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Regulation of interferon-α/β-stimulated gene expression through the gamma-activated transcriptional element

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Abstract

Interferons (IFNs) stimulate gene expression to mediate their biological actions. A multimeric transcription factor consisting of STAT1, STAT2 and p48, a DNA binding protein, regulates IFN-α/β stimulated gene expression. Since the cellular level of p48 is also increased by pre-treatment of cells with IFN-γ, it is also known as ISGF3γ. To understand how IFN-γ regulates the expression of the p48 gene, we have previously isolated and characterized the promoter of murine p48 gene and identified a novel γ-IFN activated transcriptional element (GATE). In this study using several mutant constructs of p48 promoter we have determined that the same element responds to IFN-α/β treatment. Relatively high doses of IFN-α/β compared to IFN-γ are required for the induction of p48 promoter. This ability of IFN-α/β to regulate GATE dependent gene expression is linked to the activation of a factor induced by IFN-α. However, IFN-γ induces the binding of two γ-IFN inducible factors (GIFs) to GATE. The IFN-α inducible GATE binding factor is not recognized by specific antibodies raised against the known IFN-regulated factors. It is likely IFN-γ is a stronger inducer of this gene because it activates two GIFs. GATE-like elements present in hither to undefined IFN-stimulated genes may control IFN-responses in a unique manner.

Keywords: Interferon-α/β; Stimulated gene expression; Transcriptional elements

1. Introduction

Interferons regulate antiviral, antitumor and immune functions of the host (Kalvakolanu and Borden, 1997). Type I IFNs (α, β) bind to a specific cell surface receptor and stimulate gene expression using JAK–STAT pathway (Sen and

Abbreviations: GATE, γ-IFN activated transcriptional element; GIF, γ-IFN inducible factor; IFN, Interferon; ISRE, IFN stimulated response element; ISGF3, IFN-stimulated gene factor 3; JAK, Janus tyrosine kinase; STAT, Signal transducing activator of transcription.

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Ransohoff, 1997; Stark et al., 1998). Ligand activated IFN-α/β receptor recruits Janus tyrosine kinases (JAK), JAK1 and TYK2, which in turn induce the tyrosine phosphorylation of signal transducing activators of transcription (STAT) protein-1 and protein-2. Activated STAT1 and STAT2 migrate to the nucleus and associate with a 48-kDa DNA binding protein (p48 or ISGF3γ) and form ISGF3 (IFN-stimulated gene factor 3), to form the active transcription factor (Sen and Ransohoff, 1997; Stark et al., 1998). ISGF3 binds to the IFN-stimulated response element (ISRE) of various cellular genes and induces transcription (Dale et al., 1989; Levy et al., 1990). Type II IFN (γ) binds to a distinct receptor, which recruits JAK1 and JAK2 to induce the tyrosine phosphorylation of STAT1 alone (Sen and Ransohoff, 1997; Stark et al., 1998). STAT1 dimer translocates to the nucleus, and binds to γ-IFN activated site (GAS). GAS-like palindromic elements are present in several gene promotes including those encoding growth-regulatory molecules IRF-1 (IFN gene regulatory factor-1) and ICSBP (IFN consensus sequence binding protein; Kanno et al., 1993; Pine et al., 1994). The JAK–STAT pathway is rapidly activated and deactivated within an hour after the initial stimulus in many cell types, probably due to the activation of phosphatases (David et al., 1993).

The DNA binding subunit of ISGF3, p48, is regulated in a unique manner. In several variant cells that do not respond to IFN-α/β, IFN-γ pre-treatment enhances the formation of ISGF3 (Bandyopadhyay et al., 1990; Levy et al., 1990). Consequently, the transcriptional activation of genes involved in antiviral and antitumor pathways (Lewis et al., 1989) are robustly induced by IFN-α/β in IFN-γ pre-treated cells. However, the p48 gene induction by IFN-γ is much slower (12–18 h) than many IFN-stimulated genes (Bandyopadhyay et al., 1990; Levy et al., 1990) and occurs while the JAK–STAT pathway is turned off. Furthermore, certain viruses down regulate cellular p48 levels to inhibit the action of IFNs (Ackrill et al., 1991; Gutch and Reich, 1991; Kalvakolanu et al., 1991; Leonard and Sen, 1997). Therefore, it is likely that the p48 promoter is regulated in a unique manner. Indeed, our recent studies identified a novel γ-IFN activated transcriptional element (GATE) in the promoter of p48 (Weihua et al., 1997). This element has no significant relationship to the known IFN responsive elements and binds to factors called γ-IFN inducible factors (GIF). Although p48 is induced mainly by IFN-γ, it is also inducible by IFN-α/β in some cell types (Muller et al., 1993). In this study, we have examined whether IFN-α/β induces gene expression from GATE. We have found that IFN-α/β induces the GATE-dependent gene expression, although more weakly than IFN-γ. High doses of IFN-α or IFN-β are required to mediate this effect. IFN-α induced GATE-binding transcription factors are distinct from those characterized to date.

