 can inhibit transactivating function of CBP, interaction between CBP and vIRF1 may contribute transforming activity of vIRF1.

It was previously reported that IRF3 interacts with CBP/p300 (30–32). IRF3 plays a direct role in virus-mediated signaling, and its role is critical in producing an antiviral effect. Adenovirus E1A blocks the interaction of IRF3 and CBP/p300, and inhibits IRF3-mediated transactivation through targeting CBP/p300 (38). Thus, the inhibitory activity of vIRF1 in IFN signaling could be a result of competitive binding between IRF3 and vIRF1 to CBP/p300. To escape the host immune system host cells, this strategy could be applicable in KSHV.

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REFERENCES

1. Chang, Y., Cesarman, E., Pessin, M. S., Lee, F., Culpepper, J., Knowles, D. M., and Moore, P. S. (1994) *Science* **266,** 1865–1869.
2. Chang, Y., and Moore, P. S. (1996) *Infect. Agents Dis.* **5,** 215–222.
3. Russo, J. J., Bohenzky, R. A., Chien, M. C., Chen, J., Yan, M., Maddalena, D., Parry, J. P., Peruzzi, D., Edelman, I. S., Chang, Y., and Moore, P. S. (1995) *Proc. Natl. Acad. Sci. USA* **93,** 14862–14867.
4. Burysek, L., Yeow, W. S., and Pitha, P. M. (1999) *J. Hum. Virol.* **2,** 19–32.
5. Li, M., Lee, H, Guo, J., Neipel, F., Fleckenstein, B., Ozato, K., and Jung, J. U.(1998) *J. Virol.* **72,** 5433–5440.
6. Zimring, J., Goodbourn, S., and Offermann, M. K. (1998) *J. Virol.* **72,** 701–707.
7. Gao, S., Boshoff, C., Jayachandra, S., Weiss, R. A., Chang, Y., and Moore, P. S. (1997) *Oncogene* **15,** 1979–1985.
8. Sarid, R., Flore, O., Bohenzky, R. A., Chang, Y., and Moore, P. S. (1998) *J. Virol.* **72,** 1005–1012.
9. Roan, F., Zimring, J. C., Goodbourn, S., and Offermann, M. K. (1999) *J. Gen. Virol.* **80,** 2205–2209.
10. Kwok, R. P. S., Lundbald, J. R., Chrivia, J. C., Richards, J. P., Bächinger, H. P., Brennan, R. G., Roberts, S. G. E., Green, M. R., and Goodman, R. H. (1994) *Nature* **370,** 223–226.
11. Arias, J., Alberts, A. S., Brindle, P., Claret, F. X., Smeal, T., Karin, M., Feramisco, J., and Montminy, M. (1994) *Nature* **370,** 226–229.
12. Bannister, A. J., and Kouzarides, T. (1995) *EMBO J.* **14,** 4758–4762.
13. Dai, P., Akimaru, H., Tanaka, Y., Hou, D. X., Yasukawa, R., Kenei-Ishii, C., Takahashi, T., and Ishii, S. (1996) *Genes Dev.* **10,** 528–540.
14. Yuan, W., Condorelli, G., Caruso, M., Felsani, A., and Giordano, A. (1996) *J. Biol. Chem.* **271,** 9009–9013.
15. Lee, J. S., Galvin, K. M., See, R. H., Eckner, R. Livingston, D., Moran, E., and Shi, Y. (1995) *Genes Dev.* **9,** 1188–1198.
16. Yang, X. J., Ogryzko, V. V., Nishikawa, J., Howard, B. H., and Nakatani, Y. (1996) *Nature* **382,** 319–324.
17. Perkins, N. D., Felzien, L. K., Betts, J. C., Leung, K., Beach, D. H., and Nabel, G. J. (1997) *Science* **275,** 523–527.
18. Arany, Z., Newsome, D., Oldread, E., Livingston, D. M., and Eckner, R. (1995) *Nature* **374,** 81–84.
19. Lundblad, J. R., Kwok, R. P. S., Laurance, M. E., Harter, M. L., and Goodman, R. H. (1995) *Nature* **374,** 85–88.
20. Dorsman, J. C., Teunisse, A. F., Zantema, A., and van der Eb, A. J. (1997) *J. Gen. Virol.* **78,** 423–426.
21. Kwok, R. P., Laurance, M. E., Lundbald, J. R., Goldman, P. S., Shih, H., Connor, L. M., Marriott, S. J., and Goodman, R. H. (1996) *Nature* **380,** 642–646.
22. Eckner, R., Ludlow, J. W., Lill, N. L., Oldread, E., Arany, Z., Modjtahedi, N., Decaprio, J. A., Livingston, D. M., and Morgan, J. A. (1996) *Mol. Cell. Biol.* **16,** 3454–3464.
23. Zimmermann, H., Degenkolbe, R., Bernard, H., and O'Connor, M. (1999) *J. Virol.* **73,** 6209–6219.
24. Patel, D., Huang, S., Baglia, L. A., and McCance, D. J. (1999) *EMBO J.* **18,** 5061–5072.
25. Bannister, A. J., and Kouzarides, T. (1996) *Nature* **384,** 641–643.
26. Ogryzko, V. V., Schiltz, R. L., Russanova, V., Howard, B. H., and Nakatani, Y. (1996) *Cell* **87,** 953–959.
27. Chakravarti, D., LaMorte, V. J., Nelson, M. C., Nakajima, T., Schulman, I. G., Jugulion, H., Montminy, M., and Evans, R. M. (1996) *Nature* **383,** 99–103.
28. Chakravarti, D., Ogryzko, V., Kao, H. Y., Nash, A., Chen, H., Nakatani, Y., Evans, R. M. (1999) *Cell* **96,** 393–403.
29. Burysek, L., Yeow, W., Lubyova, B., Kellum, M., Schafer, S. L., Huang, Y. Q., and Pitha, P. M. (1999) *J. Virol.* **73,** 7334–7342.
30. Weaver, B. K., Kumar, K. P., and Reich, N. C. (1998) *Mol. Cell. Biol.* **18,** 1359–1368.
31. Yoneyama, M., Suhara, Y., Fukuhara, Y., Fukada, M., Nishida, E., and Fujita, T. (1998) *EMBO J.* **17,** 1087–1095.
32. Lin, R., Mamane, Y., and Hiscott, J. (1999) *Mol. Cell. Biol.* **19,** 2465–2474.
33. Wilson, A. C., and Patient, R. K. (1991) *in* Gene Transfer and Expression Protocols (Murray, E. J., Ed.), pp. 397–404. Humana Press, New Jersey.
34. Graham, F. L., and van der Eb, A. J. (1973) *Virology* **52,** 456–467.
35. Guarante, L. (1983) *Methods Ezymol.* **101,** 181–193.
36. Adamson, A. L., and Kenney, S. (1999) *J. Virol.* **73,** 6551–6558.
37. Puri, P. L., Avantaggiati, M. L., Balsano, C., Sang, N., Graessmann, A., Giordano, A., and Levrero, M. (1997) *EMBO J.* **16,** 369–383.
38. Juang, Y. T., Lowther, W., Kellum, M., Au, W. C., Lin, R., Hiscott, J., Pitha, P. M. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 9837–9842.