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Transcriptional induction of genes by IFN-β in mouse cells is regulated by a transcription factor similar to human ISGF-3

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Abstract

Previous studies of IFN-stimulated transcription factors in murine cells have identified a variety of *trans*-acting factors that bind to the IFN-stimulated response element (ISRE) whose role in gene expression remain unclear. The present investigation was undertaken to delineate the signal transduction pathway as well as to identify the transcription factors regulated by murine IFN-β in L929 cells. Tyrosine kinase inhibitor, Genistein, abrogated gene induction and activation of transcription factors by IFN-β. As early as 5 min after IFN-β treatment, a transcription factor was activated in the cytoplasm which subsequently migrated into the nucleus. Anti-phosphotyrosine antibodies detected a specific transcription factor induced by mIFN-β. Antibodies raised against human ISGF-3 subunit proteins p48, p84, p91 and p113 recognized this factor in the cytoplasm as well as in the nucleus of IFN-β-treated L929 cells. An antibody raised against an oligopeptide of human p113 (residues 435–450) recognized the ISGF-3 complexes both in human

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and murine cells. However, a different antibody against the C-terminus of human p113 (residues 671–806) did not recognize the ISGF-3 like complex in mouse cells, indicating differences in the primary sequence of these proteins.

Keywords: IFN-β; Murine L929 cell; Gene induction; Tyrosine phosphorylation

1. Introduction

Interferons induce a number of biological effects in the target cells which include anti-viral, antitumoral and immunomodulatory effects (Sen and Ransohoff, 1993; Borden, 1993). These effects are mediated by the products of cellular genes activated by IFNs, called the IFN-stimulated genes (ISGs). Although type I IFNs (IFNs-α and IFN-β) and type II IFN (IFN-γ) bind to distinct cellular receptors, they induce overlapping as well as distinct sets of cellular genes, suggesting an overlap in their signal transduction pathways (see Sen and Ransohoff, 1993; Pelligrini and Schindler, 1993). Gene sequence comparisons among various ISG promoters have identified a unique element called IFN-stimulated response element (ISRE). This element is both necessary and sufficient for gene induction by type I IFNs (Sen and Ransohoff, 1993; Pelligrini and Schindler, 1993). Although certain IFN-γ-inducible genes have been induced via the ISRE, recent studies indicate that a distinct response element called IFN-γ activation site (GAS) is important for the induction of several cellular genes such as GBP, Ly6E, ICSBP, IRF-1 and FcγRI by IFN-γ (Lew et al., 1991; Sen and Ransohoff, 1993; Pelligrini and Schindler, 1993).

A unique factor called ISGF-3 has been activated in human cell lines in response to IFN-α treatment (Bandyopadhyay et al., 1990; Dale et al., 1989; Levy et al., 1989). Prior to IFN-treatment, ISGF-3 was a dormant cytoplasmic protein, which translocated to the nucleus following IFN-treatment (Bandyopadhyay et al., 1990; Dale et al., 1989; Levy et al., 1989). Activation of distinct tyrosine kinases which subsequently phosphorylate ISGF-3, following IFN-α treatment of human cells have been identified (Velazquez et al., 1992; Muller et al., 1993; Walting et al., 1993). ISGF-3 was a complex of 4 distinct proteins of 48, 84, 91 and 113 kDa (Kessler et al., 1990). The 48 kDa (p48) bound to ISRE, while the other three proteins, modified by tyrosine phosphorylation in the cytoplasm, migrated to the nucleus and associated with p48 to stimulate the transcription (Fu, 1992; Kessler et al., 1990; Schindler et al., 1992; Veals et al., 1992). cDNAs corresponding to human ISGF-3 proteins have been isolated recently. The p48 protein had sequence homologies to *trans*-acting factors IRF-1, IRF-2, ICSBP and the oncoprotein *myb* (Veals et al., 1992). The p113, p84 and p91 subunits of ISGF-3 belonged to a distinct class of proteins, now designated STAT (signal transducer and activator of transcription) proteins (Fu et al., 1992; Schindler et al., 1992). The p84 and p91 proteins were encoded by the same cDNA (Schindler et al., 1992). The p91 protein has an additional 38 amino acid sequence at the C-terminus that was absent in the p84 (Schindler et al., 1992). p91 but not p113 was tyrosine phosphorylated in response to IFN-γ, and bound to GAS to stimulate the expression of GBP, Ly6E, IRF-1, ICSBP and FcγRI genes (Khan et al., 1993; Kanno et al., 1992; Sims et al., 1993; Pearse et al., 1993; Shuai et al., 1992).

