Inhibition of Interferon Signaling by the Kaposi's Sarcoma-Associated Herpesvirus Full-Length Viral Interferon Regulatory Factor 2 Protein

Suzanne Fuld,¹ Charles Cunningham,² Kevin Klucher,³ Andrew J. Davison,² and David J. Blackbourn^{4*}

Lab21 Limited, Unit 184, The Science Park, Cambridge, CB4 0GA, United Kingdom¹; MRC Virology Unit, Church Street, Glasgow G11 5JR, United Kingdom²; Department of Cytokine Biology, ZymoGenetics, Inc., 1201 Eastlake Ave. E., Seattle, Washington 98102³; and Cancer Research UK Institute for Cancer Studies, University of Birmingham, Birmingham B15 2TT, United Kingdom⁴

Received 18 April 2005/Accepted 28 December 2005

Interferon (IFN) signal transduction involves interferon regulatory factors (IRF). Kaposi's sarcoma-associated herpesvirus (KSHV) encodes four IRF homologues: viral IRF 1 (vIRF-1) to vIRF-4. Previous functional studies revealed that the first exon of vIRF-2 inhibited alpha/beta interferon (IFN- α/β) signaling. We now show that full-length vIRF-2 protein, translated from two spliced exons, inhibited both IFN- α - and IFN- λ -driven transactivation of a reporter promoter containing the interferon stimulated response element (ISRE). Transactivation of the ISRE promoter by IRF-1 was negatively regulated by vIRF-2 protein as well. Transactivation of a full-length IFN- β reporter promoter by either IRF-3 or IRF-1, but not IRF-7, was also inhibited by vIRF-2 protein. Thus, vIRF-2 protein is an interferon induction antagonist that acts pleiotropically, presumably facilitating KSHV infection and dissemination in vivo.

Alpha interferon (IFN- α) and IFN- β are produced as part of an immediate response by mammalian cells to virus infection and act by inducing various effector genes (reviewed in reference 16). The regulation of these genes and the IFN- α and - β genes involves the IFN regulatory factor (IRF) family of transcription factors, which in humans contains at least nine members (reviewed in references 18, 34, 35, and 46). IRFs bind to cognate DNA sequences, including the IFN-stimulated response element (ISRE) present in the promoters of IFN- α - and IFN- β -responsive genes, and positive regulatory domains (PRD) I and II in the IFN- β promoter (see references 10, 20, and 45). When IFN- α/β bind to their receptors, the IFN-stimulated gene factor-3 (ISGF-3) transcription complex is assembled from IRF-9 (p48) and posttranslationally modified signal transducer and activation of transcription 1 (STAT-1) and STAT-2 proteins. ISGF-3 drives the expression of ISRE-containing promoters (reviewed in reference 45). Other IFN-inducible genes are expressed after de novo synthesis of transcription factors, including IRF-1 and IRF-7. The most recently described family of IFNs is IFN- λ , which includes IFN- λ 1, - λ 2, and - λ 3 (22), alternatively named interleukin-28A (IL-28A), IL-28B, and IL-29, respectively (43). These cytokines share signaling similarities with IFN- α/β (11) and activate ISRE-containing promoters (reviewed in reference 48).

Upon virus infection, IFN signaling is initiated rapidly, independent of protein synthesis, by a cellular mechanism that senses the infection and triggers the IFN- α/β pathway to respond (reviewed in reference 39). Thus, IRF-3 is posttranslationally modified through C-terminal phosphorylation by a "virus-activated kinase" (VAK) (41, 44) that promotes translocation of the protein from the cytoplasm to the nucleus, where it

is assimilated into the enhancesome, a multiprotein complex that facilitates transcription of IFN and IFN-responsive genes. Assembly of the enhancesome is understood through studies of the IFN- β promoter that forms the paradigm for understanding the molecular basis of IFN-inducible gene regulation (reviewed in reference 49). The components of VAK that phosphorylate IRF-3 include the I κ B kinase homologues, I κ B kinase-epsilon (IKK ϵ), and TANK-binding kinase 1 (12, 42). These kinases were previously implicated in NF- κ B activation, but how in turn they are regulated to phosphorylate IRF-3, the pivotal step in cellular sensing of virus infection, depends upon how the cell senses that viral threat. The cytoplasmic RNA helicases RIG1 (50) and mda5 (1) are now believed to function in this capacity through the adapter protein IPS-1, at least for RNA viruses infecting via membrane fusion at the cell surface, whereas the adaptor protein TRIF is involved if Toll-like receptor 3 binds extracellular double-stranded RNA or infection occurs by endocytosis (reviewed in references 7, 24, and 39). Which components are involved in sensing DNA virus infection is unclear.

