A Hybrid IRF9-STAT2 Protein Recapitulates Interferon-stimulated Gene Expression and Antiviral Response*

Received for publication, December 19, 2002, and in revised form, February 4, 2003
Published, JBC Papers in Press, February 5, 2003, DOI 10.1074/jbc.M2129722000

Thomas A. Kraus‡§, Joe F. Lau‡, Jean-Patrick Parisien, and Curt M. Horvath¶
From the Immunobiology Center, The Mount Sinai School of Medicine, New York, New York 10029

Type I interferon (IFN) signaling induces the heterotrimeric transcription complex, IFN-stimulated gene factor (ISGF) 3, which contains STAT1, STAT2, and the DNA binding subunit, interferon regulatory factor (IRF) 9. Because IRF9 is targeted to the nucleus in the absence of IFN stimulation, the potential of IRF9 protein for gene regulation was examined using a GAL4 DNA binding domain fusion system. GAL4-IRF9 was transcriptionally active in reporter gene assays but not in the absence of cellular STAT1 and STAT2. However, the in vivo IRF9 protein was readily converted to a constitutively active ISGF3-like activator by fusion with the C-terminal transcriptional activation domain of STAT2 or the acidic activation domain of herpesvirus VP16. The IRF9 hybrids are targeted to endogenous ISGF3 target loci and can activate their transcription. Moreover, expression of the IRF9-STAT2 fusion can recapitulate the type I interferon biological response, producing a cellular antiviral state that protects cells from virus-induced cytopathic effects and inhibits virus replication. The antiviral state generated by regulated IRF9-STAT2 hybrid protein expression is independent of autocrine IFN signaling and inhibits both RNA and DNA viruses.

Type I interferons (IFNα and IFNβ, referred to here collectively as IFN)1 provide the first wave of innate immune defense against virus infection and can also modulate the adaptive immune response (1, 2). Several studies have examined the use of IFN as a therapeutic agent, either by direct administration or by gene therapy-based IFN gene expression approaches (3–8). Despite the high potential as a pharmacologic agent for both infectious diseases and cancer, a variety of shortcomings have limited the widespread use of IFN, including the inability to obtain clinically relevant concentrations in vivo, the short half-life of IFN in circulation, limited access of exogenous IFN to the site of infection, and the evolution of viruses that can antagonize enzymes involved in the antiviral response. Novel strategies for activation of IFN biological responses by targeting transcriptional regulators to specific cells might circumvent these problems and ultimately prove to be an effective therapeutic option.

Most of the immediate actions of IFN can be attributed to the activity of an IFN-inducible transcriptional regulator, the IFN-stimulated gene factor 3 (ISGF3), and its IFN-stimulated gene (ISG) targets. As a trimeric complex, the ISGF3 transcription factor is mechanistically unique compared with other STAT-dependent transcription pathways. The cytoplasmic tyrosine kinase-based signaling events leading to STAT protein activation and STAT1:STAT2 dimerization are dissociable from the nuclear transcription functions of ISGF3 that require obligatory interactions between STATs and interferon regulatory factor 9 (IRF9). IRF9 is essential as the DNA binding component of ISGF3 that binds to consensus IFN-stimulated response elements (ISRE) and provides specific protein interaction sites for the IFN-activated STAT1 and STAT2 proteins (9–11). In the nucleus, the modular STAT2 C-terminal transcriptional activation domain (TAD) provides the essential signals for the induction of target gene transcription (12).

Although it is generally accepted that IRF9 functions as an adaptor molecule for tethering a STAT1:STAT2 dimer to the ISRE DNA element, an emerging body of evidence suggests non-ISGF3 roles for IRF9 in the regulation of gene expression. For instance, the human IP-10 gene requires an IRF9 signal transduction domain (13). IRF9 is efficiently targeted to the nucleus via an intrinsic bipartite localization signal that functions in the absence of IFN signaling (14), and ectopic expression of IRF9 confers resistance to anti-microtubule agents in breast cancer cell lines independent of IFN signals (15). Interestingly, IRF9 mRNA levels are increased in half of the breast and uterine cancer samples tested (15), suggesting a potential role for this factor in cancer biology. Therefore, the potential of IRF9 to act as a direct transcriptional regulator independent of its role as the ISGF3 DNA binding adaptor was examined. Results demonstrate that whereas IRF9 lacks intrinsic transcriptional capacity, IRF9-TAD fusion proteins effectively mimic the activity of the trimeric ISGF3 complex. The IRF9 hybrids function in the absence of endogenous IFN signaling and provide a novel strategy for enhancing endogenous antiviral responses.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection—Human fibrosarcoma 2fTGH, 2fTGH-derived cell lines U3A (STAT1-deficient) and U6A (STAT2-deficient), 293T, CV-1, Vero, and the 293 Tet-On (purchased from Clontech) were adapted to and maintained in Dulbecco’s Modified Eagle’s medium supplemented with 10% Cosmic calf serum (HyClone). Transfection of cells with cDNAs was carried out by standard CaPO4 procedures (16).

