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Characterization of a Novel Human Herpesvirus 8-Encoded Protein, vIRF-3, That Shows Homology to Viral and Cellular Interferon Regulatory Factors

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The genome of the human herpesvirus 8 (HHV-8) contains a cluster of open reading frames (ORFs) encoding proteins with homology to the cellular transcription factors of the interferon regulatory factor (IRF) family. Two of these homologues, vIRF-1 and vIRF-2, were previously identified and functionally analyzed. In this study, we have characterized a novel gene, designated vIRF-3, encoded within the previously predicted ORF K10.5 and our newly identified ORF K10.6. Northern blotting of RNA extracted from BCBL-1 cells with a vIRF-3-specific probe and reverse transcription-PCR analyses revealed a single transcript of 2.2 kb with a splice present in the coding region. The vIRF-3 mRNA levels in BCBL-1 cells were increased upon 12-*O*-tetradecanoylphorbol-13-acetate treatment, with kinetics of expression similar to those of the early immediate genes. The vIRF-3 ORF encodes a 73-kDa protein with homology to cellular IRF-4 and HHV-8-encoded vIRF-2 and K11. In transient transfection assays with the IFNACAT reporter, vIRF-3 functioned as a dominant-negative mutant of both IRF-3 and IRF-7 and inhibited virus-mediated transcriptional activity of the IFNA promoter. Similarly, the overexpression of vIRF-3 in mouse L929 cells resulted in inhibition of virus-mediated synthesis of biologically active interferons. These results suggest that by targeting IRF-3 and IRF-7, which play a critical role in the activation of alpha/beta interferon (IFN) genes, HHV-8 has evolved a mechanism by which it directly subverts the functions of IRFs and down-regulates the induction of the IFN genes that are important components of the innate immunity.

Viral infection induces expression of cellular genes encoding early inflammatory proteins, like cytokines and chemokines, that can modulate humoral and cellular immunity. Among these, a group of proteins with a direct antiviral effect, the interferons (IFNs), play a critical role in the innate immunity to viral infection. To overcome the inhibitory effect of IFNs, some viruses have developed a variety of strategies by which they can antagonize effects of cytokines that are involved in viral clearance (15, 33). DNA viruses of the poxvirus and herpesvirus families have captured and modified cellular genes that encode cytokines or chemokines as well as their receptors (20, 25). The human herpesvirus 8 (HHV-8)–Kaposi's sarcoma-associated herpesvirus contains a cluster of open reading frames (ORFs) encoding proteins with homology to the cellular transcription factors of the interferon regulatory factor (IRF) family (22).

The molecular mechanism by which viruses activate expression of IFN genes in infected cells is unclear. However, it has been shown that members of the IRF family of transcription factors play a critical role in the regulated expression of both alpha/beta IFN genes (IFNA and IFNB) and IFN-stimulated genes (ISGs) (28). All of the cellular IRFs identified show a high degree of homology in the amino-terminal region of the molecule that consists of the DNA binding domain, characterized by five conserved tryptophan (W) repeats. Three of these tryptophan residues contact DNA by recognizing a GAAA sequence (8). The carboxy-terminal parts of these proteins are diverse. The first IRF, IRF-1, was assumed to serve as a positive regulatory activator of both IFNA and IFNB genes in infected cells, whereas IRF-2, which binds to the same DNA element, was characterized as a repressor (10). Recently, two members of the IRF family, IRF-3 and IRF-7, were identified as transducers of the virus-mediated signaling pathway in infected cells (2, 3, 11, 17, 19, 38–40, 45, 46).

The HHV-8–Kaposi's sarcoma-associated herpesvirus has been established as an important factor in the pathogenesis of Kaposi's sarcoma and AIDS-associated body cavity-based lymphoma (23, 26). Analyses of HHV-8 genomic sequences (36) showed that this virus, as well as the other gamma herpesviruses, Epstein-Barr virus and herpesvirus saimiri, contain a unique set of nonstructural genes that may be involved in viral mimicry and may be essential for viral replication in vivo and for viral pathogenicity. HHV-8 contains several homologues of cellular IRFs (22), which are clustered in the 83- to 95-kb region of the HHV-8 genome. The ORF K9-encoded vIRF (designated vIRF-1) is a 449-amino-acid (aa)-long protein that shows partial sequence homology with cellular IRFs (22). In contrast, vIRF-2 encodes only a 163-aa-long protein with 40% identity to vIRF-1 in the amino-terminal region (5). The amino-terminal regions of vIRF-1 and vIRF-2 contain four tryptophan residues; however, only two of these are present in the same configuration as in cellular IRFs. Lytic reactivation of HHV-8 in BCBL-1 tumor cell line and expression of vIRF-1 can be induced by treatment with 12-*O*-tetradecanoylphorbol-13-acetate (TPA). In a transient expression assay, vIRF-1 inhibits virus-mediated transcriptional activation of the IFNA gene promoter and IFN-stimulated activation of ISG promot-

