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Vesicular Stomatitis Virus Infection Induces a Nuclear DNA-Binding Factor Specific for the Interferon-Stimulated Response Element

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Vesicular stomatitis virus (VSV) has a broad host range. It replicates in the cytoplasm and causes rapid cytopathic effects. We show that following VSV infection, a nuclear factor that binds to a select set of interferon-stimulated responsive elements (ISRE) is induced in many cell types. This factor, tentatively called VSV-induced binding protein (VIBP), was estimated to have an approximate molecular mass of 50 kDa and was distinct from known members of the interferon regulatory factor family, that are known to bind to the ISRE. Induction of VIBP required tyrosine kinase activity but did not require cellular transcription. Treatment of cells with cycloheximide, which inhibits translation, only partially inhibited induction of VIBP. However, type I interferons and staurosporine, both of which inhibit VSV transcription, inhibited VIBP induction. Moreover, a double-stranded RNA analog, poly(I)-poly(C) also induced a DNA-binding activity very similar to that of VIBP. These results indicate that a preexisting cellular protein is activated upon VSV infection and that this activation requires primary viral transcripts. The functional activity of VIBP was analyzed in cells stably transfected with a herpesvirus thymidine kinase-luciferase reporter gene that is under control of the ISRE. While activity of the control promoter without ISRE was strongly inhibited following VSV infection (as a result of virus-mediated transcriptional shutdown of the host cell), the inhibition was reversed by the ISRE-containing promoter, albeit partially, which suggests that VSV infection differentially affects transcription of host genes. Although VIBP was induced in all other cells tested, it was not induced in embryonal carcinoma cells after VSV infection, suggesting developmental regulation of VIBP inducibility.

Vesicular stomatitis virus (VSV) is a rhabdovirus and contains a negative-strand RNA genome. It infects a wide variety of vertebrate and invertebrate cells and causes rapid cytopathic effects (57). Upon infection, the approximately 11-kb VSV genome is sequentially transcribed into a small AU-rich leader RNA and the five viral mRNAs encoding the structural proteins of the virus in the order of leader RNA-N-P-M-G-L (1, 46). Viral replication takes place entirely in the cytoplasm, and viral gene expression causes an inhibition of cellular and RNA polymerase II- and III-dependent transcription (58, 63), as well as cellular protein synthesis (32, 60). The viral gene product that has been associated with the shutoff of host cell transcription and with cytopathogenesis is the VSV matrix protein, M (3, 4). M protein is also essential for viral assembly (57). Coexpression of VSV M protein alone has been shown to be sufficient to inhibit transcription of a reporter gene in DNA transfection assays (3), as well as from chromosomal DNA, with the integrated HIV-1 provirus as a template (41). M protein is the only viral protein that has been detected in the nucleus (27). In addition, M protein in the absence of the other viral proteins causes cell rounding after VSV infection (4). Like M protein, the VSV leader RNA has also been detected in the nuclei of infected cells (24). The addition of leader RNA to whole-cell extracts or isolated cell nuclei has been shown to inhibit nuclear transcription (15, 35). Supporting evidence for the inhibitory effect of leader RNA in intact cells, however, is still missing; in fact, some in vivo data seem to exclude its role (11, 62).

Despite these rapid cytopathic effects and transcriptional and translational shutdown of the cell, some VSV-infected cells are still able to induce type I (α/β) interferon (IFN) synthesis, and the level of IFN expression depends on the viral strain used (32). Furthermore, type I IFNs confer strong protection against VSV infection (29), which may involve induction of the MxA protein (51). IFN appears to inhibit VSV largely by inhibiting both viral transcription and translation (2, 30, 33, 52). In IFN-treated cells, the rate of primary transcription of VSV is initially inhibited even in the presence of cycloheximide (2, 30). Furthermore, it has been found that besides being able to induce IFN, some VSV strains were also able to suppress IFN expression (14).

Transcriptional activation of type I IFNs following viral infection is mediated by *cis* elements homologous to the interferon-stimulated response element (ISRE), although other elements in the promoter are also involved in activation (16, 28, 53). Proteins belonging to the IFN regulatory factor (IRF) family bind to the ISRE and homologous sequences. This family includes IRF-1, IRF-2, ISGF3γ, and ICSBP (10, 16, 17, 25, 34, 56). ISGF3γ associates with three ISGF3α subunits to form a functional complex, ISGF3 (25, 47). Several lines of evidence indicate that IRF-1 and IRF-2 are involved in regulating IFN genes, the former by activating and the latter by repressing transcription (13, 16). Type I IFNs, in turn, stimulate transcription of a series of cellular genes bearing the ISRE, including 2-5A synthetase, 2-5A-dependent protein kinase (PKR), and 2-5A-dependent RNase, which are involved in conferring antiviral activities (36, 49, 65). In addition to

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IRF-1 and IRF-2, ISGF3 and ICSBP bind to the ISREs of these and other IFN-inducible genes (10, 56). The requirement of ISGF3 for rapid induction of these genes has been extensively documented (39). ICSBP, expressed mostly in the immune system (5, 10), is shown to repress transcription of IFN-inducible genes (40, 59). Some IFN-inducible genes are directly activated following viral infection (12, 18, 43, 50), as mechanisms of transcription mediated by viruses share those mediated by IFN. The present study was undertaken with the aim of analyzing mechanisms of transcriptional regulation in the host cell following VSV infections, mechanisms relevant to the activation of IFN and IFN-dependent gene expression that occurs despite the overall shutdown of cellular transcription by the viral M protein. We show that VSV-induced binding protein (VIBP), a nuclear factor that binds to the ISRE, is rapidly induced in mouse and human cells following VSV infection. Our results indicate that VIBP is induced as a result of tyrosine kinase-dependent activation of a preexisting cellular factor. Analysis with several inhibitors points to the role of VSV primary transcripts for triggering VIBP activation. Furthermore, evidence that VIBP plays a role in cellular response to VSV infection is presented.

MATERIALS AND METHODS

Cells. Murine L929 cells were maintained in minimum essential medium; murine PU5-1.8, NIH 3T3, human FS4 (a gift from Anderson, Fordham University, New York), and HeLa cells were maintained in Dulbecco's modified Eagle's medium; and murine EL4 and human HL-60 cells were maintained in RPMI 1640 medium (all media from Gibco) supplemented with 10% fetal bovine serum and antibiotics. Murine P19 cells were grown in α minimum essential medium (Gibco) and were treated with all-*trans* retinoic acid (Sigma) at 1 μM for 3.5 days (37).

Viruses and infection. The wild-type VSV strains (Indiana and New Jersey serotypes) were propagated in L929 or baby hamster kidney (BHK-21) cells, and titers were determined on monolayers of L929 (48). Semiconfluent cells were infected with VSV at various multiplicities of infection (MOI). After 1 h of adsorption in serum-free medium at 37°C, cells were rinsed and then incubated with fresh complete medium for 6 h, unless otherwise indicated. Simian virus 40 (SV40) (55) and the WR strain of vaccinia virus (6) were gifts from M. Avantaggiati, National Institute of Child Health and Human Development, and M. Buller, National Institute of Allergy and Infectious Diseases, respectively. Semiconfluent L929 cells were incubated with various MOIs of SV40 or vaccinia virus at 37°C for 120 or 90 min, respectively. Cells were rinsed and incubated with fresh complete medium for the indicated periods. Cytopathic effects (cell rounding and lysis) were monitored as described previously (4). Extracts used for electrophoretic mobility shift assay (EMSA) were prepared as described previously (5).

Reagents used in this work were obtained from the following sources: murine natural IFN-α/β was from Lee BioMolecular Research Laboratories; cycloheximide (CHX), actinomycin D, 2-aminopurine, and staurosporine were from Sigma; and geldanamycin (64) and genistein (7) were from Bethesda Research Laboratories. Cells were treated with IFN-α/β overnight and washed, and then infection and the subsequent incubation were performed in the absence of IFNs. Other reagents were added 30 min prior to infection and were present continuously during infection and the subsequent period of incubation.

Transfection of poly(I)-poly(C). Suspended L929 cells (10^7 cells in 250 μl of complete medium) were transfected with 5 μg of poly(I)-poly(C) (Pharmacia) per ml or 10 μg of plasmid DNA, pSKII (Stratagene), by electroporation with a Cellporator (800 μF, 210 V; Bethesda Research Laboratories) at room temperature. Cells were replated in fresh complete medium and incubated for 5 h. CHX (50 μg/ml) was added 30 min prior to and during transfection, as well as during the subsequent 5-h incubation.