Plasmids—GAL4-IRF9 and GAL4-S1C were constructed by insertion
of PCR-amplified ORF into the GAL4-DBD plasmid pSG424 (17). Inserts for the IRF9-S1C, IRF9-S2C, and IRF9-VP16 hybrid cDNA constructs were created by standard four-primer PCR technique (18). Briefly, the first step involves generation of two PCR fragments encompassing full-length IRF9 and the STAT2 104 C-terminal amino acids, the 38 C-terminal amino acids of STAT1, or the 80 C-terminal amino acids of the VP16 TAD (17). These products are then combined for use as templates for a second PCR reaction, with external 5' and 3' primers to create IRF9-S1C, IRF9-S2C, or IRF9-VP16 hybrid inserts with BamHI and NotI restriction sites. Primers used were as follows: T IRF9, 5'-CCCGGATCCTCGACGAGGAGGAGCGGACG-3'; S2-REV, 5'-GCGGCGCGGCGGCTGATAGAACTGGTCAAGGG-3'; TIRF9-S2C T, 5'-GGATGGATGATCCTCGAGGAGCCAGC-3'; IIRF9-S2C B, 5'-GGCATTGTGCTGCCTGATAGAACTGGTCAAGGG-3'; IIRF9-S1C B, 5'-GCGGCGCGGCGGCTGATAGAACTGGTCAAGGG-3'; IRF9-VP16 B, 5'-GGCGGCGGCGGCTGATAGAACTGGTCAAGGG-3'; IRF9-VP16 F, 5'-GGCGGCGGCGGCTGATAGAACTGGTCAAGGG-3'; GAL4-DBD T, 5'-GCGGCGCGGCGGCTGATAGAACTGGTCAAGGG-3'; GAL4-DBD B, 5'-GCGGCGCGGCGGCTGATAGAACTGGTCAAGGG-3'; GAL4-TAD T, 5'-GCGGCGCGGCGGCTGATAGAACTGGTCAAGGG-3'; GAL4-TAD B, 5'-GCGGCGCGGCGGCTGATAGAACTGGTCAAGGG-3' (Fig. 1A). These IRF9-TAD hybrid inserts were subcloned into the tetracycline-regulated plasmid pBI (Clontech). All constructs were verified by DNA sequencing.

**Reporter Gene Assays**—For detection of GAL4 constructs in a reporter gene assay, 293T, U3A, and U6A cells were transiently transfected with either vector alone or GAL4-IRF9 fusion constructs, along with a reporter gene containing five GAL4 upstream activation sequence elements fused to the luciferase gene (17). For detection of IFN-activated STATs and thereby unresponsive to IFN (24, 25). In the absence of STAT1 and STAT2 proteins, the activity of GAL4-IRF9 fusion constructs was tested in sister cell lines deficient in one of the STAT proteins. The activity of IRF9 localized to the STAT protein interaction region, the role of STAT1 and STAT2 proteins in activation by GAL4-IRF9 was tested in sister cell lines deficient in one of the IFN-activated STATs and thereby unresponsive to IFN (24, 25). In the absence of STAT1 (U3A cells), GAL4-IRF9 has a low constitutive level of transcriptional activity, as observed in the STAT-intact cells, that does not change upon IFNγ stimulation (Fig. 1C). In contrast, in the absence of STAT2 (U6A cells), no basal activity was observed, but IFNγ stimulation activated the transcriptional activity of IRF9 and IFN with simian virus 5 (SV5, W3A strain), type II human parainfluenza virus (HPIV2; Greer strain), and VSV infection were performed as follows: 293T cells carrying the Tet-induced IRF9-S2C transgene were treated with Dox or IFN for 24 h. Cells were then washed with serum-free medium, infected with virus at a multiplicity of 1.0 and/or 0.1 pfu/cell for 2 h, washed with serum-free medium, and cultured for an additional 48 h (18 h for VSV) in the presence or absence of Dox or IFN in Dulbecco's modified Eagle's medium with 2% serum. For SV5 antiviral assays performed in the presence of IFN neutralizing antibodies, cells were pre-treated with Dox in the presence or absence of 400 neutralizing units of anti-IFNα and anti-IFNβ antibodies (PBL Biomedical Laboratories), a concentration sufficient to prevent the antiviral activity of exogenous IFN (data not shown). Cells were infected as above and then cultured for 48 h in the continued presence of either Dox or IFN and the anti-IFN antibodies as indicated. Supernatants were then titered in plaque assays using simian CV-1 cells with an overlay containing 0.5% agar with Dulbecco's modified Eagle's medium and 10 mM HEPES, pH 7.2. The monolayer was fixed in 3.7% formaldehyde and stained with 0.1% crystal violet (Sigma) dissolved in 20% EOH. Anti-viral assay using herpes simplex virus (HSV-1, gift from John Blahos) was performed similarly, except cells were infected at a multiplicity of 5 pfu/cell. Supernatants were harvested from infected Dox-inducible IRF9-S2C cell lines 24 h after infection and titered on Vero cells. No antiviral effects or virus replication inhibition were observed in Dox-treated control cells or IRF9 cells.