2. Materials and methods

2.0.1. Cell culture and reagents

Murine macrophage RAW (RAW264.7) cell line was grown in RPMI-1640 supplemented with 10% fetal bovine serum (Weihua et al., 1997). Rabbit antibodies specific for STAT1 (p84/p91), and STAT2 were provided by Chris Schindler, Columbia University (Schindler et al., 1992). Rabbit antibody raised against ISGF3γ (p48) was provided by David Levy, New York University. Rabbit antibodies against IRF-1, IRF-2, and ICSBP were gifts from Keiko Ozato, National Institute of Health (Kanno et al., 1993). Rabbit anti-IRF-3 antibody was a gift from Paula Pitha-Rowe, Johns Hopkins University School of Medicine (Au et al., 1995). Recombinant murine (Boehringer Mannheim) IFN-γ, IFN-α BDBB and murine IFN-β (Toray Industries, Tokyo, Japan) were used for induction. All other reagents were purchased from Sigma Chemical Company.

2.0.2. Gene expression analyses

Northern blot, transfection and electrophoretic mobility shift analyses (EMSA) were performed as described elsewhere (Ausubel et al., 1994; Weihua et al., 1997). The characterization of the murine p48 gene promoter was described previously. Clone A6 contained sequences -1045 to $+42$ bp relative to transcription start site of p48

placed upstream of luciferase gene. Construct A8 was generated after restriction digestion of A6 and religation. These inserts were cloned in pGL3 basic vector (Promega) in which the expression of luciferase gene expression was controlled by the insert. All heterologous promoter-driven constructs contained the SV40 early promoter in pGL3-promoter vector. These vectors lacked all cryptic start sites and yielded superior luciferase activities (Promega). Deletion and point mutations were constructed using a polymerase chain reaction-based kit (Stratagene). The following oligonucleotides were used for EMSA, site directed mutagenesis, EMSA and/or reporter gene expression analyses. GATE: 5′ CCCGGAGAGAATTGAAACTTAGGG 3′; GATE-Mu: 5′ CCCGGAGAGAAT TACTCCTTAGGG 3′; MSE *pm*: 5′ AGACCACGGAGTTTC 3′; Short GATE 5′ GAGAGAA TTGAAACTT 3′; and ISRE: 5′ TAGTTTCACTTTCCC 3′. Mutated bases are underlined. All constructs were confirmed by DNA sequencing. These mutants were described in our previous publication (Weihua et al., 1997).

3. Results

3.1. Induction of p48 gene promoter by IFN-α/β

We first determined whether IFN-α induced the expression of p48 mRNA. Treatment of RAW cells with a low dose of IFN-α (150 U/ml) for 15 h did not induce the expression of p48 mRNA (Fig. 1, lane 2), as compared to equivalent dose of IFN-γ, which strongly induced it (lane 4). However, a high dose of IFN-α (1500 U/ml) caused a 3-fold increase (lane 3) in p48 mRNA expression, compared to untreated cells (lane1). All lanes had a comparable amount of β-actin mRNA, suggesting a specific effect of IFN-α on p48 mRNA.

To examine the induction more quantitatively, we transfected RAW cells with a reporter gene A6, which contained the wild type promoter of murine p48 gene (Fig. 2A). These cells were treated with low doses of IFN-α or IFN-β (150 U/ml). At this concentration, IFN-α and IFN-β did not induce the reporter gene (Fig. 2B, bars 2 and 3), although IFN-γ caused a significant increase in luciferase expression (Fig. 2C, bar 2). A higher dose of IFN-γ (1500 U/ml) increased gene expression further. A high dose of either IFN-α (1500 U/ml) or IFN-β (1500 U/ml) also induced luciferase gene expression in transfected cells. A 2.8- to 3-fold increase in luciferase expression occurred upon treatment with both IFNs (Fig. 2B, bars 5 and 6). These results show that IFN-α and IFN-β are capable of inducing p48 gene expression, albeit weakly as compared to IFN-γ.