Stable transfection of reporter genes and luciferase assays. The control luciferase reporter gene (*tk-luc*) was under control of the herpes thymidine kinase promoter (37). Three copies of ISG15 ISRE oligomers were inserted into the *Bgl*II site of *tk-luc* to produce ISRE-*tk-luc*. L929 cells were cotransfected with 10 μg of the above reporter and the neomycin resistance gene under the control of Rous sarcoma virus (pRSV-neo; 1 μg) by the liposome-mediated gene transfer method with Lipofectin (Bethesda Research Laboratories). Cells were selected with G418 at 400 μg/ml for 2 weeks. Resistant cells were pooled and infected with VSV at a MOI of 10 or 100 as above. Luciferase activities were measured as described previously (37) and normalized according to protein concentrations.

EMSA. EMSAs were performed as described previously (5) with the following modifications. Sodium acetate at 1.5 M (pH. 5.2) was added to the lower reservoir buffer used for electrophoresis, which improved the resolution of retarded bands. The ISRE oligonucleotides used as a probe were from the ISG15 gene (5), GAT CCT CGG GAA AGG GAA ACC GAA ACT GAA GCC. The oligonucleotides used as competitors were as follows: ISG54 ISRE (25), gat cct TTC ACT TTC TAG TTT CAC TTT CCC TTg; IFN-β PRDI (53), AGG AAA ACT GAA AGG GAG AAG TGA AAG TGG G; 9-27 ISRE (42), TTT ACA AAC AGC AGG AAA TAG AAA CTT AGG AAA TAG AAA CTT AGG AGAAAT ACA; 6-16 ISRE (42), GGA GCT GGG AGA GAG GGG AAA ATG AAA CTG CAG AGT GCA; GBP ISRE (26), TAG AGT AAT ATG AAA CTG AAA GTA; major histocompatibility complex class I (*H-2L^d*) ICS (10), gat cGA TTC CCC ATC TCC TCA GTT TCA CTT CTG Cac cgc atg; and ISCBP pIRE (9, 21), AGT GAT TTC TCG GAA AGA GAG. Rabbit antibodies against IRF-1 and IRF-2 were described previously (5). Rabbit antibodies against ISGF3γ and P91 (ISGF3α) were prepared with synthetic peptides TPEQQAAILSLV and EFDSMMNTV, which correspond to the C-terminal region of the above proteins, respectively. Specific reactivities of these antibodies have been confirmed by immunoprecipitation and EMSA with in vitro translation IRF products. These antibodies were added to nuclear extracts at a 1:15 dilution 15 min before the addition of the probe.

UV cross-linking. For UV cross-linking (38), nuclear extracts (50 μg) from L929 cells that had been infected with VSV at a MOI of 100 for 10 h were mixed with 5×10^5 cpm of ^{32}P-labeled ISG15 ISRE oligonucleotides, and EMSA was performed as above. The gels were then exposed to 312-nm UV light for 30 min. Appropriate gel lanes were cut out, equilibrated in a loading buffer, and resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (PAGE) (10% polyacrylamide) in the second dimension.

RESULTS

Induction of VIBP in L929 cells following VSV infection: effect of viral doses and the kinetics of induction. Murine L929 fibroblasts were infected with various doses of VSV (MOI from 1 to 100, Indiana serotype) for 1 h and further incubated for 6 h. Nuclear extracts were tested for DNA-binding activity for the ISRE with a ^{32}P-labeled ISG15 probe (5). Results of EMSA are shown in Fig. 1A. Extracts from control, mock-infected cells produced two bands, both of which represent IRF-2 (lane 1, see below in Fig. 2). The upper IRF-2 band is presumably a multimerized form of IRF-2. In contrast, extracts from cells infected with VSV at all doses generated another band, tentatively designated VIBP (lanes 2 to 6 in Fig. 1A), which migrated just above the lower IRF-2 band. The intensity of the VIBP band increased with increasing MOIs. VSV infection at a MOI lower than (and equal to) 10 led to induction of another band with slower migration, later identified to be ISGF3 (lanes 2 to 4). ISGF3 was, however, not detected when cells were infected with VSV at higher MOIs (lanes 5 and 6). The time course of VIBP induction was examined with extracts prepared from immediately after infection to 10 h after infection at a MOI of 10 (Fig. 1B, lanes 2 to 7) or 100 (lanes 8 to 13). VIBP induction was not detected up to 1 h after infection but became evident 2 h after infection at both doses of VSV. The VIBP band intensity increased during the subsequent 10 h postinfection. In contrast, the IRF-2 band intensity decreased during the postinfection period. This decrease was accounted for by degradation of the IRF-2 protein, rather than the loss of DNA-binding activity, since Western immunoblot assays showed reduced IRF-2 levels following VSV infection (results not shown). ISGF3 was detected 4 to 6 h after infection, when cells were infected at a MOI of 10 (Fig. 1B, lanes 5 to 7) but was seen only immediately following infection when infection was performed at a MOI of 100 (lanes 8 to 13). VIBP was detected mostly in the nuclear fraction, not in the cytoplasmic fraction (results not shown). The induction of the VIBP activity was specific for the ISRE and was not due to a global change in host cell DNA-binding activities caused by VSV infection. No inducible band was detected when the same sets of extracts were tested with the pIRE (GAS) probe to which the stat P91 (a subunit of ISGF3α) binds (9, 21, 47). These results demonstrate that in addition to ISGF3, a unique nuclear factor-binding activity is induced upon infection with

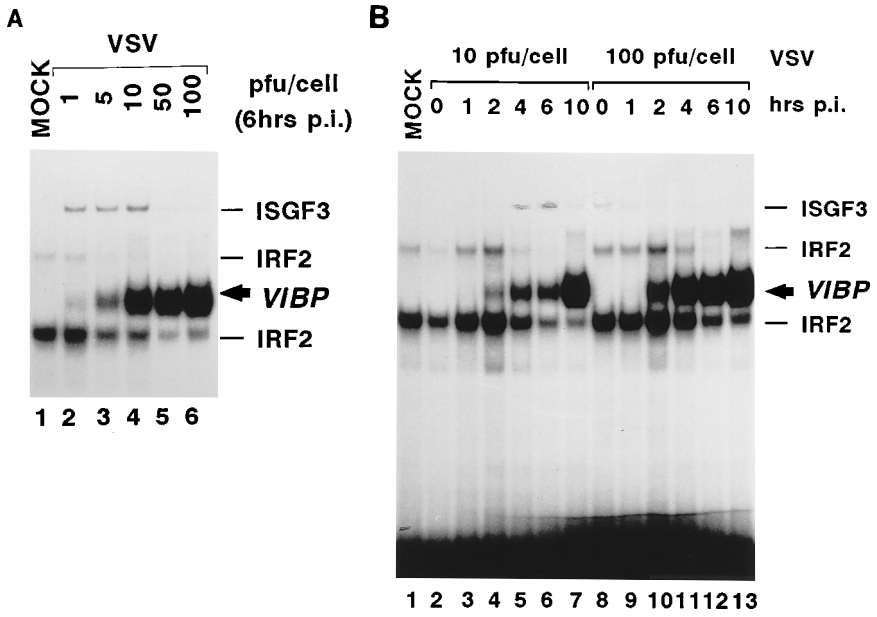

FIG. 1. Induction of VIBP in L929 cells infected with VSV. (A) VSV dose dependence. L929 cells were infected with VSV at MOIs from 1 to 100 and were incubated for 6 h after infection (hrs p.i.). Nuclear extracts from infected cells were analyzed in EMSA with ^{32}P-labeled ISG15 ISRE oligonucleotide as a probe (5). (B) Kinetics of VIBP induction. L929 cells were infected with VSV at a MOI of 10 (lanes 1 to 7) or 100 (lanes 8 to 13) and then incubated for various periods. Nuclear extracts were tested in EMSA.

VSV in L929 cells. Infection with a New Jersey strain of VSV also led to induction of VIBP (results not shown).

Although we did not pursue this observation further, we observed that NF-κB activity was also induced in L929 cells following VSV infection (results not shown), which may be attributed to activation of PKR (23, 36).