KSHV (5), etiologically linked with Kaposi's sarcoma (KS), primary effusion lymphoma, and multicentric Castleman's disease (reviewed in reference 6), encodes four viral IRF (vIRF) genes. They are located in the 83- to 95-kb region of the KSHV genome between open reading frames (ORFs) 57 and 58 (8). Three of the four (vIRF-1 to -3) have been cloned and functionally characterized, while vIRF-4 (ORF10/K10.1) has been detected by gene array analysis (19), Northern blotting, and reverse transcription-PCR (8), but the protein has yet to be characterized. The product of ORF K9 is the vIRF-1 protein, the first viral member of the IRF family to be described (32) and studied in detail. vIRF-1 has transforming activity: it reduced intracellular levels of the inducible cyclin-dependent kinase inhibitor p21^{WAF1/CIP1}, transforming NIH 3T3 cells to become tumorigenic in nude mice (15). In addition, this protein negatively regulated IFN signaling in the cell. Thus, in

* Corresponding author. Mailing address: Cancer Research UK Institute for Cancer Studies, University of Birmingham, Vincent Dr., Birmingham B15 2TT, United Kingdom. Phone: 44 (0)121 415-8804. Fax: 44 (0)121 414-4486. E-mail: d.j.blackbourn@bham.ac.uk.

reporter gene studies, vIRF-1 inhibited IFN signaling from IFN- α / β - and IFN- γ -responsive reporter genes, although not by a mechanism that involved DNA binding (13, 15, 51). The mechanism behind vIRF-1 inhibiting IFN induction of responsive genes is apparently by suppressing the transcriptional activity of IRF-1 and IRF-3, either interacting with them directly and/or competing for their binding to the transcriptional coactivator p300 (3, 27). The vIRF-1 protein may also inhibit the histone acetyltransferase activity of p300, restricting chromatin remodeling and therefore the transcriptional activity of cellular genes, including those encoding cytokines (25). Nevertheless, whether the kinetics of K9 expression in KSHV-infected cells are consistent with an effective anti-IFN response is debatable (36). Moreover, vIRF-1 suppressed the transcription and proapoptotic activities of p53 (33, 40). The multifunctional nature of vIRF-1 can be appreciated from other studies indicating that, in addition to its role in inhibiting transcription (15), this protein can act as a transcriptional activator (38). The vIRF-3 protein has been named latency-associated nuclear antigen 2 (LANA-2), consistent with its expression kinetics and its cellular location and to distinguish it from ORF73-encoded LANA (37). These authors found LANA-2 in the nuclei of B cells of subjects with primary effusion lymphoma and multicentric Castleman's disease; it was not expressed in KS. These researchers showed that LANA-2 inhibited p53-induced transcription and apoptosis. However, it may either decrease the transcription of the IFN- α / β genes by targeting IRF-3 and IRF-7 (30) or transactivate them (29).

The subject of the present study was vIRF-2. The first functional studies of this protein were performed with a 163-amino-acid residue ORF, representing the first exon (ORFK11.1) of vIRF-2 derived from a direct PCR product of the KSHV genome (4). These authors identified that K11.1 bound to a consensus NF- κ B binding site, but not the ISRE and that it suppressed IRF-1- and IRF-3-driven activation of an IFN- α reporter promoter in cells infected with Newcastle disease virus. In pull-down assays this fragment of vIRF-2 protein also interacted with cellular IRF-1 and weakly with p300/CBP, p65, IRF-2, and IFN consensus sequence binding protein/IRF-8; it did not bind IRF-3. This group went on to show that K11.1 is a 20-kDa protein that exerts its anti-IFN effect in part by binding to, and suppressing, double-stranded RNA-activated protein kinase R (2). Kirchoff et al. showed that K11.1, like vIRF-1, inhibited apoptosis by the transcriptional repression of CD95L (21).

More recently, we found that the vIRF-2 gene encodes a 2.2-kb spliced transcript representing the two exons of ORFs K11.1 and K11 (8), from which the full-length vIRF-2 protein is translated. Jenner et al. (19) made similar observations. In the present study, we determined whether this full-length vIRF-2 protein, expressed from an amplification product of KSHV cDNA, inhibits IFN signaling.

vIRF-2 protein inhibits IFN- α -induced ISRE signaling. Full-length vIRF-2 protein was expressed in 293 cells by subcloning the spliced cDNA of vIRF-2 into the pcDNA4/HisMax vector (Invitrogen). The size of the protein, with contiguous amino-terminal polyhistidine and Xpress epitope tags, was approximately 160 kDa (Fig. 1). To measure the impact of vIRF-2 protein on IFN- α signaling, reporter gene studies were performed with the pISRE-luc vector (Stratagene), in which firefly luciferase gene (*luc*) ex-