We next examined whether IFN-α induced expression of p48 gene promoter was mediated through previously described GATE (Weihua et al., 1997) or other elements. A promoter construct A8, which contained a 351-bp upstream region of the p48 promoter but lacked the regions corresponding to GATE and the adjacent myc-stimulated element (MSE), was transfected into cells and treated with IFN-α and IFN-β (Fig. 3A). Luciferase activity was not induced after IFN-α or IFN-β treatment in cells transfected with A8 construct (Fig. 3B, bars 4–6). Construct A6 was induced normally under these conditions (Fig. 3B,

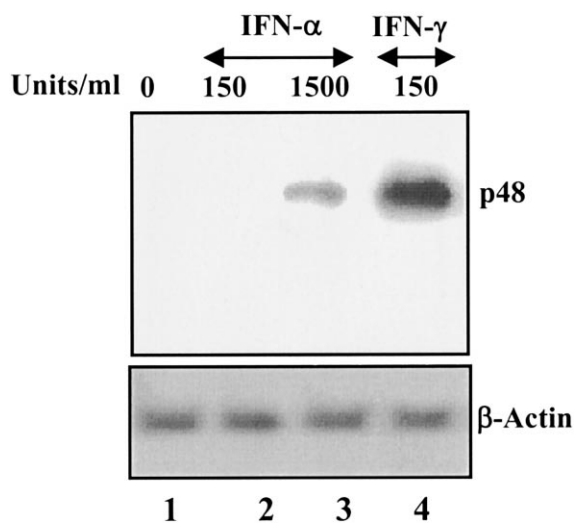

Fig. 1. Northern blot analysis of p48 gene expression in RAW cells (A). Poly A + RNA (4 μg) from IFN-treated cells (for 18 h) was hybridized with a ^{32}P-labeled human p48 cDNA (4). The same blot was probed with labeled human β-actin cDNA to ensure the presence of equal amounts of RNA in each lane. Blots were exposed for 24 and 6 h to detect p48 and β-actin mRNAs, respectively.

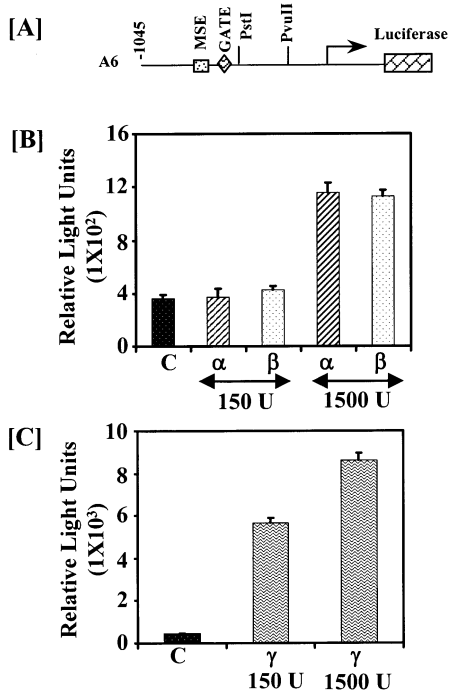

Fig. 2. Panel A shows the structure of the A6 construct used for transfection in panel B. Various restriction enzyme sites were indicated. The number of the nucleotide furthest upstream from the +1 site is indicated on the left. GATE, gamma IFN-activated transcriptional element; MSE, myc-stimulated element; arrow, transcription start site. Reporter plasmid (10 μg) was electroporated into cells and luciferase activity was measured using equal amounts (70 μg) of whole cell extracts prepared after treatment with indicated doses of IFNs. C, no treatment; α, IFN-α; β, IFN-β; and γ, IFN-γ. Each bar represents the mean ± SEM of triplicate measurements. Panel B shows the induction by IFN-α and IFN-β. Panel C represents the data obtained with IFN-γ treatment in the same experiment. Note the differences between the y-axes of panels B and C.

bars 1–3). To determine whether IFN-α response could be conferred to a heterologous promoter, a fragment derived from p48 promoter (−351 to −1045 bp) was cloned upstream of the SV40 early promoter in pGL3 promoter vector (Fig. 4A). The resultant construct was transfected into RAW cells and checked for IFN-α inducibility (Fig. 4B). Expression of luciferase gene was induced by IFN-α. Similar results were obtained with IFN-β

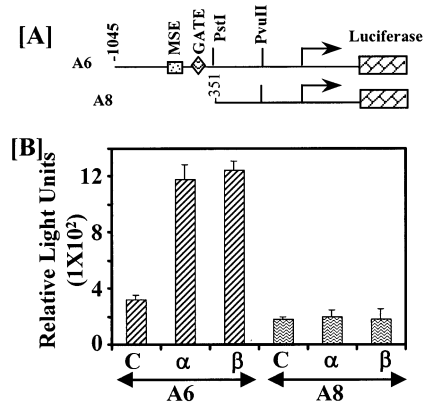

Fig. 3. IFN-α or IFN-β-induced expression from p48 promoter and its mutant A8. See (A) for the structures of these constructs. Transfection was performed as described in Fig. 2. Where indicated, cells were treated with 1500 U/ml of IFNs for 20 h prior to protein extraction and luciferase assay.

treatment (data not presented). These results confirmed that the IFN-α and IFN-β responsive element was present in this 694-bp fragment.