We and others have shown that activation of ISGF-3 is inhibited in human cells expressing adenoviral oncogene E1A and Hepatitis B viral terminal protein (Ackrill et al., 1991; Gutch and Reich, 1991; Kalvakolanu et al., 1991). As a result, these cells failed to express IFN-inducible genes and the resultant biological actions of IFNs. A cellular E1A-like activity present in undifferentiated murine embryonal carcinoma cells also appears to be a potential inhibitor of a ISGs (Kalvakolanu and Sen, 1993). Differentiation of these cells with retinoic acid-induced IFN-β responses. An ISRE-binding *trans*-acting factor also appeared in response to IFN-β in these cells. However, the identity of this factor is unknown.

IFN-responses in murine cells are relatively less understood. One study suggested the involvement of arachidonic acid metabolism in the gene induction by IFN-α in mouse cells (Hannigan and Williams, 1991). A number of factors that potentially bind to ISRE have been isolated from murine sources (Haque et al., 1991; Kumar et al., 1991; Yan and Tamm, 1991; 1992). However, to date, the role of these factors in regulation of ISGs in murine cells has not been understood. In the present studies, we investigated the factors that respond to IFN-β in mouse cells. Our studies show that mouse IFN-β induces tyrosine phosphorylation of a *trans*-acting factor that bound to ISRE and this factors is very similar to ISGF-3 of human cells. The murine p113 appears to be antigenically different from that human cells.

2. Materials and methods

Cell culture. Mouse L929 and human HeLa cells were grown in DMEM supplemented with 10% fetal bovine serum. Natural murine IFN-β (Lee Biomolecular) was employed for treating murine cells, whereas a recombinant IFN-β (Serono Inc.) was used for treating human cells. Murine IFN-γ was from Genentech Inc. Probes for human p84/91; p113 were provided by Dr. Chris Schindler, Columbia University, New York.

Antibodies. Antibodies against the ISGF-3 proteins were raised in rabbits injected with synthetic oligopeptides conjugated to keyhole limpet hemocyanin according to standard protocols (Coligan et al., 1992). These oligopeptides were prepared on the basis of the published sequences: p48 (the N-terminal 8 amino acids); p91 (aa 701–713); p113 (aa 435–450). Two other antibodies raised against the ISGF-3 components described elsewhere (Fu et al., 1992; Schindler et al., 1992) were kindly provided by Dr. Chris Schindler, Columbia University, New York. One of these antibodies that detects both p84 and p91 was raised against a GST fusion protein containing the amino acids 598–705 of these proteins. Anti p113c was raised against a GST fusion protein consisting of human p113 residues 671–806. Pooled normal rabbit serum was used as a negative control. Monoclonal anti-phosphotyrosine antibody was obtained from Upstate Biotechnologies Inc.

Transcriptional analyses. Northern blot and nuclear run off transcription analyses were performed as described previously (Kalvakolanu et al., 1991). Murine PKR probe was a gift from Dr. Bryan Williams, Cleveland Clinic Foundation.