FIG. 1. Expression of full-length epitope-tagged vIRF-2 protein. 293 cells were separately transiently transfected with either 500 ng of pcDNA4/HisMax DNA (lane 1) or 500 ng of pcDNA4/vIRF-2 plasmid DNA (lane 2). Lysates were prepared after 48 h and analyzed by Western blotting with anti-Xpress primary antibody and horseradish peroxidase-conjugated secondary antibody. The position of the epitope-tagged vIRF-2 protein is indicated. M, molecular weight protein marker.

pression is regulated through the IFN-stimulated gene (ISG) 56K ISRE element. Dose-dependent induction of *luc* expression peaked at 800-fold with 200 U of rIFN- α 2b/ml in 293 cells transiently transfected with pISRE-luc. When 293 cells were transiently cotransfected with pISRE-luc and increasing amounts of vIRF-2 expression vector (pcDNA4/vIRF-2) and then treated with this concentration of rIFN- α 2b, *luc* expression was inhibited by up to 80% (Fig. 2A).

vIRF-2 protein inhibits IL-28A- and IL-29-induced ISRE signaling. The promoter of pISRE-luc is also activated by the IFN- λ family members IL-28A and IL-29 (43). We therefore determined whether vIRF-2 protein could inhibit the transcriptional activation of this promoter when driven by the newly described recombinant cytokines. Dose-dependent induction of *luc* expression was obtained in wild-type 293 cells (i.e., cells not engineered to overexpress the receptor IL-28R α) transiently transfected with pISRE-luc and treated with either rIL-28A or rIL-29. Induction peaked at 20- and 17-fold with the addition of 1,000 ng of either rIL-28A or rIL-29/ml, respectively. When 293 cells, transiently transfected with pISRE-luc, were treated with this concentration of either rIL-28A or rIL-29, *luc* expression was inhibited by 62% (Fig. 2B) and 55% (Fig. 2C), respectively, by cotransfecting them with 500 ng of pcDNA4/vIRF-2 plasmid DNA.

vIRF-2 protein inhibits IRF-1-induced ISRE signaling. IFN- α or IFN- λ treatment of cells induces formation of the ISGF-3 transcription complex that activates ISRE-containing promoters. IRF-1 can also transcriptionally activate ISRE-containing promoters since this site overlaps with the IRF-E element, to which IRF-1 binds within the IFN- β promoter (see reference 47). Therefore, we determined whether vIRF-2 protein also regulates IRF-1 activation of the ISRE-containing promoter. Dose-depen-

FIG. 2. Inhibition of rIFN-driven expression of pISRE-luc by vIRF-2 protein expression. (A) Inhibition of rIFN- α . 293 cells were transfected with pISRE-luc (250 ng) and increasing amounts of pcDNA4/vIRF-2 plasmid. At 5 h posttransfection the cells were treated with rIFN- α 2b (200 U/ml) and harvested 16 h later. The empty parental plasmid backbone, pcDNA4/HisMax, was added as a “stuffer” plasmid to equalize the amount of DNA in each transfection to 800 ng. The pRLSV40 plasmid (10 ng) constitutively expressing *Renilla* luciferase was added as an internal control to which firefly luciferase levels were normalized. Transfections where pcDNA4/vIRF-2 plasmids were not included were calculated to have 100% luciferase activity. The data represent the mean $\pm$ the standard deviation of two independent experiments, each performed in duplicate, but the differences between the experiments are too low for the error bars to be clearly distinguished in this figure. (B) Inhibition of rIL-28A (IFN- λ 1). The experiment was performed as described in Fig. 2A, except that the cells were treated with rIL-28A (1,000 ng/ml). The data are presented as mean normalized firefly luciferase activity ($\pm$ the standard deviation) for three independent experiments. (C) Inhibition of rIL-29 (IFN- λ 3). The experiment was performed as described in Fig. 2A, with the exception that cells were treated with rIL-29 (1,000 ng/ml). The data are presented as mean normalized firefly luciferase activity ($\pm$ the standard deviation) for three independent experiments.

dent induction of luciferase gene expression from pISRE-luc peaked at approximately 3 orders of magnitude, with the addition of 50 ng of pIRF-1, in transiently transfected 293 cells. This induction was not due to the activity of endogenously produced IFN- α or IFN- β , since polyclonal neutralizing antibodies directed against human IFN- α and IFN- β did not affect the level of induction of pISRE-luc (Fig. 3A). To ensure that sufficient antibodies had been added to the cotransfected cells to neutralize endogenous IFN, the antibodies were added to cells that were then treated with excess recombinant IFN- α 2b. Recombinant IFN- α 2b