### RESULTS

**Transcriptional Activity of IRF9**—A heterologous DNA binding domain fusion system was used to test the intrinsic transcriptional capacity of IRF9. Hybrid cDNA constructs were designed that fuse the yeast GAL4-DBD to IRF9, and the resulting fusion proteins were assayed for the ability to activate transcription of a GAL4-responsive luciferase reporter gene (17, 21). No reporter gene activity was observed when the GAL4-DBD alone was expressed, but fusion of the full-length IRF9 to GAL4 resulted in a fusion protein that mediated a 5–10-fold greater transcription than GAL4 alone (Fig. 1A). As a positive control, the activity of GAL4-S1C, a fusion with the well characterized 38-amino acid TAD of STAT1 (21–23), was assayed in parallel. GAL4-S1C activated transcription over 100-fold in this system, indicating that the intrinsic activity of IRF9 was relatively weak (Fig. 1A). To determine the specific IRF9 amino acids responsible for this modest transcriptional activity, smaller GAL4-IRF9 fusions were engineered and expressed (Fig. 1B). The N-terminal portion that encompasses the DNA binding and nuclear localization domain of IRF9 (amino acids 1–200) was transcriptionally inactive as a GAL4 fusion, and the central region of the molecule (amino acids 100–300) produced only a minor stimulation of the reporter gene above basal levels. The C-terminal IRF9 domain (amino acids 200–393) was the only region to exhibit significant transcriptional activity, giving rise to reporter gene activity that was even greater than the full-length IRF9 fusion (Fig. 1B). These data indicate that the IRF9 transcriptional activity localizes to its C-terminal half, previously determined to be the STAT1 and STAT2 binding region (10).

**Role of STAT1 and STAT2 in IRF9 Transcriptional Activity**—A current model of ISGF3 signaling assumes that IRF9 is activated by IFN-activated STATs and thereby unresponsive to IFN (24, 25). In the absence of STAT1 (U3A cells), GAL4-IRF9 has a low constitutive level of transcriptional activity, as observed in the STAT-intact cells, that does not change upon IFNγ stimulation (Fig. 1C). In contrast, in the absence of STAT2 (U6A cells), no basal activity was observed, but IFNγ stimulation activated the transcriptional activity of IRF9 and IFN with simian virus 5 (SV5, W3A strain), type II human parainfluenza virus (HPIV2; Greer strain), and VSV infection were performed as follows: 293T cells carrying the Tet-induced IRF9-S2C transgene were treated with Dox or IFN for 24 h. Cells were then washed with serum-free medium, infected with virus at a multiplicity of 1.0 and/or 0.1 pfu/cell for 2 h, washed with serum-free medium, and cultured for an additional 48 h (18 h for VSV) in the presence or absence of Dox or IFN in Dulbecco's modified Eagle's medium with 2% serum. For SV5 antiviral assays performed in the presence of IFN neutralizing antibodies, cells were pre-treated with Dox in the presence or absence of 400 neutralizing units of anti-IFNα and anti-IFNβ antibodies (PBL Biomedical Laboratories), a concentration sufficient to prevent the antiviral activity of exogenous IFN (data not shown). Cells were infected as above and then cultured for 48 h in the continued presence of either Dox or IFN and the anti-IFN antibodies as indicated. Supernatants were then titered in plaque assays using simian CV-1 cells with an overlay containing 0.5% agar with Dulbecco's modified Eagle's medium and 10 mM HEPES, pH 7.2. The monolayer was fixed in 3.7% formaldehyde and stained with 0.1% crystal violet (Sigma) dissolved in 20% EOH. Anti-viral assay using herpes simplex virus (HSV-1, gift from John Blahos) was performed similarly, except cells were infected at a multiplicity of 5 pfu/cell. Supernatants were harvested from infected Dox-inducible IRF9-S2C cell lines 24 h after infection and titered on Vero cells. No antiviral effects or virus replication inhibition were observed in Dox-treated control cells or IRF9 cells.
result of direct stimulatory effects, as no additional IFNγ induction was observed in the absence of STAT1 (U3A cells). As STAT1 dimers form in response to IFNγ in STAT2-deficient cells, this result suggests that STAT1 is responsible for the activity of GAL4-IRF9 in the IFNγ-treated cells. To test this hypothesis, the reporter gene assay was carried out in the absence of both STAT1 and STAT2 by using STAT2-deficient cells (U6A) and dominant negative STAT1 mutants to block the activity of the remaining endogenous STAT1 (Fig. 1D). Expression in STAT2-deficient U6A cells of dominant negative forms of STAT1 that either fail to activate and dimerize (STAT1 Y701F) or lack transcriptional activity (STAT1β) results in a situation where neither STAT1 nor STAT2 is functional. GAL4-IRF9 failed to activate the reporter gene in this virtual null cellular environment, in support of the conclusion that association of GAL4-IRF9 with endogenous STAT2 is required for basal transcription, but association with STAT1 is responsible for the observed IFNγ-inducible transcription. Together, the results with the GAL4-IRF9 system indicate that IRF9 lacks intrinsic transcriptional activity, providing experimental evidence that supports a model where IRF9 functions to integrate STAT1 or STAT2 transcriptional activation signals to the ISRE element on target promoters.