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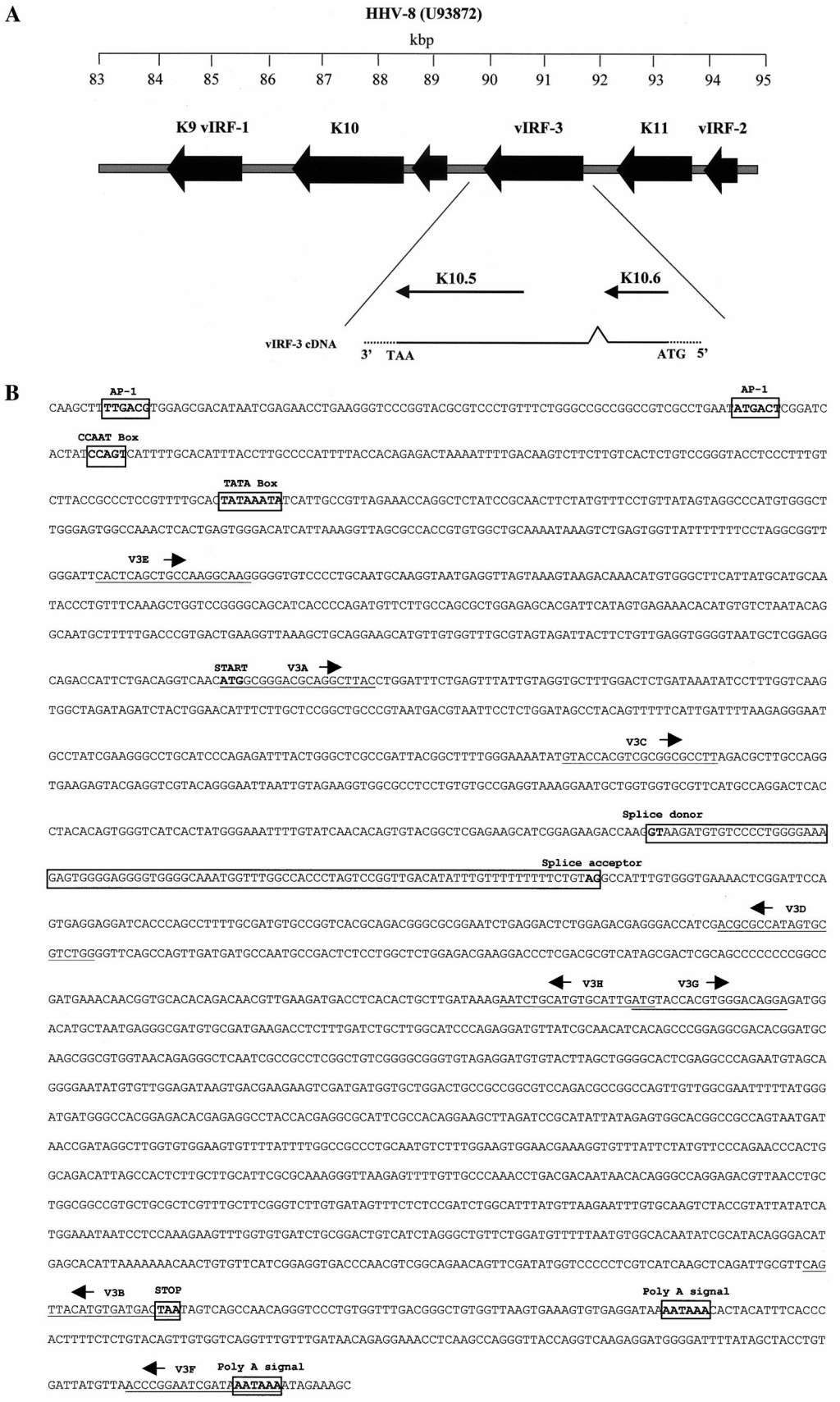

FIG. 1. Genomic organization of HHV-8-encoded vIRF-3. (A) Schematic diagram of the 83- to 95-kb region of the HHV-8 genome (GenBank accession no. U93872) showing the cluster of ORFs with homology to cellular IRFs. Diagram of vIRF-3 ORF is shown below. (B) Map of the vIRF-3 ORF. A putative CCAAT box, TATA box, AP-1 binding sites, and poly(A) signals are boxed and in boldface. The nucleotide sequence corresponding to the intron is boxed; the splice donor (GT. . .) and splice acceptor (. . .AG) sites are in boldface. The primers (V3A, V3B, V3C, V3D, V3E, V3F, V3G, and V3H) used for RT-PCR analyses are underlined. The arrows indicate the orientations of the primers.

ers (6, 9, 16, 32, 47). However, neither vIRF-1 nor vIRF-2 bind to DNA with the same specificity as cellular IRFs, indicating that if vIRFs are DNA binding proteins, their binding has a pattern distinct from that of the cellular IRFs. In this context, we have recently shown that vIRF-2 can bind to oligodeoxynucleotides corresponding to the NF-κB site (5). Consequently, vIRF-2 down-modulates RelA-stimulated transcriptional activity of the human immunodeficiency virus long terminal repeat promoter. Thus, both vIRF-1 and vIRF-2 show properties distinct from those of cellular IRFs and from each other, and, therefore, each of these vIRFs may have a unique role in HHV-8 pathogenicity.

The aim of this study was to clone and characterize another HHV-8-encoded protein, vIRF-3, that shows homology to cellular and viral IRFs. We demonstrate that the TPA stimulation of the BCBL-1 tumor cell line induces expression of vIRF-3 mRNA with kinetics similar to that of the HHV-8 lytic genes. Functional analysis has shown that vIRF-3 down-regulates virus-mediated activation of IFNA gene promoters. These results suggest that, by targeting cellular IRFs, the HHV-8-encoded vIRF-3 effectively modulates their functions and consequently diminishes the early inflammatory response.