FIG. 3. Inhibition of IRF-1-driven expression of pISRE-luc by vIRF-2 protein expression. (A) IRF-1 induces pISRE-luc in a mechanism distinct from rIFN- α induction. 293 cell transfections contained pISRE-luc (250 ng) reporter plasmid. The pIRF-1 plasmid (50 ng) was cotransfected where indicated. After transfection for 5 h, rIFN- α 2b (200 U/ml) and/or 400 neutralizing units of each rabbit polyclonal antibody against human IFN- α and IFN- β /ml was added to the cells as indicated. The pRLSV40 plasmid (10 ng) constitutively expressing *Renilla* luciferase was added as an internal control to which firefly luciferase levels were normalized. The empty parental plasmid backbone, pcDNA4/HisMax, was added as a “stuffer” plasmid to equalize the amount of DNA in each transfection to 800 ng. The data represent the mean $\pm$ the standard deviation of two independent experiments, each performed in duplicate. (B) Inhibition of pIRF-1-induced stimulation of pISRE-luc by vIRF-2. 293 cells were transfected with the pISRE-luc reporter plasmid (250 ng), the expression of which was driven by IRF-1, itself constitutively expressed from the cotransfected pIRF-1 plasmid (50 ng). Increasing amounts (0 to 50 ng) of the vIRF-2 expression plasmid pcDNA4/vIRF-2 were also cotransfected. A parallel transfection containing pIRF-2 (50 ng) and pISRE-luc (250 ng) was performed in the absence of pcDNA4/vIRF-2 plasmid. The pRLSV40 plasmid (10 ng) constitutively expressing *Renilla* luciferase was added as an internal control to which firefly luciferase levels were normalized. The empty parental plasmid backbone, pcDNA4/HisMax, was added as a “stuffer” plasmid to equalize the amount of DNA in each transfection to 800 ng. The cells were harvested 16 h after transfection. Transfections where pcDNA4/vIRF-2 plasmid was not included were calculated to have 100% luciferase activity. The data represent the mean $\pm$ the standard deviation of two independent experiments, each performed in duplicate, but the differences between the experiments are too low for the error bars to be clearly distinguished in this figure.

FIG. 4. Effect of vIRF-2 protein expression on IRF activation of the full-length IFN- β promoter (p125-luc). 293 cells were harvested 16 h after transfection with the full-length IFN- β promoter (p125-luc) reporter vector. The pRLSV40 plasmid (10 ng) constitutively expressing *Renilla* luciferase was added as an internal control to which firefly luciferase levels were normalized. The IRF expression plasmids were pIRF-1, pIRF-3(5D), and pIRF-7(477/479), each transfected at 200 ng, or 200 ng of both pIRF-3(5D) and pIRF-7(477/479) plasmid. Each transfection included 50 ng of either the empty parental plasmid backbone, pcDNA4, or the vIRF-2-expressing plasmid, pcDNA4/vIRF-2 and 100 ng of p125-luc.

induction of promoter activity was almost completely inhibited in the presence of the neutralizing antibodies (Fig. 3A).

When pISRE-luc and pIRF-1 were cotransfected into 293 cells in the presence of increasing amounts of vIRF-2 expression vector, *luc* activity was reduced by more than 80% with 50 ng of pcDNA4/vIRF-2, indicating that vIRF-2 inhibits IRF-1-driven transcriptional activation of the ISRE-containing promoter (Fig. 3B). Since IRF-2 expression is known to antagonize IRF-1 induction of ISRE promoters (47), parallel experiments were performed in which vIRF-2 expression vector was replaced by an IRF-2 expression vector. As expected, IRF-2 expression also suppressed pIRF-1 induction of pISRE-luc almost completely (Fig. 3B).

vIRF-2 protein inhibits IRF-3 transactivation. To determine whether the activity of other cellular IRFs was also regulated by vIRF-2, reporter gene assays were performed with expression plasmids for IRF-1, IRF-3, or IRF-7, driving the full-length IFN- β gene promoter in the firefly luciferase-containing reporter plasmid p125-luc (14). Constitutively active variants of IRF-3 [IRF-3(5D) (28)] and IRF-7 [IRF-7(D477/479) (26)] were evaluated.

Cotransfection of plasmid p125-luc with the pIRF-1 plasmid stimulated the IFN- β promoter by more than 80-fold, pIRF-3(5D) activated this promoter by approximately 1,000-fold, and pIRF-7(477/479) plasmid induced the reporter promoter by 56-fold. These induction levels were normalized to 100% for each IRF expression vector to enable the effect of vIRF-2 expression to be compared.