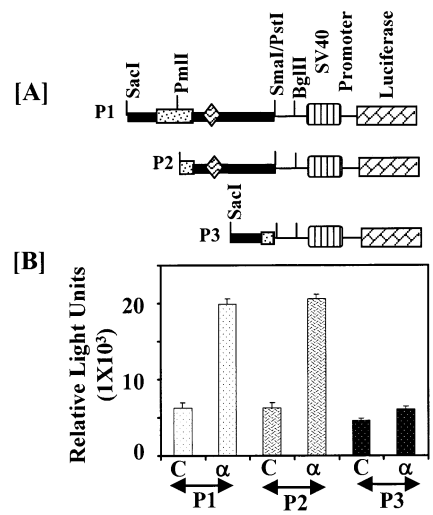

Fig. 4. Constructs with p48-derived promoter elements (indicated with thick lines) in the pGL3 promoter vector, in which SV40 early promoter drives the expression of luciferase gene. Transfection and luciferase assays were performed as in Fig. 2. Where indicated, cells were treated with 1500 U/ml of IFNs for 20 h prior to protein extraction and luciferase assay.

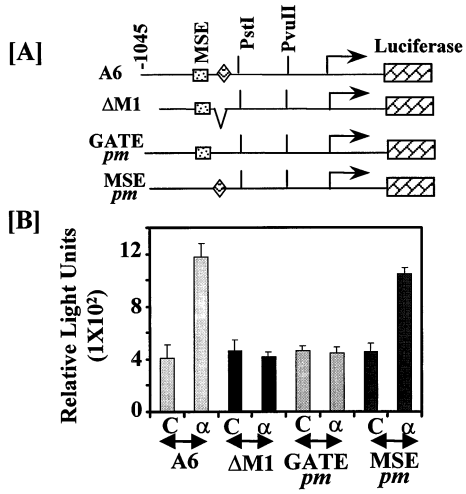

Fig. 5. GATE is required for IFN-α-induced expression of the p48 gene. Various mutant constructs were indicated (A). ΔM1, a deletion mutant that lacked GATE; *pm*, point mutant; Other notations are defined under Fig. 2. Transfection and IFN-treatment was performed as in Fig. 3.

3.2. Identification of GATE as the IFN-α response element

Because essentially similar results were obtained with both IFNs, representative data from IFN-α treated cells will be presented further. To map the location of IFN-α/β response element, we employed constructs derived from A6 in which either a deletion (ΔM1) or a site directed mutation (GATE *pm*) were introduced in the GATE (Fig. 5A). As shown in Fig. 3, IFN-α/β failed to induce the expression of GATE *pm* and ΔM1. Similarly, the promoter construct P3 did not respond to IFN-α at all. This construct was similar to P1 except that it lacked both GATE and MSE (Fig. 4B, bars 3 and 4). To rule out the possibility that MSE contributes to the IFN response, we mutated the MSE to generate construct MSE *pm* (Fig. 5A). Similarly, we also generated a mutant construct P2 in which MSE was deleted, leaving the GATE intact (Fig. 4A). Both these constructs were induced by IFN-α (Fig. 4B and Fig. 5B, lanes 5 and 6). These results indicated that only GATE was essential for IFN-α stimulated expression of p48.

Since mutations in GATE caused loss of IFN-α response, we next examined whether GATE alone responded to IFN-α. A synthetic GATE (24 bp) based on the sequence from p48 promoter, was cloned upstream of SV40 promoter (GATE-W). A control construct with a mutated GATE sequence (GATE-Mu) was also included in this experiment (Fig. 6A). A 3-fold increase in luciferase activity was observed in GATE-W transfected cells treated with IFN-α, compared to untreated cells (Fig. 6B, bars 1 and 2). However, under these conditions the GATE-Mu construct did not respond to IFN-α (Fig. 6B, lanes 3 and 4).