Relationships of VIBP with IRF family proteins: supershift analysis. ISRE-binding activities seen in various types of cells following IFN treatment or viral infection are attributed to proteins belonging to the IRF family. To determine whether VIBP is a known ISRE-binding protein belonging to the IRF family, antibodies specific for individual IRF proteins were tested for reactivity with VIBP in EMSA. Results are presented in Fig. 2. Anti-IRF-1 antibody had no effect on any of the bands seen in these extracts, presumably because IRF-1 is expressed at a very low level (Fig. 2, lane 2) (5). Anti-IRF-2 antibody "supershifted" two bands present before and after VSV induction but did not affect VIBP (lane 3). Antibody specific for ISGF3γ (the DNA-binding subunit of ISGF3), or for ISGF3α (P91) abolished the slowly migrating ISGF3 band but had no effect on VIBP (lanes 4 and 5). L929 cells do not express ICSBP at the level of both mRNA and protein, and ICSBP was hence not expected to be VIBP. In accordance with these results, anti-ICSBP antibody had no effect on VIBP induced in L929 cells or in lymphocytes and macrophages where ICSBP is expressed and is complexed with IRF-1 or IRF-2 (5) (results not shown). Thus, VIBP is not a known member of the IRF family.

Effects of actinomycin D and CHX. Actinomycin D, an inhibitor of cellular transcription, has been shown to have little effect on VSV transcription (20). To test whether cellular RNA synthesis is required for VIBP induction, actinomycin D (5 μg/ml) was added to L929 cells 30 min prior to infection (MOI of 10) and cells were infected and incubated in the presence of actinomycin D. As seen in Fig. 3 (left panel), actinomycin D had no inhibitory effect on VIBP induction: the intensity of VIBP induced in the presence of actinomycin D was greater than that in untreated cells (lane 2 versus 4). Treatment with actinomycin D alone without infection did not induce VIBP (lane 3). These results indicate that cellular transcription is not required for VIBP induction. To determine whether induction of VIBP is dependent on viral and/or cellular protein synthesis, the effect of CHX was tested. Like actinomycin D treatment as above, CHX (50 μg/ml) was added to L929 cells 30 min prior to infection and was present throughout infection (MOI of 10 or 100) and the subsequent 6 h of incubation. Treatment of L929 cells with CHX under conditions milder than those used in this work have been shown to completely block viral protein

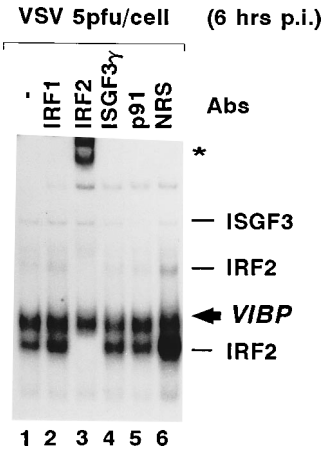

FIG. 2. Supershift analysis. Nuclear extract from L929 cells infected with VSV at a MOI of 5 were incubated with rabbit antibodies (diluted at 1:15) for 15 min prior to addition of labeled probe. Anti-IRF-1 and anti-IRF2 antibodies were described previously (5), and anti-ISGF3γ and anti-P91 antibodies are described in Materials and Methods. p.i., postinfection; NRS, control normal rabbit sera. The large amount of antisera used in the reaction was responsible for the nonspecific band migrating above the ISGF3 complex (lanes 2 to 6).

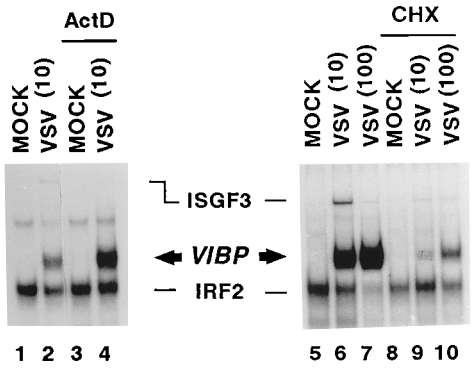

FIG. 3. Effects of actinomycin D (ActD) and CHX. Actinomycin D (5 μg/ml) (lanes 1 to 4) or CHX (50 μg/ml) (lanes 5 to 10) was added to L929 cells 30 min prior to infection and was present continuously during infection (MOIs of 10 or 100) and the subsequent 6 h of incubation. Nuclear extracts were assayed in EMSA.

synthesis (61). As seen in Fig. 3 (right panel), CHX treatment reduced the intensity of the VIBP band but did not completely inhibit its induction as tested at MOIs of both 10 and 100 (compare lanes 9 and 10 with lanes 6 and 7). Similar experiments were performed with different preparations of viruses and CHX, which confirmed incomplete inhibition of VIBP by CHX. These results indicate that viral and cellular protein synthesis is not required for primary induction of VIBP. The partial inhibition observed by CHX suggests that an activator (of either viral or cellular origin) or VIBP may also be synthesized during this period.

Effects of protein kinase inhibitors. To gain further insight into the physiological requirement of VIBP induction, effects of four kinase inhibitors, geldanamycin, genistein, staurosporine, and 2-aminopurine, were tested. These inhibitors were added 30 min before infection (MOI of 10) and were present continuously during and after infection (Fig. 4). Geldanamycin and genisteine are widely used tyrosine kinase inhibitors whose mechanisms of action may be different (7, 64). While geldanamycin had no effect, genistein completely inhibited induction

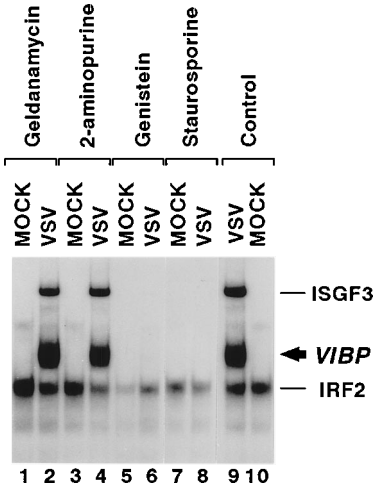

FIG. 4. Effects of protein kinase inhibitors. Geldanamycin (100 ng/ml) (lanes 1 and 2), 2-aminopurine (50 μg/ml) (lanes 3 and 4), genistein (100 μg/ml) (lanes 5 and 6), or staurosporine (1 μM) (lanes 7 and 8) was added to L929 cells 30 min prior to infection and was present continuously during infection (at a MOI of 10) and the subsequent 6 h of incubation. EMSA were performed as in Fig. 1.

of VIBP (lanes 2 and 6). In agreement with a previous study, genistein also inhibited ISGF3 (25). Staurosporine, an inhibitor of protein kinase C (and other kinases) which inhibits transcription of VSV (45), also completely inhibited VIBP induction (lane 8). Staurosporine also inhibited ISGF3 induction, as reported previously (22). 2-Aminopurine, an inhibitor of PKR (54), was tested, since this enzyme is shown to be induced upon viral infection and is capable of activating phosphorylation-dependent DNA-binding activity, including NF-κB (23). Induction of VIBP was unaffected by 2-aminopurine (lane 4). These results indicate that induction of VIBP is dependent on phosphorylation that involves tyrosine kinase as well as serine/threonine kinase activities. Whether VIBP itself is tyrosine phosphorylated is currently under investigation.

Effects of type I IFNs. Type I IFNs confer protection against VSV infection (29). The anti-VSV antiviral activity elicited by IFNs has been attributed largely to inhibition of viral transcription, although they may act on other levels as well, including translation inhibition (2, 30, 33, 51, 52). We tested whether IFN treatment interferes with induction of VIBP induction. L929 cells were pretreated with murine IFN-α/β (from 10 to 1,000 U/ml) overnight, rinsed, infected with VSV, and incubated for 6 h in the absence of IFNs. Results are shown in Fig. 5. Control cells infected with VSV at a MOI of 10 without IFN pretreatment produced a strong VIBP band (Fig. 5, lane 5). Cells pretreated with IFNs (100 U/ml) generated no detectable VIBP when infected at a MOI of 5 or 10 (lanes 2 and 3). IFN pretreatment inhibited VIBP induction even when infection was performed at a MOI of 100 (lane 4): the VIBP band intensity was much lower than that of control cells without IFN treatment (lane 4 versus lane 5). The levels of VIBP induction were dependent on the dosage of IFNs. IFNs at 100 and 1,000 U/ml virtually abrogated VIBP induction (lanes 7 and 8). Even at the lowest dose of IFNs (10 U/ml), induction of VIBP was significantly inhibited (lane 6). A prominent ISGF3 band was observed in all IFN-treated samples regardless of VSV infection: ISGF3 has been shown to be induced following type I IFN treatment (25). These data indicate that IFN treatment specifically blocks induction of VIBP. Taken together, our results show that agents that inhibit transcription of VSV also inhibit induction of VIBP.