**IRF9-TAD Fusions Activate ISRE Transcription**—The primary transcription activation function of ISGF3 has been attributed to contributions from the STATs, especially the STAT2 C terminus. Deletions from this region of STAT2 abrogate the transcriptional activity of ISGF3 (26), and fusion of the STAT2 C terminus to STAT1 or GAL4-DBD is sufficient to generate fusion proteins that activate transcription (23, 27, 28). The constitutive nuclear import of IRF9, coupled with its lack of transcriptional activity in the absence of STATs, suggests that one role of IRF9 might be to target STAT activation domains to the IFN-responsive promoter, ensuring rapid and specific activation. To test this concept, the ability of IRF9 to target STAT transcriptional activation domains to ISGF3-responsive promoters was examined. A hybrid cDNA expression vector was created that expressed IRF9 fused to the ISGF3 transcriptional activation domain that resides in the STAT2 C terminus. The C-terminal 104 amino acids of STAT2 were fused to the IRF9 ORF to create IRF9-S2C (Fig. 2A). The hybrid protein was expressed in 293T cells and subjected to ISRE-dependent luciferase assays. Expression of IRF9 protein alone resulted in weak reporter gene activity, consistent with the results observed in the GAL4-IRF9 system (Fig. 1). The IRF9-S2C fusion, however, was found to strongly activate ISRE-dependent reporter gene transcription in the absence of IFN stimulation, resulting in ~50-fold stimulation of reporter gene activity (Fig. 2A, right panel). The high level of activity of IRF9-S2C was not altered by stimulation with IFNα, but a small increase was observed following IFNγ stimulation, consistent with contributions by endogenous IFNγ-activated STAT1 dimers interacting with IRF9 to heighten transcriptional responses (29, 30). These results indicate that recruitment of the STAT2 TAD to the ISRE DNA element is sufficient for transcriptional activity.

To determine whether the activity of the IRF9-S2C chimeric activator requires endogenous STAT1 or STAT2, similar ISRE-dependent luciferase reporter gene assays were performed in STAT1-deficient U3A cells, STAT2-deficient U6A cells, or parental 2F1GH cells (Fig. 2B). As a control, a second hybrid protein fusing the IRF9 ORF to the STAT1α C-terminal 38 amino acids was also constructed (to create IRF9-S1C) and included in these assays. Although the cell-specific transfection efficiency and protein accumulation levels varied between the tested cell lines, resulting in distinct absolute activation values, all of the IRF9 expression vectors behaved similarly, re-

---

**Figure 1. Analysis of IRF9 transcriptional capacity.** A, transcriptional activity was determined for GAL4-DBD (V), GAL4-IRF9 (IRF9), and GAL4-S1C (S1C) in reporter gene assays in 293T cells. B, IRF9 residues 1–200, 100–300, and 200–393 fused to GAL4 were examined for reporter gene activation. C, control GAL4-DBD and GAL4-IRF9 were assayed in U3A cells or U6A cells, either without treatment (UNT) or following 6 h of treatment with 5 ng/ml IFNγ. D, GAL4-IRF9 is transcriptionally inert in the absence of STAT1 and STAT2. Reporter gene assays were carried out in U6A cells with co-expression of vector control, wild-type STAT1, or two dominant negative forms of STAT1 (STAT1β and STAT1 Y701F). Luciferase values were read from untreated (−) cells or cells treated with IFNγ or IFNα (1000 units/ml) for 6 h.