Transfection and reporter gene assays. L929 cells were transfected with chimeric plasmids in which expression of bacterial chloramphenicol acetyl transferase (CAT) gene is placed under the control of ISG-promoters as described in our previous publications (Kalvakolanu et al., 1991). After 30 h, the appropriate plates were treated with genistein (100 μg/ml) and allowed to incubate for an additional 10 h. IFN-β was then added to the plates at 200 U/ml and incubated further for 15 h. Extracts were prepared by freeze-thaw lysis and equal amounts of total cellular proteins were assayed for CAT activity.

Nuclear and cytoplasmic extract preparations. These methods were described previously in our publications (Kalvakolanu et al., 1991). Protein concentrations were determined and equal amounts of nuclear and cytoplasmic extracts were used in the electrophoretic mobility shift assays (EMSA).

Electrophoretic mobility shift assays. Nuclear and cytoplasmic extracts were analyzed as described earlier (Kalvakolanu et al., 1991). A synthetic ^{32}P-labeled ISRE double-stranded oligonucleotide was incubated with nuclear and cytoplasmic extracts and the complexes were subsequently analyzed on 6% polyacrylamide gels. Gels were then dried and autoradiographed. In the case of supershift assays, extracts were incubated with either normal rabbit serum or immune sera raised against the indicated proteins in the binding buffer for 30 min at room temperature prior to the addition of radiolabeled probe.

3. Results

3.1. Gene induction by IFN-β in L929 cells is inhibited by Genistein

In order to study the regulation of ISGs by mouse IFN-β, we incubated mouse L929 cells with IFN-β (200 U/ml) both in the presence and absence of tyrosine kinase inhibitor Genistein (Fig. 1A). Total cellular RNAs were extracted and 20 μg of RNA was northern blotted and probed with radiolabeled cDNAs specific to mISG-15 and PKR. While IFN-β strongly induced the expression of these genes, it failed to activate gene expression in the presence of Genistein. Similar results were obtained with a mouse 2–5 (A) synthetase probe (data not shown). Thus Genistein inhibited mIFN-β-induced expression of ISGs in a manner similar to IFN-α induction of these genes in human cells. Consistent with the northern analysis data, transcriptional induction of ISGs by mIFN-β was inhibited by Genistein (Fig. 1B). Expression of ISGs 202 and Protein kinase R (PKR) was induced normally in L929 cells treated with IFN-β. However, when cells were preincubated with Genistein, IFN-β failed to induce the transcription of these genes. Thus, inhibition of tyrosine kinase activities impaired the transcriptional induction of ISGs in mouse cells. Such impairment of transcription was not global because transcription of β-actin gene is unaffected irrespective of the presence or absence of Genistein. These observations are similar to those described for human cells (Fu, 1992; Schindler et al., 1992).

Transcriptional inhibition of ISGs was also assessed by another method. Cells were transfected with CAT reporter genes driven by ISG-202 promoter and then treated with