Functional role of VIBP: reporter analysis. To evaluate the functional role of VIBP in cellular transcription regulated by the ISRE, reporter analysis was performed in L929 cells infected with VSV. Since VSV infection caused rapid cytopathic effect in these cells, transient transfections were ineffectual. Thus, we analyzed cells stably transfected with a luciferase reporter gene. This reporter gene was under control of the *tk* promoter connected to the ISRE from the ISG15 gene (ISRE-*tk-luc*). As a control, cells transfected with the *tk-luc* reporter without ISRE were tested. When stable transfectants were treated with IFN-α/β, the ISRE-*tk* reporter gave a >10-fold increase in luciferase activity while *tk-luc* gave no increase (Fig. 6B), confirming that the stably transfected ISRE reporter responds to IFN. To test the effect of VSV infection on the reporter activity, transfectants were infected with VSV at a MOI of 10 or 100 and luciferase activities were measured 6 h after infection (Fig. 6A). VSV infection led to a marked reduction in *tk-luc* reporter activity, whose levels fell approximately to 20% that of control mock-infected cells. This reduction was most certainly caused by inhibition of host cell transcription following VSV infection by the VSV matrix protein (3, 41). In contrast, ISRE-*tk-luc* activity was not reduced as much during the same period of infection, which measured approximately 80 and 50% that of control uninfected cells after VSV infection at MOIs of 10 and 100, respectively. Inhibition

of ISRE-*tk-luc* activity was consistently less than that of control *tk-luc* activity, as measured 3, 6, and 8 h after VSV infection in several independent transfections (results not shown). These results indicate that the ISRE is able to reverse inhibition of reporter transcription caused by VSV infection. That the reversed inhibition was seen even with infection at a MOI of 100, when induction of ISGF3 is not observed (Fig. 1) suggests that VIBP takes part in enhancing ISRE-mediated transcription.

Double-stranded RNA analog poly(I)-poly(C) induces ISRE-binding activity similar to that of VIBP. Experiments illustrated in Fig. 3 to 5 point to the possibility that VSV transcripts are involved in inducing VIBP. Daly and Reich (8) reported that the double-stranded RNA analog poly(I)-poly(C) activates at least two novel ISRE-binding activities, DRAF1 and DFRAF2. To investigate whether poly(I)-poly(C) induces a factor-binding activity similar to VIBP, L929 cells were transiently transfected with poly(I)-poly(C) or plasmid DNA (tested as a control) and ISRE binding activity was examined 5 h after transfection. Figure 7A compares EMSA results obtained by poly(I)-poly(C) transfection with that by VSV infection. Poly(I)-poly(C) induced a DNA-protein complex that migrated to a position very similar to that of VIBP (Fig. 7A, lanes 2 and 4). The poly(I)-poly(C)-induced binding activity was resistant to CHX treatment (lane 7), as was found for VIBP (Fig. 3). In addition, transfection with poly(I)-poly(C) induced ISGF3, as was induced by VSV infection. The induction of binding activity by poly(I)-poly(C) was not due to nonspecific perturbation of cells caused by the transfection procedure, since transfection of plasmid DNA produced no induced band in the presence or absence of CHX (Fig. 7A, lanes 5 and 8). Judging from the position of migration, the poly(I)-poly(C)-induced band may correspond to DRAF2 reported by Daly and Reich (8). Under these conditions, however, no band resembling DRAF1 was observed, which should migrate to a position above ISGF3. To compare VIBP with poly(I)-poly(C)-induced binding activity for their DNA-binding specificity, competition EMSAs were performed with oligomers corresponding to various ISREs (Fig. 7B). ISRE oligomers for the ISG15, ISG54 (22, 25), and guanylate-binding protein (GBP) (26) genes completely competed for both VIBP and the poly(I)-poly(C)-induced binding activity (lanes 2, 3, and 6 and lanes 11, 12, and 15, respectively). On the other hand, ISREs from the major histocompatibility complex class I (ICS) (10), 9-27, and 6-16 genes (42) and PRD1 from the IFN-β gene (53) were ineffective in competing for either binding activity (lanes 4, 5, 7, and 8 and lanes 13, 14, 16, and 17, respectively). This competition pattern was distinct from that by IRF-2 which was inhibited by all competitors (except for the negative control). Similarly, binding of ISGF3 was inhibited by all ISRE oligomers except for PRD1 and GBP, consistent with previous reports (25). As expected, oligomers for the pIRE that binds to stat P91 (9, 21), used as a control, failed to compete for either binding activity (lanes 9 and 18). These results indicate that VIBP and the poly(I)-poly(C)-induced factor are very similar to each other in their electrophoretic mobility as well as their DNA-binding specificity, raising the possibility that they are the same factor.

Size estimation by UV cross-linking. UV cross-linking experiments were carried out to assess the molecular size of VIBP. The VIBP band from VSV-infected cells was first separated by EMSA, UV cross-linked in situ, and then resolved by PAGE under denaturing conditions (38). As seen in Fig. 8, VIBP migrated approximately to a position corresponding to 70 kDa, which paralleled the position of IRF-2. Assuming that molecular mass of the DNA probe is about 20 kDa, VIBP is estimated to have an apparent molecular mass of ~50 kDa. This estimate is in agreement with the size of IRF-2 (16). A similar estimate has been obtained with a bromodeoxyuridine-substituted ISRE probe (results not shown). It should be stressed here that VIBP may be a multipeptide complex and that the estimate obtained in this experiment may represent the apparent molecular mass of a DNA-binding subunit.

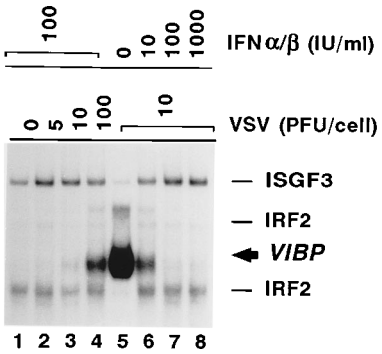

FIG. 5. Effects of IFN-α/β. L929 cells were treated with murine natural IFN-α/β overnight at various doses (10 to 1,000 U/ml). After removal of IFN, cells were infected with VSV at indicated MOIs (5 to 100) and incubated for 6 h. Control untreated cells were infected with VSV at a MOI of 10 (lane 5).

VSV induction of VIBP in various human and mouse cells: the lack of induction in embryonal carcinoma cells. VSV infects a wide variety of cells in tissue culture. To study whether induction of VIBP also occurs in a wide variety of cells, various murine and human cell lines were infected with VSV and induction of VIBP was tested in EMSA, as above. Results are summarized in Table 1. Induction of VIBP was observed in human and mouse fibroblasts, epithelial cells, monocytic cells, and T lymphocytes, whose migration position was essentially the same as that seen in L929 cells. The intensity of the VIBP band produced in murine cells was in general higher than that in human cells. Interestingly, the kinetics of VIBP induction varied among cells, in that EL4 and HL-60 cells produced the VIBP band immediately or 2 h after infection but this band was no longer detectable 6 h after infection. VIBP induction in other cells tested followed the kinetics similar to those of L929 cells.

In contrast to cells tested above, VIBP was not induced in P19 EC cells after VSV infection (Fig. 9). The absence of

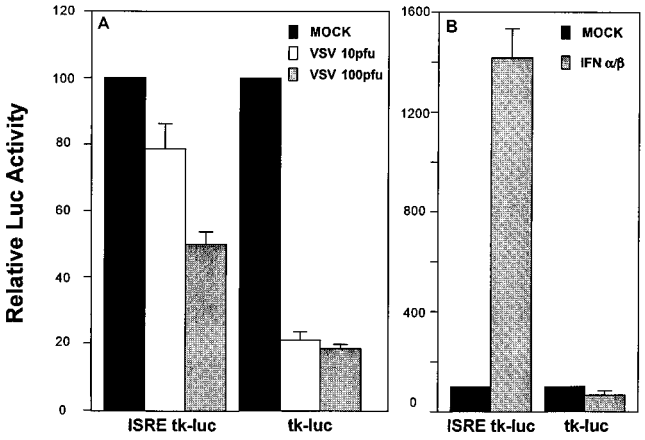

FIG. 6. ISRE reporter activity following VSV infection. (A) L929 cells stably transfected with *tk-luc* or ISRE-*tk-luc* were infected with VSV at a MOI of 10 or 100, and luciferase activity was measured 6 h after infection (37). Luciferase activity by VSV-infected cells is expressed relative to the activity by mock-infected cells. (B) To verify IFN inducibility of the ISRE reporter, transfected cells were treated with murine natural IFN-α/β (300 U/ml) for 6 h and luciferase activity was measured.