Cloning and characterization of vIRF-3. Sequence analysis of the HHV-8 genome has identified a cluster of ORFs that show partial homology to cellular transcription factors of the IRF family. These ORFs are localized in the 83- to 95-kb region of the HHV-8 genome (Fig. 1A). Two of them, vIRF-1 (K9) and vIRF-2, were previously cloned and functionally characterized (5, 6, 9). Two additional ORFs, designated K10.5 and K10.6, are located on the HHV-8 genomic sequence (GenBank accession no. U93872) at bp 90841 to 89900 and 91694 to 91236, respectively. Both K10.5 and K10.6 show amino acid homology to HHV-8-encoded K11 and vIRF-2. The expression of K10.5 ORF was examined in the HHV-8-positive B-cell line, BCBL-1. These cells are latently infected with HHV-8, and treatment with butyrate or TPA has been shown to result in expression of lytic viral genes and vIRF-1 (22, 30, 37). Northern blot analysis of the total cellular RNA from untreated or TPA-treated BCBL-1 cells with a cDNA probe corresponding to K10.5 ORF showed the presence of a single transcript of approximately 2.2 kb (Fig. 2A). This transcript appeared to be longer than the predicted K10.5 ORF (0.942 kb); therefore, we hypothesized that both ORFs, K10.5 and K10.6, are transcribed on the same mRNA. In order to confirm this hypothesis, we performed reverse transcription-PCR (RT-PCR) analysis on RNA extracted from TPA-treated (24 h) BCBL-1 cells using primers corresponding to the 5′ end of K10.6 (primer V3A) and 3′ end of K10.5 (primer V3B) (see Fig. 1B for primer sequences). As shown in Fig. 2B, amplification of RNA by RT-PCR as well as DNA isolated from BCBL-1 cells resulted in fragments of similar sizes that were subsequently cloned and sequenced. The sequence comparison of the RT-PCR product with the corresponding genomic region revealed a presence of a 94-bp intron sequence with typical splice donor-acceptor site (GT. . .AG) present in the intron (Fig. 1B). The splicing removed the stop codon (TAA) of K10.6 ORF together with additional sequence further downstream, thus generating a 1,701-nucleotide-long ORF with a predicted translation product of 566 aa containing both K10.6 and K10.5 ORFs. We have designated this ORF vIRF-3. The first ATG of K10.6 ORF is considered to be a translation start site due to its strong match to Kozak consensus (13). Furthermore, multiple in-frame stop codons can be found 5′ of this ATG. Potential poly(A) addition signals (AATAAA) are present 63 and 208 nucleotides downstream of the stop codon. To confirm our sequencing data, RT-PCR was carried out with RNA extracted from BCBL-1 cells using the primers (V3C and V3D) flanking the intron sequence (Fig. 2C). Extracted DNA served as a control against detection of genomic DNA. Amplification of HHV-8 genomic DNA with these primers yielded a 440-nucleotide-long fragment, as predicted by HHV-8 sequence (Fig. 2C, lane 5); however, a fragment of only 346 nucleotides was amplified by RT-PCR from RNA extracted from TPA-treated and untreated BCBL-1 cells (Fig. 2C, lanes 1 and 3). These data further confirm that the vIRF-3 present in the BCBL-1 cells is spliced.

To map the 5′ end of the vIRF-3 transcript, RT-PCR analysis was performed with primers V3E and V3D (see Fig. 1B for primer sequences). Similarly, the 3′ end of the vIRF-3 transcript was analyzed by using primers V3C and V3F. As can be seen in Fig. 2D, RT-PCR analysis with V3E and V3D primers yielded DNA products of expected sizes, 906 bp in the case of RNA and 1,000 bp in the case of DNA. When a similar analysis was performed with primers V3C and V3F, the resulting PCR products amplified from RNA and DNA of BCBL-1 cells were 1,670 and 1,764 bp, respectively. Sequencing analysis of these PCR products did not detect any splice site in the untranslated region.

In the search for potential regulatory sequences in the DNA region upstream of V3E primer, we used the TRANSFAC database. Scanning of the 200-nucleotide-long DNA sequence revealed the presence of potential TATA and CCAAT boxes and AP-1 binding sites (Fig. 1B). Therefore, we suggest that the vIRF-3 transcription starts approximately 30 nucleotides downstream of the potential TATA box. However, the precise localization of the transcription start site remains to be determined.

To examine the kinetics of vIRF-3 expression, we performed a quantitative RT-PCR analysis of the vIRF-3 ORF in untreated or TPA-induced BCBL-1 cells at 2, 4, 8, 16, and 24 h postinduction (Fig. 3). This experiment revealed that the levels of vIRF-3 transcript increased gradually 2 h after TPA treatment, reaching maximal levels 16 to 24 h postinduction; the levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA were not modulated by TPA treatment. These data indicate that expression of vIRF-3 increases during the lytic cycle of HHV-8 infection, together with the expression of vIRF-1 and the other lytic viral genes (22). The low levels of vIRF-3 mRNA detected in uninduced cells may reflect those few cells (1 to 5%) that are productively infected in BCBL-1 cell cultures. In contrast, vIRF-2 expression is constitutive in BCBL-1 and in other HHV-8-positive B-cell lines (unpublished observation). The role of vIRF-3 in the HHV-8 replication cycle has not been addressed in this study and remains to be established.