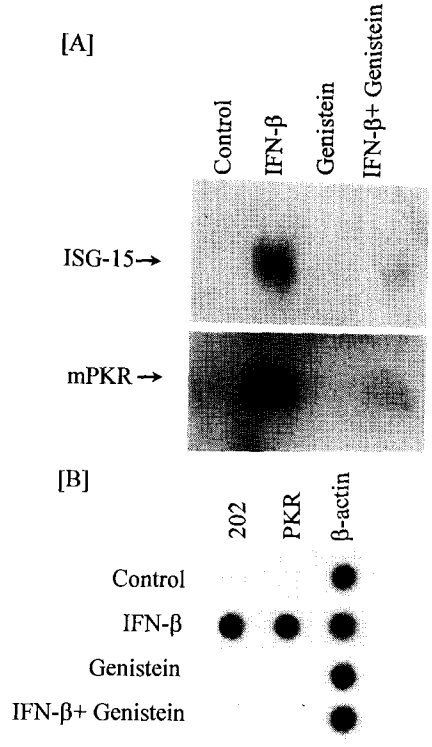

Fig. 1. Transcriptional stimulation of genes by murine IFN-β is inhibited by Genistein in L929 cells. (A) Northern blot analysis of ISG-induction. Cells were pre-incubated with genistein (100 μg/ml) for 10 h, where indicated, and then treated with mIFN-β (200 U/ml) for 6 h. Total RNA was extracted and 20 μg of RNA was northern blotted (Kalvakolanu et al., 1991). The blots were probed with random primer labeled cDNA probes of the indicated gene. Blots were autoradiographed after stringent washing. (B) Nuclear run off transcription. L929 cells were treated as indicated under Panel A, except that IFN-treatment was given for 1 h. Nuclei were isolated and run off transcription reactions were performed in the presence of α-^{32}P-UTP at room temperature for 30 min (Kalvakolanu et al., 1991). Nascently-labeled nuclear RNAs were extracted and hybridized to the cDNAs of ISGs 202, PKR and β-actin immobilized on nylon membranes. A negative control of pUC18 (not shown in the figure) was also included in these blots. After stringent washing, they were autoradiographed to detect the transcription of genes.

Genistein or IFN-β (Fig. 2A). A constitutive reporter construct expressing bacterial β-galactosidase gene under the control of β-actin promoter was employed to correct for variations that may arise due to transfection efficiency. Similar to the endogenous genes, expression of CAT gene was strongly induced by IFN-β but was inhibited in the presence of Genistein. Similar data were obtained with CAT genes driven by the promoters of ISGs 561, 6–16 and 2–5 A synthetase. Additionally, IFN-β-induced expression of a stably transfected CAT gene driven by ISG 6–16 in a L929 cell line was also inhibited by Genistein. These data demonstrate that both endogenous and exogenously transfected gene induction by IFN-β was suppressed by Genistein. Expression of IFN-γ inducible IP-10 CAT gene was also inhibited by Genistein (Fig. 2B). Normal

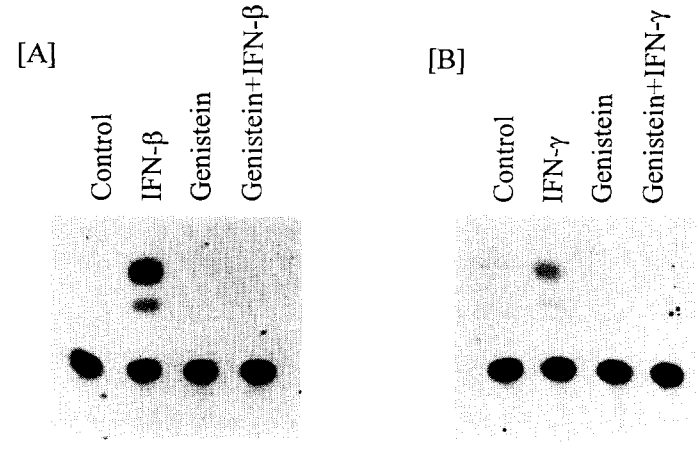

Fig. 2. Induction of ISG-CAT genes is inhibited by Genistein. L929 cells were transfected with the indicated CAT gene driven by either the promoter of 202 gene (A) or the IP-10 gene (B). Cells were treated as indicated under Section 2. IFN-β and IFN-γ were used at 200 U/ml. Chloramphenicol acetyl transferase activity was determined using 50 μg transfected cell extracts (Kalvakolanu et al., 1991). Percent acetylation values are 0.6, 27, 0.7 and 0.8 in panel A and 0.9, 8.4, 0.6 and 0.9 in panel B in the indicated order.

induction of CAT gene by IFN-γ was observed in these cells but was inhibited in the presence of Genistein. Thus, both IFN-β and IFN-γ inducible gene expression is dependent on tyrosine phosphorylation in mouse cells.