3.3. Binding of IFN-α induced transacting factors to GATE

Since IFN-α induced GATE dependent gene expression and GATE was a novel element, we examined whether GATE bound to specific factors in response to IFN-α. Electrophoretic mobility shift assay (EMSA) was employed with ^{32}P-labeled GATE as probe to determine the binding of transacting factors. Nuclear extracts from IFN-α treated cells were incubated with

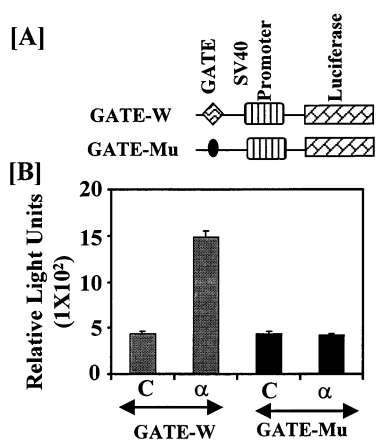

Fig. 6. Minimal GATE is sufficient for conferring IFN-α inducibility to a heterologous promoter. Constructs used in the experiment were indicated in panel A. A single copy of GATE was present in each of these constructs. GATE-W, wild type GATE; GATE-Mu, mutant GATE. The experiment was conducted as in Fig. 2. Where indicated, cells were treated with 1500 U/ml of IFNs for 20 h prior to protein extraction and luciferase assay.

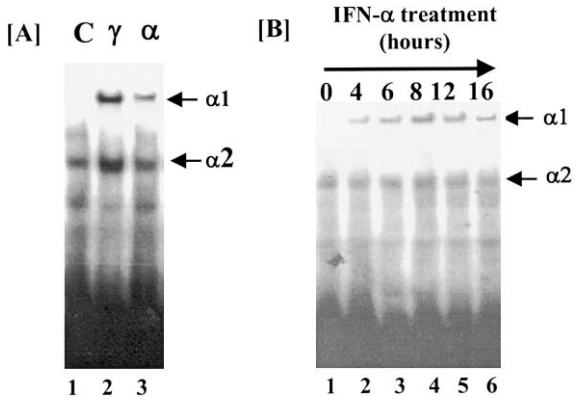

Fig. 7. IFN induced binding of transacting factors to GATE (panel A). RAW cells were stimulated for 10 h with the indicated agents. Nuclear extracts (20 μg) were analyzed in EMSA using a ^{32}P-labeled GATE probe. C, none; γ, IFN-γ (150 U/ml); α, IFN-α (1500 U/ml). Panel B shows the kinetics of GIF activation by IFN-α (1500 U/ml). The arrows show the position of specific complexes.

GATE probe and the resultant complexes were separated on non-denaturing polyacrylamide gels (Weihua et al., 1997). These assays detected the binding of two transacting factors (Fig. 7A) denoted as α1 and α2. However, only the DNA binding of α1 was induced by IFN-α treatment. In several experiments, binding of α2 to GATE did not change significantly with IFN-α treatment. In contrast, two complexes formed with IFN-γ treated cell extracts and both were strongly induced by the cytokine. The mobility of these IFN-α inducible complexes is similar to those induced by IFN-γ. Thus, unlike IFN-γ, IFN-α induces the binding of only one factor that binds to GATE. We next determined the kinetics of α1 induction. Although the α1 complex was maximally induced at 8 h, it could be detected at 4 h post IFN-α treatment (Fig. 7B). Thus, α1 is induced slowly, similarly to GIF-1.

The specificity of GATE binding of α1 and α2 was examined by competition assays. In these assays, excess (from 10- to 20-fold) wild type or mutant GATE oligonucleotide was incubated with the nuclear extracts, prior to the addition of labeled probe. A normal pattern of α1, α2 complex formation was seen with GATE (Fig. 8, lanes 1 and 2). Although some weakly interacting complexes were seen, they were not consistently seen in other experiments. Furthermore, these bands were competed out by mutant GATE and ISRE oligonucleotides. Therefore, they might not be specifically interacting with GATE. Wild type GATE, but not the mutant, was able to compete for the binding of α1, and α2 (Fig. 8; compare lanes 7 and 8 to lanes 5 and 6). As a result, α1 and α2 bands were not seen where wild type GATE was used as competing oligonucleotide (lanes 7 and 8). Although at high concentrations ISRE slightly competes with GATE for binding α1 and α2, it is not nearly as efficient as GATE (lanes 3 and 4). Such competition could be due to a weak homology of GATE to ISRE (Weihua et al., 1997).