Comparison of the amino acid sequence of vIRF-3 with those of the previously characterized HHV-8-encoded vIRF-2 and its close homologue K11 (Fig. 4A) shows that the amino-terminal region of the vIRF-3 protein (aa 1 to 150) has a high degree of amino acid identity with vIRF-2 (25% identities, 44% similarities) while the carboxy-terminal part (aa 170 to 566) contains domains that are homologous with K11 (26% identities, 47% similarities). In the N-terminal region, vIRF-2 and vIRF-3 contain four and five tryptophan repeats, respectively; however, only two of these repeats are in the same configuration as in the DNA binding domain of cellular IRFs. Neither vIRF-1 nor vIRF-2 is able to bind the oligodeoxynucleotides corresponding to the IRF-E or ISRE domain present in the promoters of IFN or ISGs, respectively. Whether vIRF-3 can bind to DNA with the same specificity as cellular IRFs is currently being examined. BLASTp database searches with vIRF-3 amino acid sequence detected a significant homology

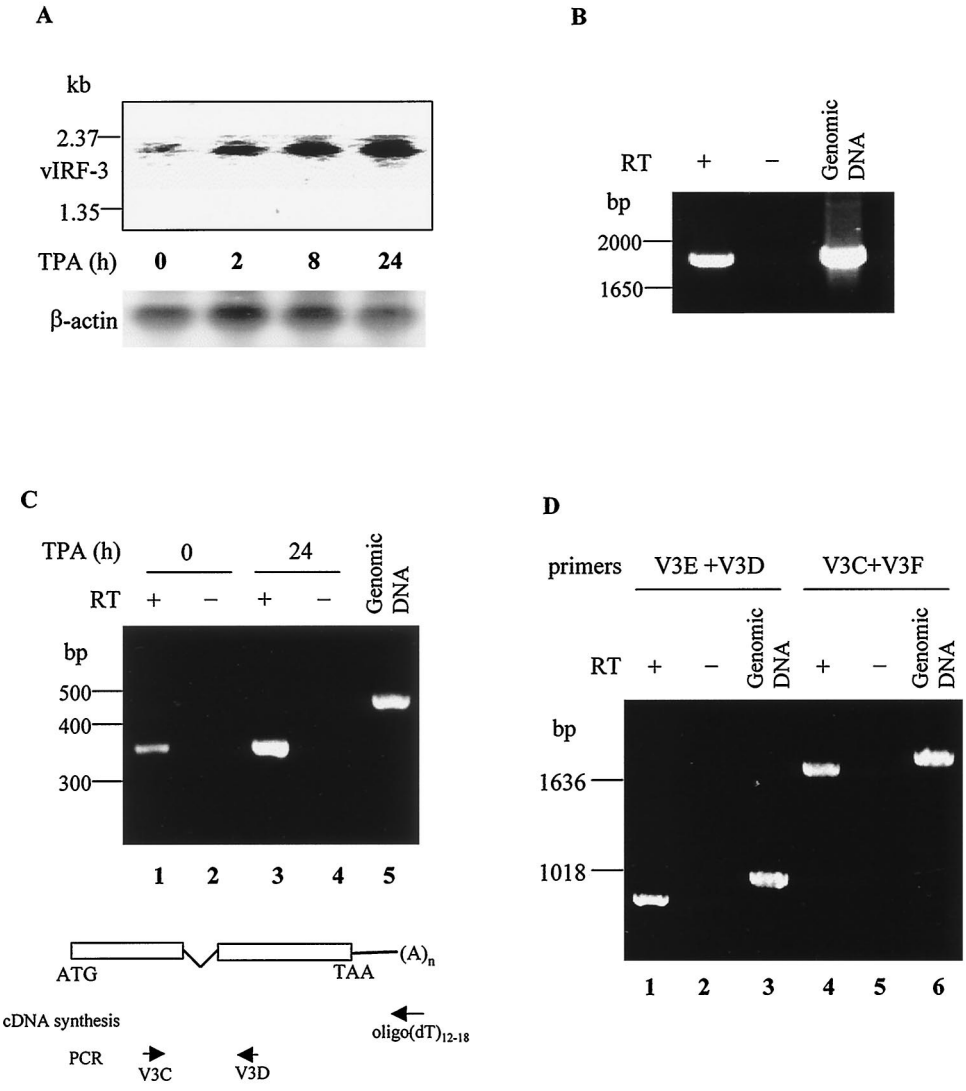

FIG. 2. Expression of the vIRF-3 gene in BCBL-1 cells. (A) Northern blot analysis of total RNA isolated from BCBL-1 cells treated with TPA (50 ng/ml) for 0, 2, 8, and 24 h. The vIRF-3-specific transcript was detected by using K10.5 ORF cDNA as a probe. The levels of β-actin mRNA at different times postinduction are shown for comparison. (B) Expression of the sense strand of vIRF-3 ORF. Total RNA was isolated from BCBL-1 cells treated with TPA for 24 h and was reverse transcribed (RT+). The primer (V3B) used for cDNA synthesis was complementary to the sense strand at the 3′ end of the vIRF-3 ORF. The cDNA was amplified by PCR using the primers V3A and V3B. The RT-PCR reaction in the absence of reverse transcriptase (RT−) and PCR amplification of genomic vIRF-3 ORF (Genomic DNA) were used as controls. (C) RT-PCR was carried out with RNA extracted from uninduced or TPA-induced (24 h) BCBL-1 cells; extracted DNA served as a control against detection of genomic DNA. Amplification of HHV-8 genomic DNA yielded a 440-nucleotide fragment (lane 5). However, a fragment of only 346-nucleotides was amplified by RT-PCR from RNA extracted from TPA-treated and untreated BCBL-1 cells (lanes 1 and 3). The RT-PCR reactions in the absence of reverse transcriptase (RT−) were used as controls (lanes 2 and 4). Schematic representation of the primers used in the assay is shown in the lower panel. (D) RT-PCR analysis of RNA extracted from BCBL-1 cells after 24 h of TPA treatment. Total RNA was reverse transcribed by using oligo(dT)$_{12–18}$ primers and PCR amplified with primers (V3E and V3D, and V3C and V3F) located in the 5′ and 3′ untranslated regions. PCR amplification of BCBL-1 DNA served as a control.