3.2. Activation of transcription factors by IFN-β in L929 cells

We next tested the activation of transcriptional factors in the presence of IFN-β in L929 cells. Cytoplasmic activation and nuclear migration of ISGF-3 were described in human cells treated with IFN-α (Bandyopadhyay et al., 1990; Dale et al., 1989; Levy et al., 1989). We then tested whether ISGF-3 like factor was activated in these cells. Using a ^{32}P-labeled ISRE, we performed electrophoretic mobility shift assays to detect the factors that may interact with this element. Two constitutive factors were present in the cells. These bands may correspond to ISGFs-1 and -2 of human cells. A novel band with a slower mobility, designated ISGF-a, appeared in IFN-β-treated cells (Fig. 3A). All these complexes could be competed out by an excess unlabeled ISRE oligonucleotide, but not by an unrelated oligonucleotide, suggesting their authenticity (data not shown). A similar factor with ISGF-3 characteristics appeared in a differentiated murine embryonal carcinoma cell line treated with IFN-β (Kalvakolanu and Sen, 1993).

The novel band appearing in response to IFN-β had similar characteristics as human ISGF-3. This factor was activated in the cytoplasm as early 5 min after IFN-treatment, and was translocated into the nucleus. Fig. 3B shows the cytoplasmic activation of this factor and Fig. 3C shows its nuclear accumulation. These features were similar to our previous observations in differentiated embryonal carcinoma cells (Kalvakolanu and Sen, 1993). Thus, in two independent cell types, mIFN-β activates *trans*-acting factors in a similar manner and their activation characteristics are consistent with those of ISGF-3 of human cells.

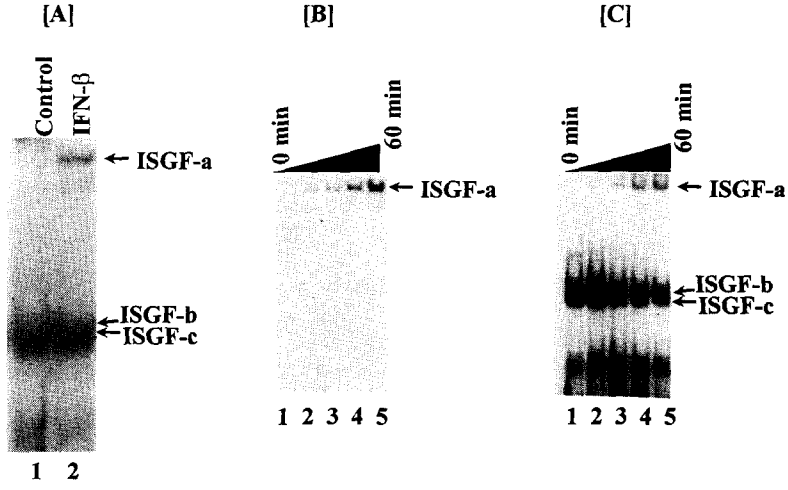

Fig. 3. Induction of ISRE-binding factors in L929 cells by IFN-β. (A) Nuclear extracts were prepared (Kalvakolanu et al., 1991) from the cells after appropriate treatments for 1 h. Equal amounts of protein (1.5 μg) from each extract were incubated with a ^{32}P-labeled ISRE probe and the products were separated on EMSA gels (42,44). Gels were dried and autoradiographed. Positions of various complexes are indicated. (B) Cytoplasmic activation of ISGF-a. Cytoplasmic extracts (1.2 μg) from various cells L929-treated IFN-β for indicated periods were used in EMSA as above. Lanes 1: 0 min; 2: 5 min; 3: 15 min; 4: 30 min; and 5: 60 min. (C) Nuclear localization of ISGF-a. Nuclear extracts (1.2 μg) were from the same cells in panel B were used in the EMSA. Lane notations are similar to panel B.