Since GATE was distinct from previously described IFN response elements, we determined whether the α1 and α2 that bind to it were distinct from the known transacting factors. In these experiments, cell extracts were pre-incubated with specific antibodies raised against several IFN-stimulated factors and used for EMSA with

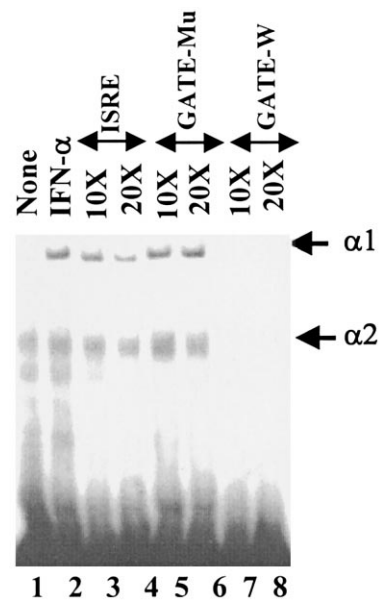

Fig. 8. Specific binding of α1 and α2 to GATE. EMSA was carried out as in Fig. 7, except that nuclear extracts were incubated with indicated molar excesses of various unlabeled double stranded oligonucleotides as competitors for 20 min prior to addition of the labeled GATE probe.

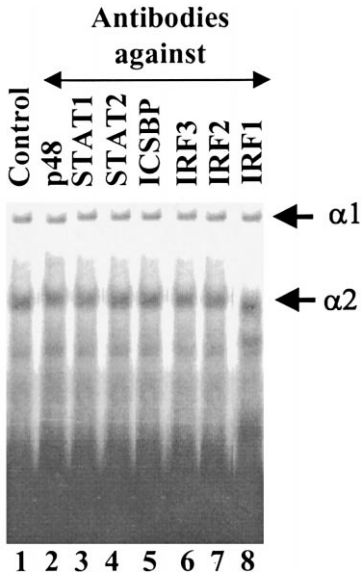

Fig. 9. The effect of antibodies against various IFN-regulated proteins on the binding of α1, and α2 to GATE. Nuclear extract (20 μg) from each sample was incubated with the indicated antibodies for 40 min at room temperature prior to addition of EMSA reagents and ^{32}P-labeled GATE.

GATE. None of the antibodies interfered with the binding of α1 and α2 to GATE or 'super-shifted' the complexes (Fig. 9). However, these antibodies super-shifted complexes formed with ISRE or pIRE probes (data not presented). These observations suggested that like IFN-γ, IFN-α also induced the binding of novel factors to GATE. Since GIF specific reagents are not currently available, it can not be ruled out that α1 and α2 are same as GIF-1 and GIF-2.

Because IFNs induce the phosphorylation of transacting factors, we examined the effects of protein kinase inhibitors on the binding of α1 and α2 to GATE (Schindler et al., 1992). Cells were pre-incubated with various protein kinase inhibitors and then stimulated with IFN-α. These included a tyrosine kinase inhibitor, genistein; a double-stranded RNA dependent serine threonine kinase inhibitor, 2-aminopurine; and a protein kinase C inhibitor staurosporine. Among these inhibitors, only staurosporine inhibited the α1 and α2 binding to GATE (Fig. 10; compare lanes 2–4 to lane 1). This characteristic paralleled that of IFN-γ induced formation of GIF complexes.

4. Discussion

The IFN-α induced transcription of IFN-stimulated genes and the resultant antiviral actions of IFNs are dependent on the nuclear levels and the activated DNA binding of transcription factor ISGF3 (Sen and Ransohoff, 1997; Stark et al., 1998). Although the functional activity of ISGF3 is modulated by IFN-α, its levels are further enhanced by the treatment of cells with IFN-γ (Dale et al., 1989; Bandyopadhyay et al., 1990; Levy et al., 1990). Indeed, IFN-γ enhances the synthesis of one of the components of ISGF3, p48 (Veals et al., 1992). As a result, this protein is also known as ISGF3γ (the IFN-γ inducible component of ISGF3). We have previously characterized the regulation of p48 gene by IFN-γ. These studies identified a novel response element, GATE, which responded to IFN-γ treatment (Weihua et al., 1997). GATE was distinct from other known IFN-response elements as analyzed by sequence comparison and functional assays.

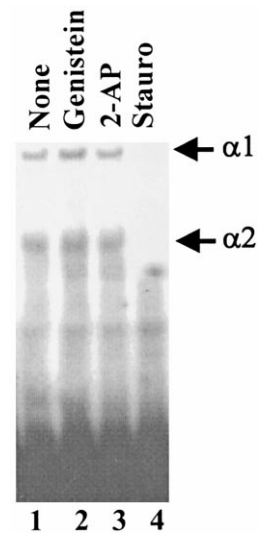

Fig. 10. The effect of protein kinase inhibitors on α1 and α2 activation. RAW cells were incubated with various protein kinase inhibitors prior to IFN-γ treatment and EMSA was performed with GATE. Genistein (100 μg/ml) was added to cells at least 10 h prior to IFN-α (1500 U/ml) treatment for 6 h. Other inhibitors, 10 mM 2-aminopurine (2-AP) and 0.5 μM staurosporine, were added 30 min prior to incubation with IFN-α. EMSA with the GATE probe was performed as above. The position of specific complexes is indicated.