to the lymphoid cell-specific IRF-4 (4, 7, 27). Alignments of vIRF-3 and IRF-4 homologous regions are shown in Fig. 4B. It was previously reported that IRF-4 can form heterodimers with the hematopoietic cell-specific transcription factor PU.1 (7, 31, 41), which increases their DNA binding affinity. The domain of IRF-4 which associates with PU.1 is located near the carboxyl terminus, between residues 245 and 412 in the region designated IRF association domain (43) which shows homology to other IRFs. Since vIRF-3 also shares similarity in this region, we suggest that vIRF-3 might exert its activity via the formation of complexes with cellular IRFs. In addition, BLAST search analysis revealed significant similarity between the vIRF-3 and vIRF homologues of two isolates of the rhesus monkey rhadinovirus (RRV26-95 and RRV17577) (1, 42). The overall organization of the rhesus monkey rhadinovirus genome was found to be very similar to that of human Kaposi's sarcoma-associated herpesvirus.

To characterize the polypeptide produced by vIRF-3 ORF, we transfected HeLa cells with vectors expressing a FLAG-tagged vIRF-3 from either a cDNA clone or a genomic fragment. A comparison of the proteins expressed from these two vIRF-3 constructs revealed no obvious difference in mobility analyzed by Western blotting after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 5B, lanes 1, 4, and 5). The apparent molecular mass of the vIRF-3 polypeptide is approximately 73 kDa. In addition, transcription and transla-

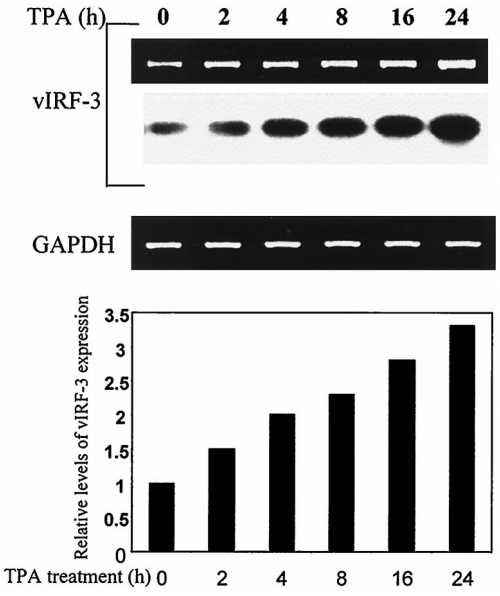

FIG. 3. Quantitative RT-PCR analysis of the vIRF-3 ORF. Total RNA isolated from BCBL-1 cells at different time points of TPA treatment (0, 2, 4, 8, 16, and 24 h) was reverse transcribed by using oligo$(dT)_{12-18}$ primers and was subsequently PCR amplified with primers complementary to the 5′ and 3′ regions of the vIRF-3 ORF. Products were visualized by agarose gel electrophoresis (upper panel). The middle panel represents the Southern blot of RT-PCR products diluted 25 times and probed with vIRF-3 cDNA. The levels of GAPDH expression are shown for comparison (lower panel). Kinetics of vIRF-3 transcript expression is summarized in the graph.

tion of FLAG-vIRF-3 cDNA in a coupled transcription-translation system in vitro yielded a protein with the same molecular mass (Fig. 5C, lane 1). We also inserted the FLAG-tagged amino-terminal (aa 1 to 254) and carboxy-terminal (aa 254 to 566) parts of vIRF-3 (vIRF-3-N′ and vIRF-3-C′, respectively) into an expression vector (Fig. 5A). When transfected into HeLa cells or translated in vitro, vIRF-3-N′ and vIRF-3-C′ encoded polypeptides with molecular masses of approximately 35 and 38 kDa, respectively (Fig. 5B and Fig. 5C, lanes 2 and 3).