3.3. Tyrosine phosphorylation of ISGF-3-like factor in response to IFN-β in L929 cells

We next asked whether activation of the ISGF-3-like factor, in response to IFN-β, involved tyrosine phosphorylation in L929 cells. Nuclear and cytoplasmic extracts were obtained from cells pre-incubated with 100 μg of Genistein for 12 h followed with mIFN-β (200 U/ml) for 1 h. EMSA assays were performed as described previously (under Fig. 3). A normal activation of ISGF-a was observed in the cytoplasm of IFN-β-treated cells and it subsequently appeared in the nucleus (Fig. 4A). Pre-incubation of cells with genistein followed by IFN-β resulted in the inhibition of activation of this factor in the cytoplasm and as a result, was absent in the nuclear fraction.

We also established the tyrosine phosphorylation of ISGF-a in another manner. Incubation of IFN-β-treated cell extracts with anti-phosphotyrosine antibody resulted in the supershift of the ISGF-3-like complex (Fig. 4B). Thus mIFN-β stimulated the tyrosine phosphorylation of the ISGF-a transcription factor in a manner similar to that of ISGF-3 of human cells. Identical inhibition of ISGF-a activation by IFN-β in the presence of Genistein was observed in mouse embryonal carcinoma F9 cells differentiated with retinoic acid (data not shown). Therefore, IFN-β induces the tyrosine phosphorylation of *trans*-acting factors in two different mouse cell types.

3.4. ISRE-binding factors in mouse cells are similar to those of human cells

Having established the ISGF-3-like features of the mIFN-β-induced complex, e.g., cytoplasmic activation, subsequent nuclear translocation of the factor and its tyrosine

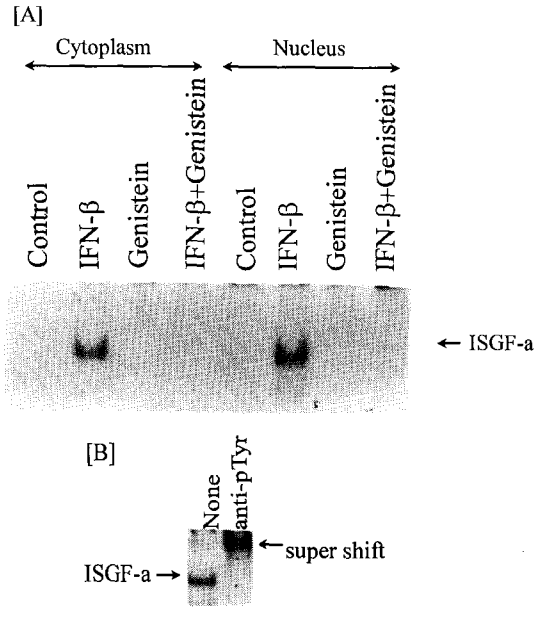

Fig. 4. ISGF-a is tyrosine phosphorylated in response to mIFN-β in L929 cells. Cells were incubated with IFN-β for 30 min. Genistein treatment is described under Fig. 1. Cytoplasmic and nuclear extracts (1.5 μg) from the same cells were isolated and used in EMSA. Only the relevant part of the autoradiogram is shown. Position of ISGF-a is indicated. (B) ISGF-a is recognized by anti-phosphotyrosine antibodies. Monoclonal anti-pTyr antibody (0.5 μg) was added to the EMSA 30 min before the addition of ISRE probe. Extracts from IFN-β-treated nuclei were used in these experiments. Positions of ISGF-a and supershifted complex are indicated.

phosphorylation, we asked whether proteins similar to ISGF-3 subunits were present in this complex. We preincubated the gel shift reactions with various antibodies raised against human ISGF-3 subunits. These antibodies were raised against various synthetic peptides derived from the published DNA sequences of the 48 kDa, 91 kDa and 113 kDa subunits of ISGF-3 (see Section 2).