Cotransfection of pcDNA4/vIRF-2 with p125-luc and either pIRF-1 or pIRF-3(5D), inhibited reporter activity by more than 50% (Fig. 4). In contrast, pIRF-7(D477/479) activity was not modulated by vIRF-2 protein. Indeed, the activity of the IFN- β promoter was increased slightly in the presence of pcDNA4/vIRF-2. Dual transfection of plasmids pIRF-3(5D) and pIRF-7(D477/479) with p125-luc activated the IFN- β promoter, but the level of inhibition by pcDNA4/vIRF-2 was intermediate (37% decrease) between the effect on either pIRF-

3(5D) or pIRF-7(D477/479) when each was added individually (Fig. 4).

Since vIRF-1 can transform NIH 3T3 cells (15) and mitigate IFN- γ signaling (51), we investigated these activities for vIRF-2. NIH 3T3 cells were transfected with pcDNA4/vIRF-2, and stable transfectants were selected by their growth in zeocin-containing culture medium. The vIRF-2 protein was expressed at early passage in these cultures as determined by Western blotting, but with increasing passages the number of cells expressing the protein declined, as determined by immunofluorescence assay (data not shown). This expression was completely lost with continuous passage, although zeocin resistance continued. These data revealed that, unlike vIRF-1, vIRF-2 is unable to transform these cells and suggest that constitutive expression of full-length vIRF-2 protein at the levels achieved with pcDNA4/vIRF-2 is detrimental to the mouse cells for reasons we do not yet understand. To determine the ability of vIRF-2 to inhibit IFN- γ signaling, reporter gene studies were performed. In this experiment, human 2fTGH cells (a gift from I. Kerr, Cancer Research UK, London, United Kingdom; see reference 9) were transfected with the IFN- γ -responsive pGAS-luc plasmid (Stratagene), in the presence of increasing amounts of pcDNA4/vIRF-2, using the experimental rationale described in Fig. 2A but activating the reporter plasmid by treatment of the cells with recombinant IFN- γ (R&D Systems). No inhibition of pGAS-*luc* activation was observed by pcDNA4/vIRF-2 (data not shown).

The induction of the IFN- α/β genes in virus-infected cells represents the most immediate antiviral response in the host (reviewed in reference 16). The newly described IFN- λ family members may also play a significant role in this innate antiviral immune response. KSHV encodes four vIRF genes whose products could deregulate this response as a mechanism of immune evasion. Indeed, both vIRF-1 (3, 15, 25, 27, 51) and vIRF-3 (30) proteins have anti-IFN activity. In the present study, full-length, epitope-tagged, vIRF-2 protein inhibited transcriptional activation of a reporter promoter containing an

ISRE element derived from the promoter of ISG 56K, when transactivation was induced with either rIFN- α or recombinant forms of the IFN- λ family members (Fig. 2). Thus, our data implicate vIRF-2 in inhibiting either the assembly, or the activity, of the ISGF-3 complex.

During the innate immune response, IRF-1 protein expression is upregulated either upon virus infection or after stimulation with IFN- α/β or IFN- γ (17, 31). In the present study, ectopic expression of IRF-1 activated the ISRE reporter promoter directly and not via inducing expression of endogenous IFN- α and IFN- β (Fig. 3A). The vIRF-2 protein inhibited this transactivation of IRF-1 (Fig. 3B). Therefore, vIRF-2 protein inhibits transactivation of ISRE-containing genes by either IFN- α/β treatment (via ISGF-3) or IRF-1 expression. Moreover, the effects of vIRF-2 protein are pleiotropic, since it also inhibited transactivation of the IFN- β promoter by IRF-3 but not IRF-7 (Fig. 4).

Thus, the KSHV full-length vIRF-2 protein inhibits the expression of IFN-inducible genes, including those that are expressed early (i.e., dependent upon IRF-3 activity) and those with delayed kinetics (IRF-1- and ISGF-3-dependent genes). Taken together, our data suggest that vIRF-2 shares with vIRF-1 the activity, but not necessarily the mechanism, of inhibiting IFN- α/β signaling but that the two proteins are divergent in their abilities to transform cells and repress IFN- γ signaling. Mechanistic studies of vIRF-2 function are in progress. These functions are consistent with the expression of the vIRF-2 gene being detectable as early as 2 h (the earliest time point studied) after experimental infection of cells (23). Hence, the vIRF-2 protein provides a strategy through which KSHV evades the innate immune response, contributing to the establishment and dissemination of the virus in the infected individual.

We thank Friedemann Weber for invaluable advice during the course of these studies, John Hiscott for the generous provision of many reagents, and Karl Burgess for technical help.