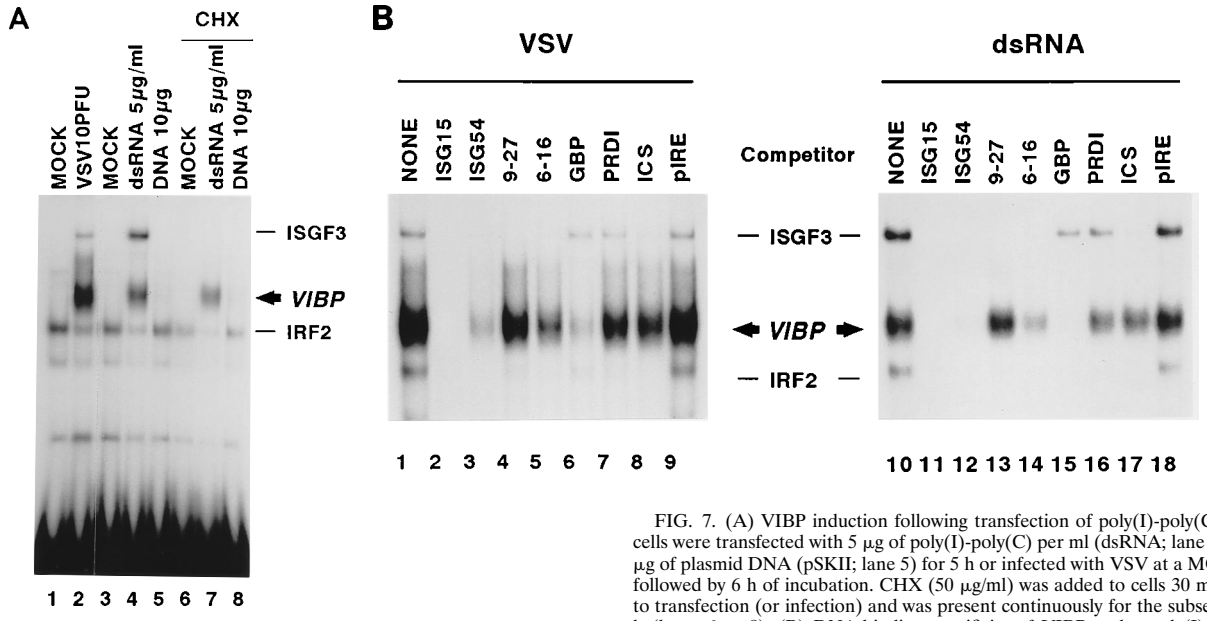

FIG. 7. (A) VIBP induction following transfection of poly(I)-poly(C). L929 cells were transfected with 5 μg of poly(I)-poly(C) per ml (dsRNA; lane 4) or 10 μg of plasmid DNA (pSKII; lane 5) for 5 h or infected with VSV at a MOI of 10 followed by 6 h of incubation. CHX (50 μg/ml) was added to cells 30 min prior to transfection (or infection) and was present continuously for the subsequent 6 h (lanes 6 to 8). (B) DNA-binding specificity of VIBP and a poly(I)-poly(C) induced factor: competition assay. Nuclear extracts from cells infected with VSV at a MOI of 10 (lanes 1 to 9) or cells transfected with 5 μg of poly(I)-poly(C) per ml (lanes 10 to 18) were incubated with an approximately 100-fold molar excess of unlabeled oligonucleotides (see Materials and Methods) for 15 min prior to addition of labeled ISG15 ISRE probe and were analyzed in EMSA. dsRNA, double-stranded RNA.

VIBP induction in P19 EC cells was not due to the lack of productive infection by VSV, since strong cytopathic effects were observed within 16 h following infection. P19 and other undifferentiated EC cells do not express IRF-1 or IRF-2 (17), nor do they express ISGF3 in response to IFN (19). Consistent with these reports, no discernible ISRE-binding activity was seen prior to and following VSV infection in P19 cells (Fig. 9, lanes 1 and 2). These factors are shown to be expressed when EC cells are allowed to differentiate in response to retinoic acid (17, 19). As seen in Fig. 9, however, VIBP was not induced in P19 cells that had been treated with retinoic acid for 3 days, even though this retinoic acid treatment led to expression of IRF-2, irrespective of VSV infection (lanes 3 to 6). These results suggest that, similar to other IRF proteins, induction of VIBP is developmentally controlled, although the timing of VIBP induction appears distinct from that of IRF-2 (see Discussion).

Lack of VIBP induction by SV40 and vaccinia virus. To assess the generality of VIBP induction, we investigated the induction of ISRE-binding activity by two other viruses, SV40 and vaccinia virus. The former is a DNA tumor virus (55), and the latter is a poxvirus with a double-stranded DNA genome (6). L929 cells were infected with either virus and VIBP induction was examined in EMSA immediately or up to 48 h after infection. Results are summarized in Table 2. No induction of ISRE-binding activity was observed with either virus during these periods, although infection with these viruses caused prominent morphological changes or cytopathic effects. These results indicate that VIBP induction is not a universal cellular reaction to viral infections but occurs in a virus-specific manner. Further studies will determine whether VIBP induction is specific for RNA viruses.

DISCUSSION

This paper describes a unique ISRE-binding factor, called VIBP, that is induced in a variety of cells following VSV infection. VIBP induction was dependent on the dosage of VSV and was seen in fibroblasts and in monocytes/lymphocytes with somewhat variable kinetics. VIBP or its DNA-binding component is estimated to be about 50 kDa in size. VIBP induction most probably results from activation of a preexisting cellular protein, for which a phosphorylation process plays a role. Molecular and cellular events that follow VSV infection have been extensively studied in many types of cells so far. However, this report, to our knowledge, is the first to demonstrate induction of a nuclear ISRE-binding activity upon VSV infection in a wide variety of cells.

Mechanism of VIBP induction. VIBP is not likely to be a viral protein itself. Most viral proteins (G, N, L, and P) may be excluded, as they are synthesized and localized in the cytoplasm whereas VIBP activity is seen mostly in the nucleus. The

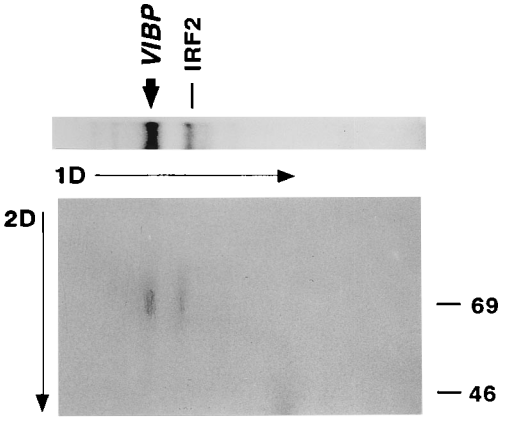

FIG. 8. Estimation of the VIBP molecular mass by UV cross-linking. L929 cells were infected with VSV at a MOI of 100, and a gel slice with the VIBP band generated in EMSA (upper lane) was UV cross-linked (38; see Materials and Methods) and resolved by sodium dodecyl sulfate-PAGE (10% polyacrylamide) (lower panel). 1D, first dimension; 2D, second dimension. The numbers at right indicate approximate molecular mass.

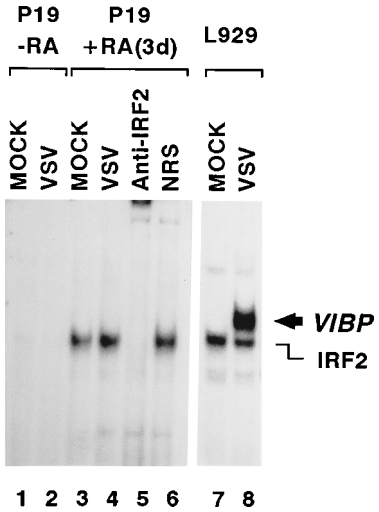

FIG. 9. Absence of VIBP induction in P19 EC cells. Undifferentiated P19 EC cells (lanes 1 and 2) or cells treated with all-*trans* retinoic acid (RA; 1 μM) for 3 days (3d) (37) were infected with VSV at a MOI of 10 followed by 6 h of incubation (lanes 3 to 6). Anti-IRF-2 antibody or control normal rabbit sera were added as in the experiment in Fig. 2. As a control, L929 cells were infected with VSV at a MOI of 10 followed by 6 h of incubation (lanes 7 and 8).