Preincubation of cytoplasmic (Fig. 5A) and nuclear extracts (Fig. 5B) with antibodies against the human ISGF-3 subunits p48, p91 and p113 resulted in the supershifting of the ISGF-a complex in L929 cells. Normal rabbit serum, however, did not supershift these complexes. Thus, the same protein that is activated in the cytoplasm is also present in the nuclear fraction and is in complex with ISRE in mouse cells (Fig. 5A and 5B). All three antibodies supershifted the ISGF-a complex. An antibody that recognizes both p84 and p91 was also able to supershift the ISGF-a complex (Fig. 5C). Taken together, these results indicate that mouse IFN-β induced an ISGF-3-like complex that was comprised of similar, if not identical, proteins to those of human cells.

We were also able to detect certain differences in the nature of p113 subunit of mouse cells. The antibody employed in Figs. 5A and 5B was raised against a synthetic oligopeptide corresponding to amino acids 435–450. It was able to supershift ISGF-3 complexes both in mouse and human cells induced by IFN-β (Fig. 5C and 5D).

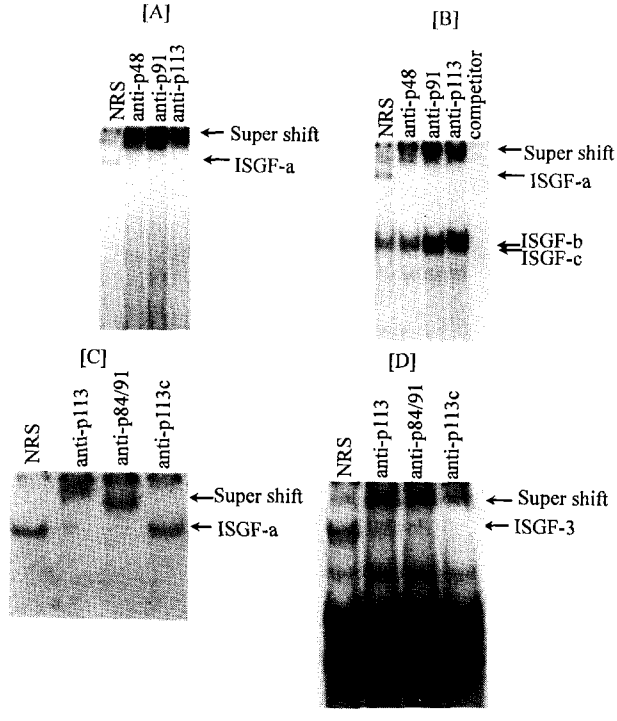

Fig. 5. ISGF-a in L929 cells is similar to human ISGF-3. Cytoplasmic (A) and nuclear (B) extracts (1.5 μg) from IFN-β-treated L929 cells were incubated with 2 μl of indicated antiserum for 30 min in the binding buffer before the addition of ISRE probe. Positions of the complexes are indicated. Where indicated with competitor, a 50-fold molar excess of unlabeled ISRE oligonucleotide was pre-incubated with anti-p113-treated extracts, prior to ISRE probe. p113 in murine cells (C) is antigenically different from human cells (D). Nuclear extracts from murine (L929) and human (HeLa) cells were incubated with the indicated antiserum. Human IFN-β-treated cell extracts (7 μg) and murine IFN-β-treated cell extracts (2.5 μg) were used in these assays. The regions against which the antibodies were raised are indicted under Section 2. In panel C, only the relevant portion of the autoradiogram is presented.

Interestingly, when we employed another antibody, p113c, raised against the C-terminal (residues 671–806) portion of p113 polypeptide, no supershifting of ISGF-3 complex in mouse cell extracts was observed. However, the same antibody was able to supershift the ISGF-3 complex in human cells. Therefore, mouse and human p113 proteins seem to be divergent at their C-termini.