vIRF-3 down-modulates the IRF-3- and IRF-7-mediated activation of IFNA promoters in infected cells. The critical role of IRF-3 and IRF-7 in the induction of IFN genes in infected cells has been previously established (3, 11, 38, 39, 45, 46). To determine whether vIRF-3 can also modulate the expression of early inflammatory genes such as alpha/beta IFN genes, we cotransfected vIRF-3-expressing plasmid into NIH 3T3 cells together with the reporter plasmid in which the promoter region of murine IFNA4 gene regulates expression of the CAT gene (34). Constitutive expression of this plasmid is very low in these cells but can be enhanced by 10-fold after infection with Sendai virus (Fig. 6A). Cotransfection of vIRF-3 with the IFNA4CAT reporter plasmid has decreased the transcriptional activity of IFNA4 promoter by twofold in infected cells. We have previously shown that in a transient transfection assay, virus-mediated stimulation of the IFNA4 gene promoter can be further enhanced by cotransfection with IRF-3 and IRF-7 (2, 3). Transfection of IRF-3-expressing plasmid increased virus-mediated activation of IFNA4 promoter by sixfold, whereas cotransfection with the IRF-7-expressing plasmid enhanced the virus-stimulated activity of this promoter by 12-fold. Cotransfection of vIRF-3 with IRF-3 inhibited IRF-3-mediated stimulation of the IFNA4 gene promoter in infected cells. Similarly, synergism between virus and IRF-7 was also inhibited in cells expressing vIRF-3. Overexpression of vIRF-3 also decreased an IRF-3 or IRF-7 activation of ISG15 promoter in cells infected with Sendai virus (data not shown).

To determine whether vIRF-3 also can inhibit induction of endogenous IFN genes, the levels of IFN synthesized in mouse L929 cells transfected with either vIRF-3 or an empty vector were compared. As shown in Table 1, overexpression of vIRF-3 decreased virus-mediated stimulation of IFN synthesis by almost twofold. It should be noted that under the conditions of transient transfection, when approximately 20 to 30% of cells are transfected, the observed inhibition is probably underestimated. These data indicate that vIRF-3 interferes with the transactivating potential of cellular IRFs that play a critical role in the induction of IFNA and IFNB genes, namely IRF-3 and IRF-7.

We next determined which part of the vIRF-3 protein confers inhibitory activity. The N-terminal (aa 1 to 254) and C-terminal (aa 254 to 566) regions of vIRF-3 cDNA were introduced into expression plasmids. Cotransfection of these plasmids with the IFNA4CAT reporter construct showed that both the N- and C-terminal parts of vIRF-3 protein retained biological activity and were able to inhibit IRF-3- and IRF-7-mediated induction of the IFNA4 promoter to the same extent as a full-size protein (Fig. 6B). Inhibition was also observed in cells that were transfected with the expression vector containing the genomic region of vIRF-3. The observed inhibition of IFNA4 activity by both N- and C-terminal parts of vIRF-3 may be due to their interaction with different regulatory factors. We have previously shown that both vIRF-1 and vIRF-2 are able to bind to IRFs or transcriptional coactivator CREB binding protein-p300 and down-regulate their transactivation of the IFNA4 promoter (5, 6).

The ability to modulate the interferon system may be essential for HHV-8 replication in vivo. It was recently reported that HHV-8 is sensitive to the antiviral effect of interferon (21). Exogenous interferon has been used clinically for treatment of Kaposi's sarcoma in AIDS patients with about a 25 to 40% response (24). In view of the finding that both vIRF-3 and vIRF-1 interfere with the IFN-mediated induction of ISGs, the sensitivity of HHV-8 replication to IFN-α indicates either that these two vIRFs are not expressed in Kaposi's sarcoma lesions or that high concentration of exogenous IFN can overcome the effect of these two vIRFs. However, the modulation of expression of the early inflammatory genes by vIRF-3 may not be limited to the interferon system. IRF-3 was shown to play a critical role in virus-mediated induction of the RANTES promoter (18), and a role for IRF-1 was implied in the activation of several inducible genes such as nitric oxide synthetase (12), PKR (29), and growth regulatory gene p21 (WAF1/CIP1) (44). Thus, vIRF-3 could have a more general effect on the expres-

TABLE 1. Inhibition of virus-mediated induction of IFN (U/ml) by vIRF-3 in mouse L929 cells

Plasmids[a]	IFN (U/ml)[b]	
	1st expt	2nd expt
Vector-NDV[c]	15,000	21,200
vIRF-3-NDV	8,250	11,520

[a] Mouse L929 cells were transfected with either vIRF-3-expressing plasmid or empty vector, and 24 h after transfection, cells were infected with NDV (multiplicity of infection = 5).

[b] The levels of IFN were determined by biological assay (35) in the medium collected 16 h after infection.

[c] NDV, Newcastle disease virus.