26-kDa matrix protein (M), the only viral protein shown to localize in the nucleus (27), is unlikely to be responsible for VIBP complex formation either, since several antibodies specific for M did not react with VIBP in EMSA or immunodeplete VIBP activity (results not shown). Furthermore, transfection of mammalian expression vectors for M into L929 cells did not induce VIBP but did inhibit transcription of a cotransfected reporter gene, as was expected of M (3, 41; data not shown). Also, it is unlikely that viral protein synthesis is required for VIBP induction. CHX treatment, known to abolish viral protein synthesis in L929 cells (61), only reduced but did not abolish VIBP induction (Fig. 3). Rather, our data appear to point to the involvement of viral transcripts in VIBP induction. VIBP induction was strongly inhibited by IFN-α/β (Fig. 5), which is shown to block viral transcription (2, 30, 33, 51). Staurosporine, also an inhibitor of VSV transcription (45), inhibited VIBP induction (Fig. 4). The lack of inhibition by actinomycin D (Fig. 3) is not inconsistent with involvement of VSV transcripts, since it selectively inhibits host cell transcription but not VSV transcription (20). Our observations that poly(I)-poly(C), a double-stranded RNA analog, induces an ISRE-binding activity similar to VIBP further support the role of RNA for inducing VIBP (Fig. 7). Upon infection, VSV RNA is sequentially synthesized from the 3′ end (1). Among VSV transcripts, the small leader RNA may be a candidate for activating VIBP. The leader RNA, the first transcript to be synthesized upon infection, is localized in the nucleus (24) and is thought to form a short "double-stranded" stem-loop structure. However, at present there is no experimental evidence to substantiate the involvement of leader RNA in VIBP induction. It is possible that other RNA species, including partially uncoated genomic RNA, become double stranded with either leader and/or mRNAs and stimulate VIBP. Further analysis to determine a component of the VSV genome involved in VIBP induction is under way.

Identity of VIBP. The lack of inhibition by actinomycin D and by CHX (Fig. 3) strongly indicates that VIBP does not require de novo synthesis of cellular RNA or protein. Thus, induction of VIBP is likely to represent activation of a latent cellular protein upon VSV infection. Our data favor the idea that this activity occurs in the nucleus and is dependent on activities of a certain cellular tyrosine kinase(s) and a serine-threonine kinase(s). VSV transcription is reported to require cellular kinase activity (45). Thus, the observed phosphorylation dependence (Fig. 4) may reflect a requirement of phosphorylation for VSV transcription and may or may not imply phosphorylation of VIBP itself.

It has been shown that very short self-complementary viral RNAs, such as human immunodeficiency virus TAR, as well as double-stranded RNA can directly activate IFN-regulated enzymes such as 2-5A synthetase, Mx (18), and PKR (12, 43, 50). Kumar et al. (23) reported that PKR, for its ability to phosphorylate Iκ-B, can activate NF-κB, a nuclear factor that binds to the IFN-β promoter. Thus, PKR may be expected to be involved in activating VIBP. However, on the basis of the lack of sensitivity to 2-aminopurine (Fig. 4) (23, 54), it is unlikely that PKR has a major role in activating VIBP.

We show that a binding activity similar to VIBP was induced upon transfection of double-stranded RNA analog poly(I)-poly(C) (Fig. 7). Like VIBP, poly(I)-poly(C)-induced binding activity was resistant to CHX, and its binding specificity was identical to that of VIBP. Recently, Daly and Reich (8) reported that poly(I)-poly(C) activates two ISRE-binding factors, designated DRAF1 and DRAF2. It is interesting that VIBP migrates to a position similar to that of DRAF2 and that both VIBP and DRAF2 are induced following activation of a latent protein, suggesting a close similarity between VIBP and

TABLE 1. VIBP inducibility in human and mouse cells[a]

Time p.i.[b]	VIBP induction in following cell type:							
	EC (P19)		T cell (EL4)	Monocytic cells		Epithelial (HeLa)	Fibroblasts	
	−RA	+RA		PU5-1.8	HL-60		FS4	NIH 3T3
Early (0–2 h)	−	−	+	−	+	−	−	−
Late (6 h)	−	−	−	+	−	+	+	+

[a] Cells were infected with VSV at a MOI of 10, and nuclear extracts prepared 0, 2, 4, 6, and 10 h after infection were tested for induction of VIBP activity in EMSA as in Fig. 1.
[b] p.i., postinfection.

TABLE 2. Absence of VIBP induction by SV40 and vaccinia virus[a]

Virus	MOI (PFU/cell)	CPE[b]	Time (h) postinfection	Inducible ISRE-binding activity
Vaccinia virus	10	+	6	−
	100	++	6	−
	1,000	+++	6	−
	10	−	0	−
	10	±	1	−
	10	+	3	−
SV40	10	±	6	−
	10	−	0	−
	10	−	3	−
	10	+	12	−
	10	+	24	−
	10	++	48	−

[a] L929 cells were infected with vaccinia virus or SV40 at the indicated MOIs for 1 h and incubated for the indicated periods. Nuclear extracts from infected cells were tested for ISRE-binding activity in EMSA as in Fig. 1.
[b] Morphological changes and cytopathic effects (CPE) were monitored by measuring cell rounding and lysis.

DRAF2. However, the EMSA pattern we have detected in our system differs from that reported by these authors: a band corresponding to DRAF1 was not detected in our assays. This difference may be due to the use of different cells and experimental conditions, but it may also suggest that different factors are induced in different cells by different RNAs. The identity of VIBP awaits its purification and sequence determination.

Biological significance of VIBP. Transfection analysis (Fig. 6) indicate that activity of the ISRE-bearing reporter is less susceptible to the transcriptional shutdown caused by VSV than is the activity of a control reporter without ISRE. These data suggest that VIBP participates in enhancing transcription of genes that bear the ISRE and homologous sequences. This enhancement of transcription may allow for expression of some genes despite the overall shutdown of cellular transcription by the VSV matrix protein. Hence, VIBP may contribute to stimulation of IFN genes, as is noted in some cells after VSV infection leads to production of type I IFNs (31). Marcus et al. reported that VSV of the New Jersey serotypes produces higher levels of IFNs than does VSV of the Indiana serotype (31). However, inducibility of VIBP was similar in the two serotypes (data not shown), suggesting the requirement of multiple components for and/or suppression of (14) IFN production. That poly(I)-poly(C) induces type I IFN and IFN-inducible genes (49) may also support the involvement of VIBP and VIBP-like proteins in regulating IFN and IFN-inducible genes. Our observations, as well as those by Dale and Reich (8), raise the possibility that there are additional ISRE-binding proteins that have not been fully studied so far, which are involved in regulating IFN and IFN-inducible genes. Some of these proteins may belong to the IRF family. The presence of additional ISRE-binding proteins may indeed be predicted from the redundancy in IFN-regulated transcription. Analyses of IRF-$1^{-/-}$ and IRF-$2^{-/-}$ mice show that these mice are capable of mounting IFNs in response to viral infection and expressing many IFN-inducible genes (34, 44).

It is interesting that P19 EC cells, despite full permissiveness to VSV infection, failed to induce VIBP. Neither IRF-1, IRF-2, nor ISGF3 is expressed in undifferentiated EC cells, including P19 EC cells. However, these factors become inducible when EC cells differentiate upon stimulation by retinoic acid, which presumably reflects developmental control of IFN-regulated transcription (17, 19). It is possible that VIBP inducibility is also developmentally controlled and that the absence of VIBP inducibility in P19 EC cells reflects the absence of the expression of a latent factor in these cells. It is of note that treatment of P19 EC cells with retinoic acid for 3 days was sufficient to induce expression of IRF-2 (17) but was not sufficient to fully induce VIBP-binding activity or ISGF3 activity (Fig. 9). Induction of both factors appeared to require a longer period of retinoic acid treatment (data not shown), suggesting that mechanisms allowing induction of VIBP are distinct from those allowing induction of IRF-2. More analysis is required for clarifying mechanisms governing developmental regulation of VIBP.