---

reporter gene (Fig. 1C). This result suggests that endogenous STAT2 may be responsible for the basal activity of GAL4-IRF9, even in the absence of STAT1, and that a STAT2-GAL4-IRF9 complex can shuttle between the cytoplasm and the nucleus. The action of IFNγ on IRF9-dependent transcription is not the
was observed for expression of IRF9 that is consistent with earlier conclusions regarding the transcriptional activity of IRF9 and may represent contributions from endogenous STAT proteins. These data indicate that the IRF9-STAT fusion is active on chromatinized endogenous loci that are normally targets of ISGF3. Based on these and previous studies (26–28), we conclude that a primary and essential TAD for ISGF3 resides within the C-terminal 104 amino acids of STAT2. Furthermore, targeting this minimal STAT2 domain to endogenous promoters is sufficient to mediate ISG transcription.

The finding that IRF9-S2C exhibited intrinsic ISGF3-like transcriptional activity but IRF9-S1C did not (Fig. 2B) was unexpected in view of the established role for the STAT1 C terminus in IFNγ-dependent transcription (reviewed in Ref. 12). To determine whether the STAT2 TAD is unique in its ability to activate IRF9, a distinct heterologous TAD was tested in this system. The well characterized acidic activation domain of herpesvirus VP16 protein was fused to IRF9 to create IRF9-VP16. Expression of this construct in 293T cells resulted in strong ISRE-directed reporter gene activity (Fig. 2D). The activity of IRF9-VP16 was also tested for ability to induce endogenous ISG transcription. Consistent with the reporter gene assays, expression of IRF9-VP16 was able to induce transcription of both ISG15 and ISG54 (Fig. 2D).

**IRF9-S2C Recapitulates IFN Biological Responses**—By the tested criteria, IRF9-TAD fusion proteins duplicate molecular aspects of ISGF3 function. To test the biological activity of this hybrid factor in IFN response assays, vectors for inducible expression of the IRF9 fusions were constructed. Tetracycline-regulated vectors with IRF9 and IRF9-S2C open reading frames were used to create mammalian cell lines that inducibly express the transgene following stimulation with the tetracycline analog, Dox. Several independent clones were isolated in 293 Tet-On cells that demonstrated similar levels of Dox-induced transgene expression (Fig. 3A, right panel). The inducible cell lines were tested for resistance to virus-induced cytopathic effects and the ability to suppress virus replication. Cells were assayed for resistance to VSV by standard methodology (19, 20). The cell lines were susceptible to VSV-induced CPE, causing death of infected cell monolayers, and no nonspecific antiviral effects were observed in Dox-treated control cells or IRF9 cells (Fig. 3A, left panels). Induction of transgene expression with Dox did not alter the susceptibility of the cells to VSV-induced CPE when IRF9 was the transgene, but cells expressing IRF9-S2C were greatly protected from viral CPE (Fig. 3A).

As VSV is well known to be susceptible to the IFN-induced antiviral state, the ability of the IRF9-S2C transgene to block the replication of an IFN-resistant virus was also tested. SV5, a member of the *Rubulavirus* genus of the Paramyxoviridae, evades the IFN signaling response by targeting STAT1 for ubiquitylation and proteasomal degradation (32–35). Significantly, SV5 replication is also inhibited by IRF9-S2C induction (see Fig. 3B and Table I). The antiviral response to RNA virus infection relies in part on autocrine and paracrine signaling through newly synthesized IFN. Virus infection can activate the synthesis and secretion of IFNβ and IFNα, which, in turn, can activate ISGF3 signaling in the infected cell, as well as adjacent cells, to amplify antiviral responses (36, 37). To determine whether the mechanism of protection provided by the IRF9-S2C expression relies on IFN production, antiviral assays for SV5 were performed in the continued presence of IFN-neutralizing antibodies (Fig. 3B). Pretreatment with Dox to express the IRF9-S2C transgene for 24 h before infection pro-
duced a cellular antiviral state, and addition of IFN-neutralizing antibodies beginning at the time of Dox treatment did not significantly alter the IRF9-S2C-induced antiviral state. These results indicate that the antiviral effects are because of IRF9-S2C transcriptional activity rather than autocrine/paracrine signaling downstream of induced IFN synthesis.