This study was supported in part by a Medical Research Council Ph.D. Studentship (S.F.) and grants from the Wellcome Trust (D.J.B., no. 059008/Z/99/Z; the Cunningham Trust (D.J.B., no. ACC/KM CT), the Association for International Cancer Research (D.J.B., no. 01-242), and Cancer Research UK (D.J.B., no. C7934).

REFERENCES

- Andrejeva, J., K. S. Childs, D. F. Young, T. S. Carlos, N. Stock, S. Goodbourn, and R. E. Randall. 2004. The V proteins of paramyxoviruses bind the IFN-inducible RNA helicase, mda-5, and inhibit its activation of the IFN-beta promoter. *Proc. Natl. Acad. Sci. USA* **101**:17264–17269.
- Burysek, L., and P. M. Pitha. 2001. Latently expressed human herpesvirus 8-encoded interferon regulatory factor 2 inhibits double-stranded RNA-activated protein kinase. *J. Virol.* **75**:2345–2352.
- Burysek, L., W. S. Yeow, B. Lubyova, M. Kellum, S. L. Schafer, Y. Q. Huang, and P. M. Pitha. 1999. Functional analysis of human herpesvirus 8-encoded viral interferon regulatory factor 1 and its association with cellular interferon regulatory factors and p300. *J. Virol.* **73**:7334–7342.
- Burysek, L., W. S. Yeow, and P. M. Pitha. 1999. Unique properties of a second human herpesvirus 8-encoded interferon regulatory factor (vIRF-2). *J. Hum. Virol.* **2**:19–32.
- Chang, Y., E. Cesarman, M. S. Pessin, F. Lee, J. Culpepper, D. M. Knowles, and P. S. Moore. 1994. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science* **266**:1865–1869.
- Cohen, A., D. G. Wolf, E. Guttman-Yassky, and R. Sarid. 2005. Kaposi's sarcoma-associated herpesvirus: clinical, diagnostic, and epidemiological aspects. *Crit. Rev. Clin. Lab. Sci.* **42**:101–153.
- Crozat, K., and B. Beutler. 2004. TLR7: a new sensor of viral infection. *Proc. Natl. Acad. Sci. USA* **101**:6835–6836.
- Cunningham, C., S. Barnard, D. J. Blackbourn, and A. J. Davison. 2003. Transcription mapping of human herpesvirus 8 genes encoding viral interferon regulatory factors. *J. Gen. Virol.* **84**:1471–1483.
- Darnell, J. E., Jr., I. M. Kerr, and G. R. Stark. 1994. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* **264**:1415–1421.
- Decker, T., P. Kovarik, and A. Meinke. 1997. GAS elements: a few nucleotides with a major impact on cytokine-induced gene expression. *J. Interferon Cytokine Res.* **17**:121–134.
- Dumoutier, L., A. Tounsi, T. Michiels, C. Sommereyns, S. V. Kutenko, and J. C. Renauld. 2004. Role of the interleukin (IL)-28 receptor tyrosine residues for antiviral and antiproliferative activity of IL-29/interferon-lambda 1: similarities with type I interferon signaling. *J. Biol. Chem.* **279**:32269–32274.
- Fitzgerald, K. A., S. M. McWhirter, K. L. Faia, D. C. Rowe, E. Latz, D. T. Golenbock, A. J. Coyle, S. M. Liao, and T. Maniatis. 2003. IKKepsilon and TBK1 are essential components of the IRF3 signaling pathway. *Nat. Immunol.* **4**:491–496.
- Flowers, C. C., S. P. Flowers, and G. J. Nabel. 1998. Kaposi's sarcoma-associated herpesvirus viral interferon regulatory factor confers resistance to the antiproliferative effect of interferon-alpha. *Mol. Med.* **4**:402–412.
- Fujita, T., G. P. Nolan, H. C. Liou, M. L. Scott, and D. Baltimore. 1993. The candidate proto-oncogene *bcl-3* encodes a transcriptional coactivator that activates through NF-kB p50 homodimers. *Genes Dev.* **7**:1354–1363.
- Gao, S. J., C. Boshoff, S. Jayachandran, R. A. Weiss, Y. Chang, and P. S. Moore. 1997. KSHV ORF K9 (vIRF) is an oncogene which inhibits the interferon signaling pathway. *Oncogene* **15**:1979–1985.