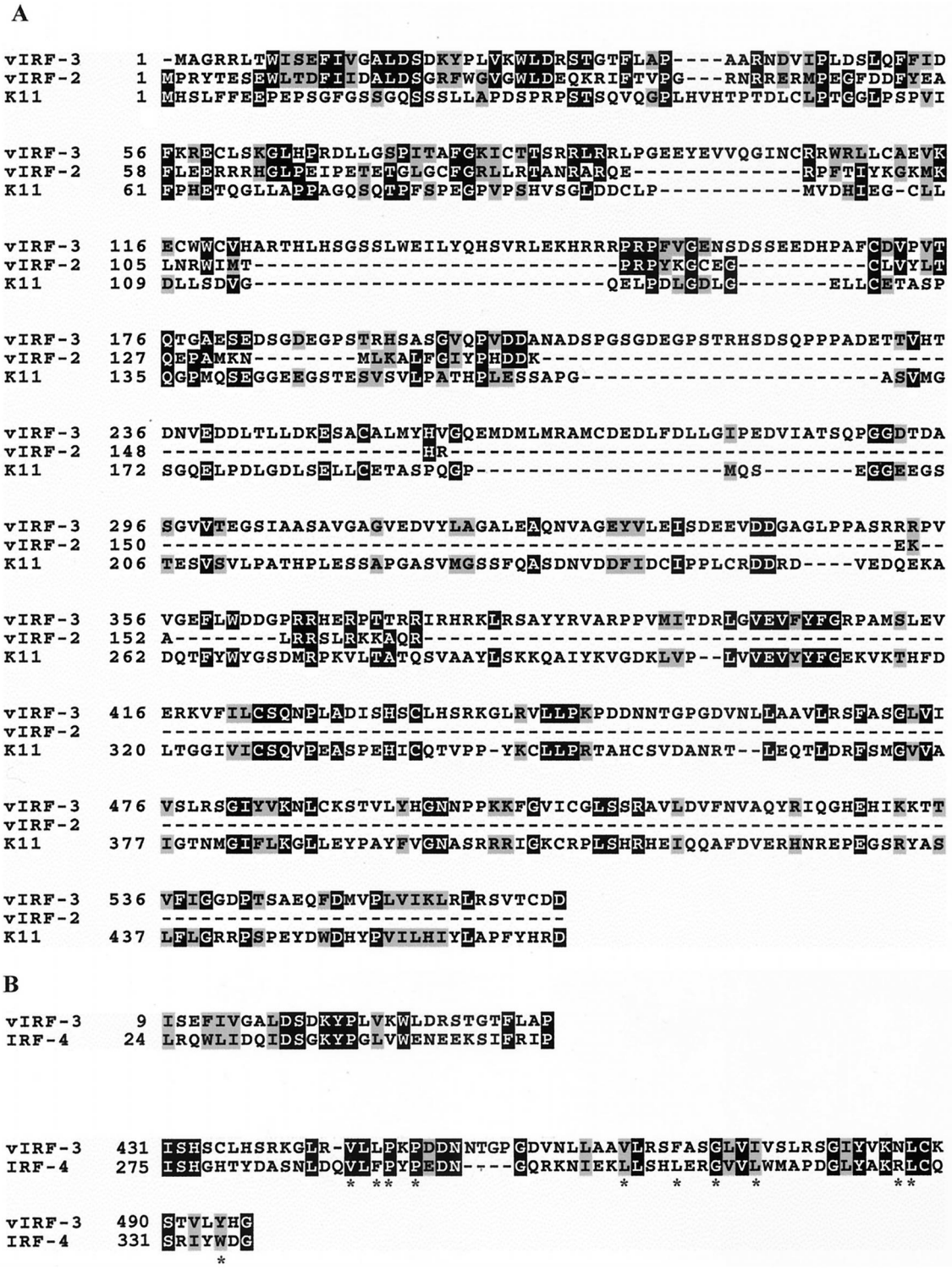

FIG. 4. (A) Multiple alignment of HHV-8-encoded IRF homologues. The alignment was constructed of vIRF-3 (accession no. AF157602), vIRF-2 (accession no. AF045550), and K11 (accession no. U93872) amino acid sequences by using CLUSTAL W software. Identical and homologous residues are shaded in black and gray, respectively. (B) Alignment of homologous regions between vIRF-3 and IRF-4 (accession no. U52682). The asterisks below the sequence indicate the residues conserved in the IRF association domain of most cellular IRFs.