To directly and quantitatively compare the activity of IRF9-S2C to exogenous IFN treatment, assays were carried out in which Dox and IFN treatments were tested for antiviral activity. The induction of ISRE-luciferase activity was used to calibrate Dox stimulation of IRF9-S2C with the ISGF3 transcriptional response to several IFN doses (Table I). Cells were transfected with the reporter gene and treated with 10 µg/ml Dox or 10, 100, or 1000 units/ml IFN prior to reporter gene assays. Results indicate that expression of IRF9-S2C induced by Dox carries a transcriptional capacity resulting in 87% of the reporter activity induced by stimulation of cells with 1000 units/ml IFN (Table I). As 100 units/ml of IFN only induced 56% of the luciferase activity, the capacity of IRF9-S2C in this particular inducible system is determined to lie between the ISGF3 activity induced by 100 and 1000 units/ml IFN. The antiviral responses induced by these same treatments were evaluated for the test viruses, VSV, SV5, and HPIV2. For VSV, 6.0 × 10^6 pfu/ml were recovered from control, unstimulated cells, but only 5.0 × 10^5 pfu/ml were collected following Dox induction of IRF9-S2C (Table I). For SV5, 2.0 × 10^7 pfu/ml were recovered from control cells, but only 1.1 × 10^5 pfu/ml were recovered from Dox pre-treated cells. Similarly, HPIV2 titers decreased from 7.3 × 10^5 to 9.2 × 10^2 pfu/ml after Dox treatment. For all the viruses tested, Dox treatment resulted in a replication inhibition level similar to that obtained following treatment with between 100 and 1000 units/ml IFN. Thus, both transcriptional response assays and biological antiviral response assays indicate that in this expression system, IRF9-S2C can induce ISGF3-like responses similar to native IFN treatment and can afford antiviral protection against several RNA viruses.

The pleiotropic IFN response is effective against a broad range of viruses. To expand our observations beyond RNA viruses, the ability of IRF9-S2C to block a DNA virus, the herpesvirus, HSV-1, was also tested. Control and Dox-treated IRF9-S2C Tet-On cells were infected with HSV-1. Virus-containing supernatants were harvested an additional 24 h later and titered by plaque assay in Vero cells. A dramatic inhibition of HSV-1 replication was observed in cells upon IRF9-S2C hybrid protein expression, reducing recovered viral titers from 2 × 10^8 to 2.6 × 10^6 pfu/ml (Table I). These results indicate that augmenting the cellular IFN response by IRF9-S2C expression can inhibit the replication of diverse virus species. Because HSV-1 has several strategies for evading IFN responses (38–42), the ability to inhibit its replication is a powerful demonstration that the IRF9-TAD fusion strategy may overcome intrinsic anti-IFN strategies encoded by viruses.

**DISCUSSION**

We have investigated the transcriptional capacity of the IRF9 component of the IFN-activated ISGF3 transcription factor. Results from studying GAL4-IRF9 fusion proteins demonstrate that IRF9 lacks the intrinsic ability to induce transcriptional responses but can participate in both basal and IFN-activated transcription through its C-terminal domain through association with either STAT1 or STAT2. Basal transcription by the GAL4-IRF9 fusion is dependent on its ability to associate with cellular STAT2 protein, reflecting the strong association between STAT2 and IRF9 demonstrated in vitro and in vivo (10, 11). In cells lacking STAT2, GAL4-IRF9 no longer induces basal activity but can still activate transcription in response to IFNγ stimulation, by association with IFNγ-activated STAT1 dimers. Independent STAT1-IRF9 contacts are in general agreement with earlier studies of STAT1-IRF9 interaction carried out in vitro and in vivo (10, 29, 30). These results collectively support a model wherein IRF9 serves as a DNA binding adapter for IFN-activated ISGF3 that must re-