Lastly, it is conceivable that VIBP is induced upon infection by other viruses and pathogens. At present, the range of viruses and pathogens capable of inducing VIBP is unknown. Our limited surveys indicate that infection by DNA viruses such as SV40 or vaccinia virus does not lead to VIBP induction in L929 cells. Screening of a wider array of viruses, and particularly viruses from other RNA virus families, may provide a better scope for the mechanism of induction and biological activity of VIBP.

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REFERENCES

1. **Banerjee, A. K.** 1987. Transcription and replication of rhabdoviruses. Microbiol. Rev. **51:**66–87.
2. **Belkowski, L. S., and G. C. Sen.** 1987. Inhibition of vesicular stomatitis viral mRNA synthesis by interferons. J. Virol. **61:**653–660.
3. **Black, B. L., and D. S. Lyles.** 1992. Vesicular stomatitis virus matrix protein inhibits host cell-directed transcription of target genes in vivo. J. Virol. **66:**4058–4064.
4. **Blondel, D., G. G. Harmison, and M. Schubert.** 1990. Role of matrix protein in cytopathogenesis of vesicular stomatitis virus. J. Virol. **64:**1716–1725.
5. **Bovolenta, C., P. H. Driggers, M. S. Marks, J. A. Medin, A. D. Politis, S. N. Vogel, D. E. Levy, K. Sakaguchi, E. Appella, J. E. Coligan, and K. Ozato.** 1994. Molecular interactions between interferon consensus sequence binding protein and members of the interferon regulatory factor family. Proc. Natl. Acad. Sci. USA **91:**5046–5050.
6. **Buller, R. M. L., and G. J. Palumbo.** 1991. Poxvirus pathogenesis. Microbiol. Rev. **55:**80–122.
7. **Cohen, P., S. Klumpp, and D. L. Schelling.** 1989. An improved procedure for identifying and quantitating protein phosphatases in mammalian tissues. FEBS Lett. **250:**596–600.
8. **Daly, C., and N. C. Reich.** 1993. Double-stranded RNA activates novel factors that bind to the interferon-stimulated response element. Mol. Cell. Biol. **13:**3756–3764.
9. **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.
10. **Driggers, P. H., D. L. Ennist, S. L. Gleason, W.-H. Mak, M. S. Marks, B.-Z. Levi, J. R. Flanagan, E. Appella, and K. Ozato.** 1990. An interferon γ-regulated protein that binds the interferon-inducible enhancer element of major histocompatibility complex class I genes. Proc. Natl. Acad. Sci. USA **87:**3743–3747.
11. **Dunigan, D. D., S. Baird, and J. Lucas-Lenard.** 1986. Lack of correlation between the accumulation of plus-strand leader RNA and the inhibition of protein and RNA synthesis in vesicular stomatitis virus infected mouse L cells. Virology **150:**231–246.
12. **Edery, I., R. Petryshyn, and N. Sonenberg.** 1989. Activation of double-stranded RNA-dependent kinase (dsl) by the TAR region of HIV-1 mRNA: a novel translational control mechanism. Cell **56:**303–312.
13. **Fujita, T., Y. Kimura, M. Miyamoto, E. L. Barsoumian, and T. Taniguchi.** 1989. Induction of endogenous IFN-α and IFN-β genes by a regulatory transcription factor, IRF-1. Nature (London) **337:**270–272.
14. **Gaccione, C., and P. I. Marcus.** 1989. Interferon induction by viruses. XVIII. Vesicular stomatitis virus-New Jersey: a single infection particle can both induce and repress interferon production. J. Interferon Res. **9:**603–614.
15. **Grinnell, B. W., and R. R. Wagner.** 1984. Nucleotide sequence and secondary structure of VSV leader RNA and homologous DNA involved in inhibition of DNA-dependent transcription. Cell **36:**533–543.
16. **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.
17. **Harada, H., K. Willison, J. Sakakibara, M. Miyamoto, T. Fujita, and T. Taniguchi.** 1990. Absence of the type I IFN system in EC cells: transcriptional activator (IRF-1) and repressor (IRF-2) genes are developmentally regulated. Cell **63:**303–312.
18. **Hug, H., M. Costas, P. Staehili, M. Aebi, and C. Weissmann.** 1988. Organization of the murine *Mx* gene and characterization of its interferon- and virus-inducible promoter. Mol. Cell. Biol. **8:**3065–3079.
19. **Kalvakolanu, D. V. R., and G. C. Sen.** 1993. Differentiation-dependent activation of interferon-stimulated gene factors and transcription factor NF-kB in mouse embryonal carcinoma cells. Proc. Natl. Acad. Sci. USA **90:**3167–3171.
20. **Kang, C. Y., and L. Prevec.** 1971. Proteins of vesicular stomatitis virus. III. Intracellular synthesis and extracellular appearance of virus-specific proteins. Virology **46:**678–690.
21. **Kanno, Y., C. A. Kozak, C. Schindler, P. H. Driggers, D. L. Ennist, S. L. Gleason, J. E. Darnell, Jr., and K. Ozato.** 1993. The genomic structure of the murine ICSBP gene reveals the presence of the gamma interferon-responsive element, to which an ISGFα subunit (or similar) molecule binds. Mol. Cell. Biol. **13:**3951–3963.
22. **Kessler, D. S., and D. E. Levy.** 1991. Protein kinase activity required for an early step in interferon-α signaling. J. Biol. Chem. **266:**23471–23476.
23. **Kumar, A., J. Haque, J. Lacoste, J. Hiscott, and B. R. G. Williams.** 1994.

Double-stranded RNA-dependent protein kinase activates transcription factor NF-Kb by phosphorylating IkB. Proc. Natl. Acad. Sci. USA **91:**6288–6292.