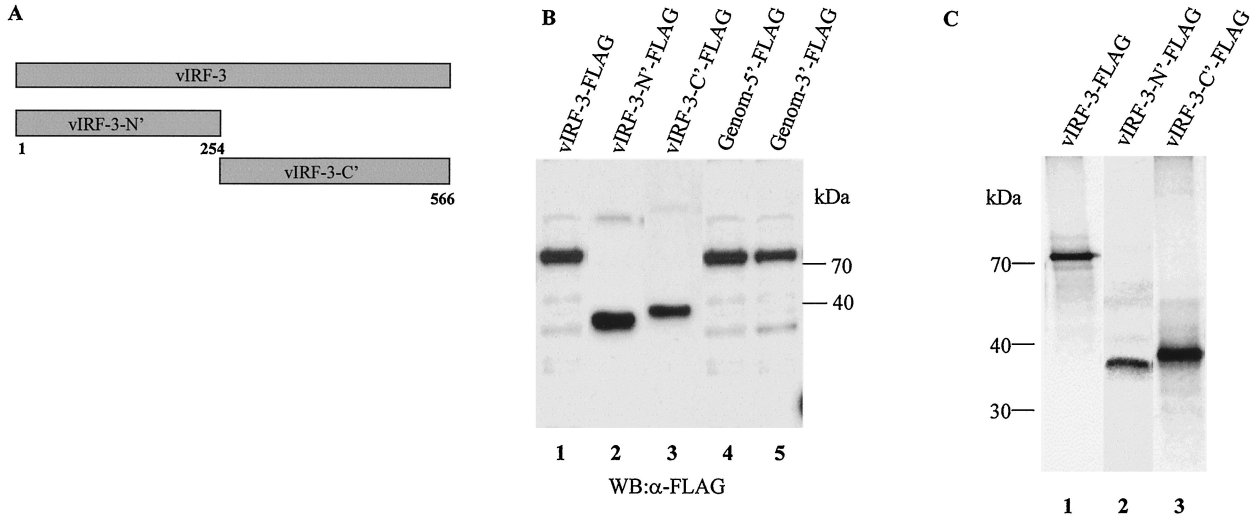

FIG. 5. Analysis of vIRF-3 protein synthesized in vivo and in vitro. (A) Schematic diagram of vIRF-3 deletion constructs. (B) Western blot analysis of cell lysates obtained from HeLa cells transfected with FLAG-tagged vIRF-3 expression constructs. To generate the vIRF-3-FLAG, vIRF-3-N′-FLAG, and vIRF-3-C′-FLAG constructs, the vIRF-3 ORF was amplified by RT-PCR from RNA of TPA-induced (24 h) BCBL-1 cells using primers V3A and V3B, V3A and V3H, and V3G and V3B, respectively (see Fig. 1B for position of primers). The primers V3A and V3G contained an *Eco*RI restriction site, and primers V3B and V3H contained a *Bam*Hi restriction site and a FLAG epitope (DYKDDDDK). The amplified products were digested and inserted into pcDNA3.1(+) (Invitrogen). To construct vIRF-3 genomic expression vectors, the viral DNA from HHV-8-harboring BCBL-1 cells was used as a template for PCR amplifications, with primers V3A and V3B containing a FLAG epitope on either the 5′ or 3′ end. The amplified products were digested with *Eco*RI and *Bam*HI and inserted into pcDNA3.1(+) vector. Transfection of vIRF-3 cDNA and vIRF-3 genomic constructs containing the intron sequence (Genom-5′-FLAG and Genom-3′-FLAG) yielded proteins of the same size of approximately 73 kDa (lanes 1, 4, and 5). The sizes of N- and C-terminal parts of vIRF-3 were 35 and 38 kDa, respectively. (C) The vIRF-3, vIRF-3-N′, and vIRF-3-C′ proteins were synthesized and labeled with [^{35}S]methionine in vitro, by using the coupled transcription-translation system (Promega, Madison, Wis.). The vIRF-3 protein migrated at approximately 73 kDa. The sizes of in vitro-translated N- and C-terminal parts of vIRF-3 were similar to those expressed in vivo.

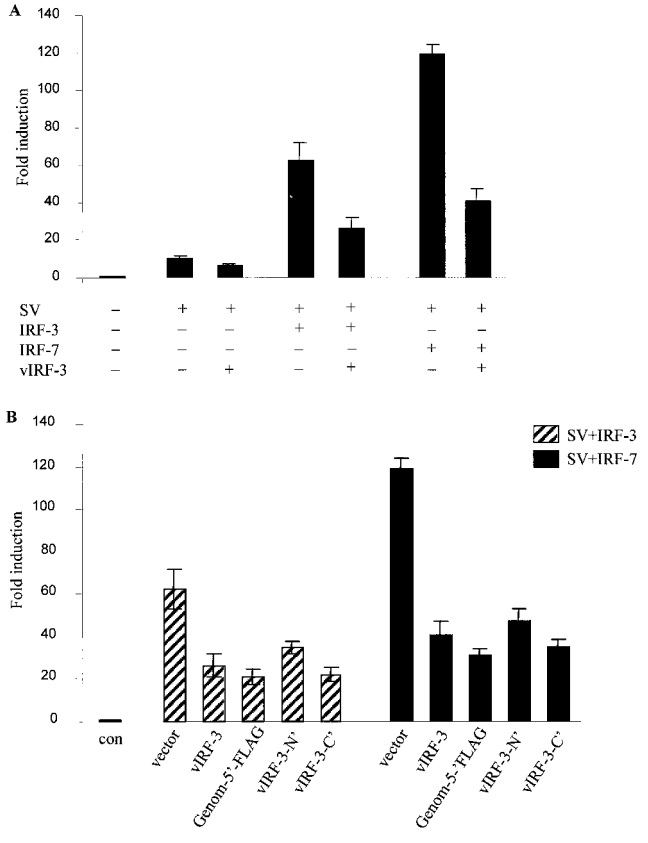

FIG. 6. Functional analysis of vIRF-3. (A) vIRF-3 protein inhibits IRF-3- and IRF-7-mediated activation of the IFNA4 promoter. The IFNA4CAT reporter plasmid (1 μg) was cotransfected with either empty vector or vectors expressing cellular IRFs (IRF-3 or IRF-7) (1 μg) and vIRF-3 (3 μg). The β-galactosidase-expressing plasmid (0.1 μg) was included as an internal standard. When indicated, 24 h after transfection, cells were infected with Sendai virus (SV) (multiplicity of infection = 5) for 16 h. The cells were harvested for CAT assay 40 h after transfection. (B) The synergistic activation of the IFNA4 promoter by Sendai virus and cellular IRF-3 or IRF-7 was inhibited by both the C-terminal and N-terminal portions of vIRF-3 protein. Transfection of genomic vIRF-3 construct (Genom-5′-FLAG) had the same inhibitory effect as vIRF-3 cDNA. Con represents cells transfected with the IFNA4CAT construct in the absence of virus infection. Error bars show standard errors for triplicate experiments.

sion of cytokine and chemokine genes. Furthermore, the apoptotic activity of IRF-1 has been well demonstrated, and overexpression of vIRF-1 in NIH 3T3 cells confers resistance to tumor necrosis factor alpha-induced apoptosis (6) that was shown to depend on both IRF-1 and PKR (14). Thus, the effects of vIRFs may extend beyond the infected cell and contribute to the HHV-8-associated pathogenicity.

Nucleotide sequence accession number. Sequence data for the vIRF-3 ORF and K10.6 ORFs have been submitted to the GenBank database under accession no. AF157602 and AF254765, respectively.

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