In murine macrophage RAW cell line pre-treatment with low doses of IFN-γ (100–200 U/ml for 18 h) significantly enhanced the levels of p48 protein. Consequently, the DNA binding of ISGF3 binding was highly enhanced compared to IFN-α alone at (200 U/ml). Higher doses of IFN-α (1000–2000 U/ml) activated ISGF3 formation albeit weakly compared to IFN-γ pre-treated cells (data not shown). Consistent with this, a low dose of IFN-γ but not of IFN-α strongly induced p48 mRNA (Fig. 1). In other cell types, IFN-α alone induces ISGF3 efficiently without IFN-γ pre-treatment (Muller et al., 1993). Therefore, we have undertaken the present study to measure the p48 gene induction quantitatively and to precisely map the location of IFN-α response element. Luciferase reporter genes driven by the wild type p48 promoter and promoter fragments placed upstream of a heterologous promoter (SV40) responded to high, but not to low, doses of IFN-α and IFN-β (Figs. 2 and 3). This observation is consistent with previous data in the literature (Bandyopadhyay et al., 1990; Levy et al., 1990). Studies with mutant promoter constructs (GATE *pm*, MSE *pm* and ΔM1) showed that GATE was essential for the IFN-α induction of p48 promoter (Figs. 4 and 5). Data obtained with synthetic GATE suggest that GATE alone is sufficient for conferring IFN-α sensitivity to the reporter genes (Fig. 6).

Since GATE was a new element, we determined whether it bound novel or known transacting factors in response to IFN-α. EMSA revealed binding of two factors α1 and α2 to GATE. Among these, the DNA binding of only α1 was enhanced by IFN-α treatment. In contrast, IFN-γ induced the binding of two factors (Weihua et al., 1997) GIF-1 and GIF-2 (GIF, γ-IFN induced factor). The mobility of the α1 and α2 complexes was similar to those of GIF-1 and GIF-2, respectively. Furthermore, the DNA binding of GIFs and, α1 and α2 was inhibited by staurosporine, indicating that protein kinase (PKC)-like activities are essential for the activation of these factors. Indeed IFN-α/β induced gene expression is inhibited by staurosporine (Reich and Pfeffer, 1990) and certain isoforms of PKC are induced by IFNs (Wang et al., 1993). The α1 complex was induced with a slower kinetics similar to GIFs (Fig. 7B), unlike the rapid activation of STATs. Like GIFs, α1 and α2 preferentially bound to GATE and their binding was not competed by ISRE, a known IFN-α/β response element (Fig. 8). Consistent with this property, factors that interact with conventional IFN responsive elements did not bind to GATE. EMSA studies suggested that the GATE binding of α1 and α2 was unaffected by specific antibodies (previously tested) raised against various known IFN regulated transcription factors (Fig. 9). Although STAT proteins did not interact with GATE, both IFN-γ and IFN-α induced expression of p48 was dependent on the presence of functional JAK1 and STAT1 in the cells, because mutant cells lacking both these components do not express p48 in response to IFN-α. Thus, it is likely that α1 and α2, like GIFs, are secondary regulatory factors under the control of JAK–STAT pathway. Although the relationship between GIFs and, α1 and α2 is unclear, they may be identical based on their biochemical characteristics. Cloning of the GIF cDNAs should define this issue. These studies are in progress; at this stage, it appears that because IFN-γ is a stronger inducer of p48 gene by virtue of its ability to induce two different transacting factors that may interact additive or synergistic manner. Although the number of genes that possess GATE, in their promoters, is uncertain at present, it is quite likely that GATE dependent genes may participate in novel IFN-regulated functions. Thus, our studies identify a novel gene regulatory mechanism for IFN-α/β.

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References

Ackrill, AM, Foster, GR, Laxton, CD, Flavell, DM, Stark, GR, Kerr, IM, 1991. Inhibition of the cellular response to interferons by products of the adenovirus type 5 E1A oncogene. Nucl Acids Res 19, 4387–4393.

Au, WC, Moore, PA, Lowther, W, Juang, YT, Pitha, PM, 1995. Identification of a member of the interferon regulatory factor family that binds to the interferon-stimulated response element and activates expression of interferon-induced genes. Proc Natl Acad Sci USA 92, 11657–11661.

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K. Current Protocols in Molecular Biology. New York: Wiley, 1994.