24. **Kurilla, M. G., H. Piwnica-Worms, and J. D. Keene.** 1982. Rapid and transient localization of the leader RNA of vesicular stomatitis virus in the nuclei of infected cells. Proc. Natl. Acad. Sci. USA **79:**5240–5244.
25. **Levy, D. E., D. S. Kessler, R. Pine, N. Reich, and J. E. Darnell, Jr.** 1988. Interferon-induced nuclear factors that bind a shared promoter element correlate with positive and negative transcription control. Genes Dev. **2:**383.
26. **Lew, D. J., T. Decker, I. Strehlow, and J. E. Darnell.** 1991. Overlapping elements in the guanylate-binding protein gene promoter mediate transcriptional induction by alpha and gamma interferons. Mol. Cell. Biol. **11:**182.
27. **Lyles, D. S., L. Puddington, and B. J. McCreedy.** 1988. Vesicular stomatitis virus M protein in the nuclei of infected cells. J. Virol. **62:**4387–4392.
28. **MacDonald, N. J., D. Kuhl, D. Maguire, D. Naf, P. Gallant, A. Goswamy, H. Hug, H. Bueler, M. Chaturvedi, J. de la Fuente, H. Ruffner, F. Meyer, and C. Weissman.** 1990. Different pathways mediate virus inducibility of the human IFN-α1 and IFN-β genes. Cell **60:**767–779.
29. **Marcus, P. I., D. L. Engelhardt, J. M. Hunt, and M. J. Sekellick.** 1971. Interferon action: inhibition of VSV RNA synthesis induced by virion-bound polymerase. Science **174:**593–598.
30. **Marcus, P. I., and M. J. Sekellick.** 1978. Interferon action. III. The rate of primary transcription of vesicular stomatitis virus is inhibited in interferon action. J. Gen. Virol. **38:**391–408.
31. **Marcus, P. I., M. J. Sekellick, and S. T. Nichol.** 1992. Interferon induction by viruses. XXI. Vesicular stomatitis virus: interferon inducibility as a phylogenetic marker. J. Interferon Res. **12:**297–305.
32. **Marvaldi, J., J. Lucas-Lenard, M. J. Sekellick, and P. I. Marcus.** 1977. Cell killing by viruses. IV. Cell killing and the inhibition of cell protein synthesis requires the same gene functions of vesicular stomatitis virus. Virology **99:**267–273.
33. **Master, P. S., and C. E. Samuel.** 1983. Mechanism of interferon action: inhibition of vesicular stomatitis virus replication in human amnion U cells by cloned human leukocyte interferon. II. Effect on viral macromolecular synthesis. J. Biol. Chem. **258:**12026–12033.
34. **Matsuyama, T., T. Kimura, M. Kitagawa, K. Pfeffer, T. Kawakami, N. Watanabe, T. M. Kundig, R. Amakawa, K. Kishihara, A. Wakeham, J. Potter, C. L. Furlonger, A. Narendran, H. Suzuki, P. S. Ohashi, C. J. Paige, T. Taniguchi, and T. W. Mak.** 1993. Targeted disruption of IRF-1 or IRF-2 results in abnormal type I IFN gene induction and aberrant lymphocyte development. Cell **75:**83–97.
35. **McGowan, J. J., A. V. Zmenon, and R. R. Wagner.** 1982. The plus strand leader RNA of VSV inhibits DNA-dependent transcription of adenovirus and SV40 genes in a soluble whole cell extract. Cell **28:**325–333.
36. **Meurs, E., K. Chong, J. Galabru, N. S. B. Thomas, I. M. Kerr, B. R. G. Williams, and A. G. Hovanessian.** 1990. Molecular cloning and characterization of the human double-stranded RNA-activated protein kinase induced by interferon. Cell **62:**379–390.
37. **Minucci, S., D. J. Zand, A. Dey, M. S. Marks, T. Nagata, J. F. Grippo, and K. Ozato.** 1994. Dominant negative retinoid X receptor β inhibits retinoic acid-responsive gene regulation in embryonal carcinoma cells. Mol. Cell. Biol. **14:**360–372.
38. **Molitor, J. A., W. H. Walker, S. Doerre, D. W. Ballard, and W. C. Greene.** 1990. NF-kB: a family of inducible and differentially expressed enhancer-binding proteins in human T cells. Proc. Natl. Acad. Sci. USA **87:**10028–10032.
39. **Muller, M., J. Briscoe, C. Laxton, D. Guschin, A. Ziemlecki, O. Silvennolnen, A. Garpur, G. Barbier, B. A. Witthuhn, C. Schindler, S. Pellegrini, A. F. Wilks, J. N. Ihle, G. R. Stark, and I. M. Kerr.** 1993. The protein tyrosine kinase JAK1 complements defects in interferon-α/β and -gamma signal transduction. Nature (London) **366:**129–135.
40. **Nelson, N., M. S. Marks, P. H. Driggers, and K. Ozato.** 1993. Interferon consensus sequence-binding protein, a member of the interferon regulatory factor family, suppresses interferon-induced gene transcription. Mol. Cell. Biol. **13:**588–599.
41. **Paik, S.-Y., A. C. Banerjea, G. G. Harmison, C.-J. Chen, and M. Schubert.** 1995. Inducible and conditional inhibition of human immunodeficiency virus proviral expression by vesicular stomatitis virus matrix protein. J. Virol. **69:**3529–3537.
42. **Parrington, J., N. C. Rogers, D. R. Gewert, R. Pine, S. A. Veals, D. E. Levy, G. R. Stark, and I. M. Kerr.** 1993. The interferon-stimulable response elements of two human genes detect overlapping sets of transcription factors. Eur. J. Biochem. **214:**617.
43. **Reich, N., R. Pine, D. Levy, and J. E. Darnell, Jr.** 1988. Transcription of interferon-stimulated genes is induced by adenovirus particles but is suppressed by E1A gene products. J. Virol. **62:**114–119.
44. **Reis, L. F., H. Ruffner, G. Stark, M. Aguet, and C. Weissmann.** 1994. Mice devoid of interferon regulatory factor 1 (irf-1) show normal expression of type I interferon genes. EMBO J. **13:**4798–4806.
45. **Rigaut, K. D., Y. Gao, and J. Lenard.** 1993. Effects of staurosporine on transcription by vesicular on transcription by vesicular stomatitis virus. Virology **194:**433–440.
46. **Rose, J. K., and M. Schubert.** 1987. Rhabdovirus genomes and their products, p. 129–166. *In* R. R. Wagner (ed.), The rhabdoviruses. Plenum Publishing Corp., New York.
47. **Schindler, C., X.-Y. Fu, R. Improta, R. Aebersold, and J. E. Darnell, Jr.** 1992. Proteins of transcription factor ISGF-3: one gene encodes the 91- and 84-kDa ISGF-3 proteins that are activated by interferon α. Proc. Natl. Acad. Sci. USA **89:**7836–7839.
48. **Sen, G. C., and R. E. Herz.** 1983. Differential antiviral effects of interferon in three murine cell lines. J. Virol. **45:**1017–1027.
49. **Sen, G. C., and P. Lengyel.** 1992. The interferon system, a bird's eye view of biochemistry. J. Biol. Chem. **267:**5017–5020.
50. **SenGupta, D. N., and R. H. Silverman.** 1989. Activation of interferon-regulated, dsRNA-dependent enzymes by human immunodeficiency virus-1 leader RNA. Nucleic Acids Res. **17:**969–978.
51. **Staehili, P., and J. Pavlovic.** 1991. Inhibition of vesicular stomatitis virus mRNA synthesis by human MxA protein. J. Virol. **65:**4498–4501.
52. **Thacore, H. R.** 1978. Effect of interferon on transcription and translation of vesicular stomatitis virus in human and simian cell cultures. J. Gen. Virol. **41:**421–426.
53. **Thanos, D., and T. Maniatis.** 1992. The high mobility group protein HMGI(Y) is required for NF-kB-dependent virus induction of the human IFN-β gene. Cell **71:**777–789.
54. **Tiwari, R. K., J. Kusari, R. Kumar, and G. C. Sen.** 1988. Gene induction by interferons and double-stranded RNA: selective inhibition by 2-aminopurine. Mol. Cell. Biol. **8:**4289–4294.
55. **Turler, H., and P. Beard.** 1994. Simian virus 40 and polyoma virus: growth titration, transformation and purification of viral component, p. 169–192. *In* B. W. J. Mahy (ed.), Virology. A practical approach. IRL Press, Oxford.
56. **Veals, S. A., C. Schindler, D. Leonard, X. Fu, J. Abersold, J. E. Darnell, Jr., and D. E. Levy.** 1992. Subunit of an alpha-interferon-responsive transcription factor is related to interferon regulatory factor and Myb families of DNA-binding proteins. Mol. Cell. Biol. **12:**3315–3324.
57. **Wagner, R. R.** 1987. Rhabdovirus biology and infection: an overview, p. 1–74. *In* R. R. Wagner (ed.), The rhabdoviruses. Plenum Publishing Corp., New York.
58. **Weck, P. K., and R. R. Wagner.** 1978. Inhibition of RNA synthesis in mouse myeloma cells infected with vesicular stomatitis virus. J. Virol. **25:**770–776.
59. **Weisz, A., P. Marx, R. Sharf, E. Appella, P. H. Driggers, K. Ozato, and B.-Z. Levi.** 1992. Human interferon consensus sequence binding protein is a negative regulator of enhancer elements common to interferon-inducible genes. J. Biol. Chem. **267:**25589–25596.
60. **Wertz, G., and J. Youngner.** 1972. Inhibition of protein synthesis in L cells infected with vesicular stomatitis virus. J. Virol. **9:**85–89.
61. **Wertz, G. W., and M. Levine.** 1973. RNA synthesis by vesicular stomatitis virus and a small-plaque mutant: effects of cycloheximide. J. Virol. **12:**253–264.
62. **Whitaker-Dowling, P., and J. S. Youngner.** 1988. Alteration of vesicular stomatitis virus L and NS proteins by UV irradiation: implications for the mechanism of host cell shut-off. Virology **164:**171–175.
63. **Wu, F. S., and J. M. Lucas-Lenard.** 1980. Inhibition of ribonucleic acid accumulations in mouse L cells infected with vesicular stomatitis virus requires viral ribonucleic acid transcription. Biochemistry **19:**804–810.
64. **Yamaki, H., S. M. M. Iguchi-Ariga, and H. Ariga.** 1989. Inhibition of c-myc gene expression in murine lymphoblastoma cells by geldanamycin and herbimycin, antibiotics of benzoquinoid ansamycin group. J. Antibiot. **42:**604–610.
65. **Zhou, A., B. A. Hassel, and R. H. Silverman.** 1993. Expression cloning of a 2-5A-dependent RNase: a uniquely regulated mediator of interferon action. Cell **72:**753–765.