- Goodbourn, S., L. Didcock, and R. E. Randall. 2000. Interferons: cell signalling, immune modulation, antiviral response and virus countermeasures. *J. Gen. Virol.* **81**(Pt. 10):2341–2364.
- Harada, H., T. Fujita, M. Miyamoto, Y. Kimura, M. Maruyama, A. Furia, T. Miyata, and T. Taniguchi. 1989. Structurally similar but functionally distinct factors, IRF-1 and IRF-2, bind to the same regulatory elements of IFN and IFN-inducible genes. *Cell* **58**:729–739.
- Harada, H., T. Taniguchi, and N. Tanaka. 1998. The role of interferon regulatory factors in the interferon system and cell growth control. *Biochimie* **80**:641–650.
- Jenner, R. G., M. M. Alba, C. Boshoff, and P. Kellam. 2001. Kaposi's sarcoma-associated herpesvirus latent and lytic gene expression as revealed by DNA arrays. *J. Virol.* **75**:891–902.
- King, P., and S. Goodbourn. 1994. The beta-interferon promoter responds to priming through multiple independent regulatory elements. *J. Biol. Chem.* **269**:30609–30615.
- Kirchhoff, S., T. Sebens, S. Baumann, A. Krueger, R. Zawatzky, M. Li-Weber, E. Meinel, F. Neipel, B. Fleckenstein, and P. H. Krammer. 2002. Viral IFN-regulatory factors inhibit activation-induced cell death via two positive regulatory IFN-regulatory factor 1-dependent domains in the CD95 ligand promoter. *J. Immunol.* **168**:1226–1234.
- Kutenko, S. V., G. Gallagher, V. V. Baurin, A. Lewis-Antes, M. Shen, N. K. Shah, J. A. Langer, F. Sheikh, H. Dickensheets, and R. P. Donnelly. 2003. IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. *Nat. Immunol.* **4**:69–77.
- Krishnan, H. H., P. P. Naranatt, M. S. Smith, L. Zeng, C. Bloomer, and B. Chandran. 2004. Concurrent expression of latent and a limited number of lytic genes with immune modulation and antiapoptotic function by Kaposi's sarcoma-associated herpesvirus early during infection of primary endothelial and fibroblast cells and subsequent decline of lytic gene expression. *J. Virol.* **78**:3601–3620.
- Levy, D. E., and I. J. Marie. 2004. RIGging an antiviral defense: it's in the CARDs. *Nat. Immunol.* **5**:699–701.
- Li, M., B. Damania, X. Alvarez, V. Ogryzko, K. Ozato, and J. U. Jung. 2000. Inhibition of p300 histone acetyltransferase by viral interferon regulatory factor. *Mol. Cell. Biol.* **20**:8254–8263.
- Lin, R., P. Genin, Y. Mamane, and J. Hiscott. 2000. Selective DNA binding and association with the CREB binding protein coactivator contribute to differential activation of alpha/beta interferon genes by interferon regulatory factors 3 and 7. *Mol. Cell. Biol.* **20**:6342–6353.
- Lin, R., P. Genin, Y. Mamane, M. Sgarbanti, A. Battistini, W. J. Harrington, G. N. Barber, and J. Hiscott. 2001. HHV-8-encoded vIRF-1 represses the interferon antiviral response by blocking IRF-3 recruitment of the CBP/p300 coactivators. *Oncogene* **20**:800–811.
- Lin, R., Y. Mamane, and J. Hiscott. 1999. Structural and functional analysis of interferon regulatory factor 3: localization of the transactivation and autoinhibitory domains. *Mol. Cell. Biol.* **19**:2465–2474.
- Lubyova, B., M. J. Kellum, A. J. Frischno, and P. M. Pitha. 2004. Kaposi's sarcoma-associated herpesvirus-encoded vIRF-3 stimulates the transcriptional activity of cellular IRF-3 and IRF-7. *J. Biol. Chem.* **279**:7643–7654.
- Lubyova, B., and P. M. Pitha. 2000. Characterization of a novel human herpesvirus 8-encoded protein, vIRF-3, that shows homology to viral and cellular interferon regulatory factors. *J. Virol.* **74**:8194–8201.
- Miyamoto, M., T. Fujita, Y. Kimura, M. Maruyama, H. Harada, Y. Sudo, T. Miyata, and T. Taniguchi. 1988. Regulated expression of a gene encoding a