Bandyopadhyay, SK, Kalvakolanu, DV, Sen, GC, 1990. Gene induction by interferons: functional complementation between trans-acting factors induced by alpha interferon and gamma interferon. Mol Cell Biol 10 (10), 5055–5063.

Dale, TC, Imam, AM, Kerr, IM, Stark, GR, 1989. Rapid activation by interferon alpha of a latent DNA-binding protein present in the cytoplasm of untreated cells. Proc Natl Acad Sci USA 86 (4), 1203–1207.

David, M, Grimley, PM, Finbloom, DS, Larner, AC, 1993. A nuclear tyrosine phosphatase downregulates interferon-induced gene expression. Mol Cell Biol 13 (12), 7515–7521.

Gutch, MJ, Reich, NC, 1991. Repression of the interferon singal transduction pathway by the adenovirus E1A oncogene. Proc Natl Acad Sci USA 88, 7913–7917.

Kalvakolanu, DV, Bandyopadhyay, SK, Harter, ML, Sen, GC, 1991. Inhibition of interferon-inducible gene expression by adenovirus E1A proteins: block in transcriptional complex formation. Proc Natl Acad Sci U S A 88, 7459–7463.

Kalvakolanu DV, Borden EC. Interferons: cellular and molecular biology of their actions. In: Bertino JR, editor. Encyclopedia of Cancer, vol. 2. San Diego, CA: Academic Press, 1997:940–951.

Kanno, Y, Kozak, CA, Schindler, C, Driggers, PH, Ennist, DL, Gleason, SL, Darnell, JE Jr, Ozato, K, 1993. The genomic structure of the murine ICSBP gene reveals the presence of the gamma interferon-responsive element, to which an ISGF3 alpha subunit (or similar) molecule binds. Mol Cell Biol 13 (7), 3951–3963.

Leonard, GT, Sen, GC, 1997. Restoration of interferon responses of adenovirus E1A-expressiing HT1080 cell lines by overexpression of p48 protein. J Virol 71, 5095–5101.

Levy, DE, Lew, DJ, Decker, T, Kessler, DS, Darnell, JE Jr, 1990. Synergistic interaction between interferon-alpha and interferon-gamma through induced synthesis of one subunit of the transcription factor ISGF3. EMBO J 9 (4), 1105–1111.

Lewis, JA, Huq, A, Shan, B, 1989. Beta and gamma interferons act synergistically to produce an antiviral state in cells resistant to both interferons individually. J Virol 63, 4569–4578.

Muller, M, Briscoe, J, Laxton, C, Guschin, D, Ziemiecki, A, Silvennoinen, O, Harpur, AG, Barbieri, G, Witthuhn, BA, Schindler, C, Pellegrini, S, Wilks, AF, Ihle, JN, Stark, GR, Kerr, I.M., 1993. The protein tyrosine kinase JAK1 complements defects in interferon-alpha/beta and -gamma signal transduction. Nature 366, 129–135.

Pine, R, Canova, A, Schindler, C, 1994. Tyrosine phosphorylated p91 binds to a single element in the ISGF2/IRF-1 promoter to mediate induction by IFN alpha and IFN gamma, and is likely to autoregulate the p91 gene. EMBO J 13, 158–167.

Reich, NC, Pfeffer, LM, 1990. Evidence for involvement of protein kinase C in the cellular response to interferon alpha. Proc Natl Acad Sci USA 87, 8761–8765.

Schindler, C, Shuai, K, Prezioso, VR, Darnell, JE Jr, 1992. Interferon-dependent tyrosine phosphorylation of a latent cytoplasmic transcription factor. Science 257, 809–813.

Sen GC, Ransohoff RM. Transcriptional regulation in the interferon system. Molecular Biology Interlligence Unit. Chapman and Hall and Landes Bioscience, Austin, TX, pp. 1–174.

Stark, GR, Kerr, IM, Williams, BRG, Silverman, RH, Schreiber, RD, 1998. How cells respond to interferons. Annu Rev Biochem 67, 227–264.

Veals, SA, Schindler, C, Leonard, D, Fu, XY, Aebersold, R, Darnell, JE Jr, Levy, DE, 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.

Wang, C, Constantinescu, SN, MacEwan, DJ, Strulovici, B, Dekker, LV, Parker, PJ, Pfeffer, LM, 1993. Interferon alpha induces protein kinase C-epsilon (PKC-epsilon) gene expression and a 4.7-kb PKC-epsilon-related transcript. Proc Natl Acad Sci USA 90, 6944–6948.

Weihua, X, Kolla, V, Kalvakolanu, DV, 1997. Interferon-γ induced transcription of the murine ISGF3γ (p48) gene is mediated by novel factors. Proc Natl Acad Sci USA 94, 103–108.