**Table I**

| Virus   | Titer (pfu/ml) | ISRE-LUC<sup>a</sup> | Control | Dox<sup>b</sup> | 1000 units/ml IFN<sup>c</sup> | 100 units/ml IFN<sup>c</sup> | 10 units/ml IFN<sup>c</sup> |
|---------|----------------|----------------------|---------|---------------|-----------------------------|-----------------------------|-----------------------------|
| SV5     | 2.0 × 10<sup>7</sup> | 1.1 × 10<sup>5</sup> | 1.8 × 10<sup>4</sup> | 1.0 × 10<sup>6</sup> | 3.0 × 10<sup>6</sup> |
| HPIV2   | 7.3 × 10<sup>5</sup> | 9.2 × 10<sup>3</sup> | 5.7 × 10<sup>2</sup> | 7.0 × 10<sup>3</sup> | 1.6 × 10<sup>4</sup> |
| VSV     | 6.0 × 10<sup>7</sup> | 5.0 × 10<sup>6</sup> | 3.5 × 10<sup>5</sup> | 5.0 × 10<sup>6</sup> | 3.0 × 10<sup>7</sup> |
| HSV-1   | 2.0 × 10<sup>5</sup> | 2.6 × 10<sup>5</sup> |           |               |                             |

<sup>a</sup>Tet-regulated IRF9-S2C cell lines were treated as indicated and then infected with SV5 or HPIV2 for 48 h, HSV-1 for 24 h, or VSV for 18 h prior to harvest and titration of supernatants. Virus titers are expressed as pfu/ml.

<sup>b</sup>Cells were pretreated with Dox (10 µg/ml) for 24 h prior to infection.

<sup>c</sup>Cells were transfected with the ISRE-reported gene and treated as indicated. Data indicate percent of luciferase activity (normalized to co-expressed β-galactosidase), with maximal response obtained with 1000 units/ml IFN as 100%.
TAD constructs (IRF9-VP16 dependent transcription, and the observed hierarchy for IRF9-TAD) can be used interchangeably for ISGF3 signaling (24, 25). STAT1 and STAT2. The unanticipated inactivity of the STAT1 tent responses both from transfected reporter genes and from functionality.


cruit the STAT protein transcriptional activation domains for

structs and good advice were provided by Aseem Ansari (University of

Sinai Summer Undergraduate Research Program. VP16 TAD con-

splice variant STAT1

individual antiviral genes.

mine the specificity of these IRF9-TAD fusions for activating

IFN stimulation with transgene expression will better deter-

on a case-by-case basis, but given the observed general imita-

efficacy of IRF9-S2C against individual viruses will be needed

can also achieve high levels of ISRE-directed transcription in

RNA and DNA viruses that are known to be IFN-resistant and

evolved by the viruses. The results presented here demon-

2 T. A. Kraus and C. M. Horvath, unpublished observations.

Acknowledgments—We are grateful to many colleagues for providing reagents and expertise for these studies. Initial experiments with IRF9 fusions were conducted in part by Adam L. Stone as part of the Mount Sinai Summer Undergraduate Research Program. VP16 TAD constructs and good advice were provided by Aseem Ansari (University of Wisconsin), and GAL4-Stat1C construct was provided by Jillian Zhang (Weill Cornell Medical School). We thank Griffith Parks (Wake Forest University) for providing HPV12 and SV5, John Blaho (Mount Sinai) for providing HSV-1, and George Stark (Lerner Institute) for 2TGH and daughter cell lines. We also thank members of the Horvath laboratory, Marcus Kretzschmar, Lloyd Mayer, and Tom Moran, for helpful discussions and reading this manuscript.

REFERENCES

1. Brassard, D. L., Grace, M. J., and Bordens, R. W. (2002) J. Leukocyte Biol. 71, 565–581

2. Samuel, C. E. (2001) Clin. Microbiol. Rev. 14, 778–809

3. Mendiratta, S. K., Quesada, A., Matar, M., Thull, N. M., Bishop, J. S., Nordstrom, J. L., and Pericle, P. (2000) Hum. Gene Ther. 11, 1851–1862

4. Qin, X. Q., Tao, N., Dergraw, A., Moy, P., Fawell, S., Davis, A., Wilson, J. M., and Horvath, J. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 14411–14416

5. Horton, H. M., Anderson, D., Hernandez, P., Barnhart, K. M., Norman, J. A., and Parker, S. E. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1553–1558

6. Ferrantini, M., and Belardelli, F. (2000) Semin. Cancer Biol. 10, 145–157

7. Coleman, M., Muller, S., Quesada, A., Mendiratta, S. K., Wang, J., Thull, N. M., Bishop, J., Matar, M., Jester, J., and Pericle, P. (1998) Hum. Gene Ther. 9, 2225–2230

8. Albin, A., Marchisone, C., Del Grosso, F., Benelli, R., Masiello, L., Tacchetti, C., Bono, M., Ferrantini, M., Rozera, C., Truni, M., Belardelli, F., Santi, L., and Noonan, D. M. (2000) Am. J. Pathol. 156, 1381–1393