- nuclear factor, IRF-1, that specifically binds to IFN-beta gene regulatory elements. *Cell* **54**:903–913.
32. Moore, P. S., C. Boshoff, R. A. Weiss, and Y. Chang. 1996. Molecular mimicry of human cytokine and cytokine response pathway genes by KSHV. *Science* **274**:1739–1744.
 33. Nakamura, H., M. Li, J. Zarycki, and J. U. Jung. 2001. Inhibition of p53 tumor suppressor by viral interferon regulatory factor. *J. Virol.* **75**:7572–7582.
 34. Nguyen, H., J. Hiscott, and P. M. Pitha. 1997. The growing family of interferon regulatory factors. *Cytokine Growth Factor Rev.* **8**:293–312.
 35. Pitha, P. M., W. C. Au, W. Lowther, Y. T. Juang, S. L. Schafer, L. Burysek, J. Hiscott, and P. A. Moore. 1998. Role of the interferon regulatory factors (IRFs) in virus-mediated signaling and regulation of cell growth. *Biochimie* **80**:651–658.
 36. Pozharskaya, V. P., L. L. Weakland, J. C. Zimring, L. T. Krug, E. R. Unger, A. Neisch, H. Joshi, N. Inoue, and M. K. Offermann. 2004. Short duration of elevated vIRF-1 expression during lytic replication of human herpesvirus 8 limits its ability to block antiviral responses induced by alpha interferon in BCBL-1 cells. *J. Virol.* **78**:6621–6635.
 37. Rivas, C., A. E. Thlick, C. Parravicini, P. S. Moore, and Y. Chang. 2001. Kaposi's sarcoma-associated herpesvirus LANA2 is a B-cell-specific latent viral protein that inhibits p53. *J. Virol.* **75**:429–438.
 38. Roan, F., J. C. Zimring, S. Goodbourn, and M. K. Offermann. 1999. Transcriptional activation by the human herpesvirus-8-encoded interferon regulatory factor. *J. Gen. Virol.* **80**:2205–2209.
 39. Sen, G. C., and S. N. Sarkar. 2005. Hitching RIG to action. *Nat. Immunol.* **6**:1074–1076.
 40. Seo, T., J. Park, D. Lee, S. G. Hwang, and J. Choe. 2001. Viral interferon regulatory factor 1 of Kaposi's sarcoma-associated herpesvirus binds to p53 and represses p53-dependent transcription and apoptosis. *J. Virol.* **75**:6193–6198.
 41. Servant, M. J., B. ten Oever, C. LePage, L. Conti, S. Gessani, I. Julkunen, R. Lin, and J. Hiscott. 2001. Identification of distinct signaling pathways leading to the phosphorylation of interferon regulatory factor 3. *J. Biol. Chem.* **276**:355–363.
 42. Sharma, S., B. R. tenOever, N. Grandvaux, G.-P. Zhou, R. Lin, and J. Hiscott. 2003. Triggering the interferon antiviral response through an IKK-related pathway. *Science* **300**:1148–1151.
 43. Sheppard, P., W. Kindsvogel, W. Xu, K. Henderson, S. Schlutsmeyer, T. E. Whitmore, R. Kuestner, U. Garrigues, C. Birks, J. Roraback, C. Ostrander, D. Dong, J. Shin, S. Presnell, B. Fox, B. Haldeman, E. Cooper, D. Taft, T. Gilbert, F. J. Grant, M. Tackett, W. Krivan, G. McKnight, C. Clegg, D. Foster, and K. M. Klucher. 2003. IL-28, IL-29, and their class II cytokine receptor IL-28R. *Nat. Immunol.* **4**:63–68.
 44. Smith, E. J., I. Marie, A. Prakash, A. Garcia-Sastre, and D. E. Levy. 2001. IRF3 and IRF7 phosphorylation in virus-infected cells does not require double-stranded RNA-dependent protein kinase R or I κ B kinase but is blocked by Vaccinia virus E3L protein. *J. Biol. Chem.* **276**:8951–8957.
 45. Stark, G. R., I. M. Kerr, B. R. Williams, R. H. Silverman, and R. D. Schreiber. 1998. How cells respond to interferons. *Annu. Rev. Biochem.* **67**:227–264.
 46. Tanaka, N., and T. Taniguchi. 2000. The interferon regulatory factors and oncogenesis. *Semin. Cancer Biol.* **10**:73–81.
 47. Taniguchi, T., and A. Takaoka. 2002. The interferon-alpha/beta system in antiviral responses: a multimodal machinery of gene regulation by the IRF family of transcription factors. *Curr. Opin. Immunol.* **14**:111–116.
 48. Vilcek, J. 2003. Novel interferons. *Nat. Immunol.* **4**:8–9.
 49. Vo, N., and R. H. Goodman. 2001. CREB-binding protein and p300 in transcriptional regulation. *J. Biol. Chem.* **276**:13505–13508.
 50. Yoneyama, M., M. Kikuchi, T. Natsukawa, N. Shinobu, T. Imaizumi, M. Miyagishi, K. Taira, S. Akira, and T. Fujita. 2004. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nat. Immunol.* **5**:730–737.
 51. Zimring, J. C., S. Goodbourn, and M. K. Offermann. 1998. Human herpesvirus 8 encodes an interferon regulatory factor (IRF) homolog that represses IRF-1-mediated transcription. *J. Virol.* **72**:701–707.