9. Lee, D. E., Kessler, D. S., Fine, R. I., and Darnell, J. E., Jr. (1989) Genes Dev. 3, 1362–1372

10. Horvath, C. M., Stark, G. R., Kerr, I. M., and Darnell, J. E. (1996) Mol. Cell. Biol. 16, 6857–6864

11. Martinez-Mozgawa, M., Gutch, M. J., French, D. L., and Reich, N. C. (1997) J. Biol. Chem. 272, 20070–20076

12. Horvath, C. M. (2000) Trends Biochem. Sci. 25, 496–502

13. Majumder, S., Zhou, L. Z., Chattevedi, P., Babcock, G., Aras, S., and Ransohoff, R. M. (1998) J. Immunol. 161, 4736–4744

14. Lau, J. F., Parisien, J.-P., and Horvath, C. M. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 7273–7283

15. Luker, K. E., Pica, C. M., Schreiber, R. D., and Pinnwala-Worms, D. (2001) Cancer Res. 61, 6540–6547

16. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Strahl, K. (1994) Current Protocols in Molecular Biology, John Wiley & Sons, Inc., New York

17. Sadowski, I., and Pleshne, M. (1989) Nucleic Acids Res. 17, 7539

18. Horvath, C. M., Wen, Z., and Darnell, J. E. (1995) Genes Dev. 9, 984–994

19. Friedman, R. M. (1981) J. Biol. Chem. 256, 9527–9534

20. Friedman, R. M. (1981) J. Biol. Chem. 256, 9527–9534

21. Zhang, J. J., Zhao, Y., Chait, B. T., Lathem, W. W., Ritzi, M., Knippers, R., and Darnell, J. E., Jr. (1998) EMBO J. 17, 6963–6971

22. Ouchi, T., Lee, S. W., Ouchi, M., Aaronsen, S. A., and Horvath, C. M. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 5208–5213

23. Shen, Y., and Darnell, J. E. (2001) J. Virol. 75, 2627–2633

24. Muller, M., Laxton, C., Briscoe, J., Schindler, C., Impota, T., Darnell, J. E., Jr., Stark, G. R., and Kerr, I. M. (1993) EMBO J. 12, 4221–4228

25. Leung, S., Qureshi, S. A., Kerr, I. M., and Darnell, J. E., Jr., and Stark, G. R. (1995) Mol. Cell. Biol. 15, 1312–1317

26. Qureshi, S. A., Leung, S., Kerr, I. M., Stark, G. R., and Darnell, J. E., Jr. (1996) Mol. Cell. Biol. 16, 288–295

27. Park, C., Lecomte, M. J., and Schindler, C. (1999) Nucleic Acids Res. 27, 4191–4199

28. Paulson, M., Paharody, S., Pan, L., Guadagno, S., Mui, A. L., and Levy, D. E. (1999) J. Biol. Chem. 274, 25343–25349

29. Bluyssen, H. A., and Levy, D. E. (1997) Proc. Natl. Acad. Sci. U. S. A. 92, 5645–5649

30. Bluyssen, H. A., and Levy, D. E. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 5928–5933

31. Kirchoff, S., Schaper, F., and Hauser, H. (1995) Nucleic Acids Res. 23, 2981–2989

32. Parisien, J.-P., Lau, J. F., and Horvath, C. M. (2002) J. Virol. 76, 4190–4198

33. Ulane, C. M., and Horvath, C. M. (2002) Virology 304, 160–166

34. Didcock, L., Young, D. F., Goodbourn, S., and Randall, R. E. (1999) J. Virol. 73, 9928–9932

35. Marie, I., Durbin, J. E., and Levy, D. E. (1998) EMBO J. 17, 6660–6669

36. Levy, D. E. (2002) J. Exp. Med. 195, F15–18

37. Cheng, G., Brett, M. E., and He, B. (2001) Virology 290, 115–120

38. Cassady, K. A., Gross, M., and Roizman, B. (1999) J. Virol. 72, 8620–8626

39. He, B., Gross, M., and Roizman, B. (1999) J. Virol. 72, 8620–8626

40. Mossman, K. L., and Smiley, J. R. (2002) J. Virol. 76, 1995–1998

41. J. Virol. 74, 11215–11221
A Hybrid IRF9-STAT2 Protein Recapitulates Interferon-stimulated Gene Expression and Antiviral Response
Thomas A. Kraus, Joe F. Lau, Jean-Patrick Parisien and Curt M. Horvath

J. Biol. Chem. 2003, 278:13033-13038.
doi: 10.1074/jbc.M212972200 originally published online February 5, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M212972200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 40 references, 28 of which can be accessed free at
http://www.jbc.org/content/278/15/13033.full.html#ref-list-1