3.5. Human ISGF-3 subunit probes detect mRNAs corresponding to ISGF subunits in mouse cells

As further evidence for the presence of similar proteins in the mouse and human cells and their activation by IFN-β, we performed northern blot analyses using the cDNA probes corresponding to human ISGF-3 subunits p84/91 and p113 (Fu et al., 1992; Schindler et al., 1992). Fig. 6 shows that mRNAs corresponding to ISGF-3 subunits were identifiable in mouse cells. mRNAs of 113, 84 and 91 kDa were inducible by

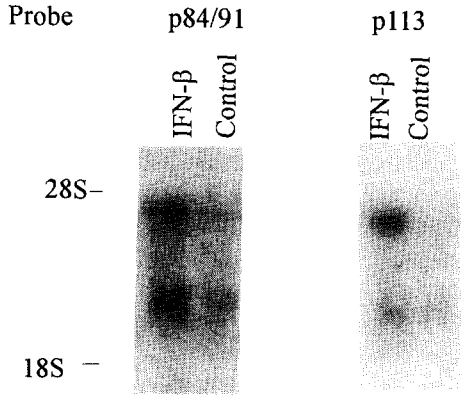

Fig. 6. ISGF-3 subunit probes detect specific mRNAs in L929 cells. Total RNAs from the cells were isolated from various treatments and 20 μg of RNA was northern blotted. Blots were probed with the indicated human cDNA probe.

IFN-β. A smaller species of p113 mRNA is also seen in these cells whose identity remains unclear.

4. Discussion

Results presented in this report provide evidence for the involvement of proteins similar to human ISGF-3 subunits, but not the previously described murine ISRE-binding proteins (Yan and Tamm, 1990; 1991; 1992) in mIFN-β-regulated gene expression. These observations also indicate that tyrosine phosphorylation of these factors is responsible for signal transduction in mouse cells unlike the previous reports which suggested arachidonic acid metabolism for IFN-signaling in mouse cells (Hannigan and Williams, 1991). Several evidences indicate that tyrosine phosphorylation is the key player in signal transduction by IFNs and other cytokines (Muller et al., 1993; Velazquez et al., 1992; Walting et al., 1993). Consistent with this, a cDNA for mouse JAK1 tyrosine kinase was able to complement a defect in a human mutant cell line that lacked this gene (Muller et al., 1993). This kinase was as efficient as the human JAK1 kinase in these cells. Furthermore, phosphorylation of JAK1 and JAK2 kinases in mIFN-α,γ-treated mouse 3T3 fibroblasts has been reported while these studies were in progress (Silvennoinen et al., 1993).

Mouse Balbc/3T3 cells that were resistant to the antiproliferative action of IFN-β were also defective in signaling by EGF, PDGF and basic FGF (Mundschau and Faller, 1991). Consistent with this, a recent study demonstrated rapid in vivo activation of p91 and p84 following injection of EGF into mice (Ruff-Jamison et al., 1993). It will be interesting to test whether these IFN-β-resistant mouse Balbc/3T3 cells lacked ISGF-3 subunits.

Previous attempts to isolate the ISRE-binding factors in mouse cells described the identification of a number of proteins (Haque et al., 1991; Kumar et al., 1991; Yan and Tamm, 1990; 1991; 1992). To date, none of these proteins have been shown to be positive activators of ISRE driven gene expression. In Balbc/3T3 fibroblasts, six complexes that bind to an ISRE sequence, derived from murine 2–5 (A) synthetase gene promoter, were identified (Yan and Tamm, 1990). Binding of one of these complexes, C4, was greatly enhanced by IFN-α/β treatment, although it was obtained from both untreated and IFN-treated cells. It was proposed that the factor yielding this complex (named IFN-α/β-stimulated response factor or ISRF) preexisted in the cytoplasm, presumably complexed with an inhibitor, and that it dissociated from the inhibitor following IFN-treatment and translocated to the nucleus (Yan and Tamm, 1990). However, we did not detect any factors with such characteristics either in L929 fibroblasts or in F9 embryonal carcinoma cells differentiated with retinoic acid. A cDNA encoding a factor called IREBF-1 was isolated, from mouse cell library, as a protein that binds ISRE sequence (Yan and Tamm, 1991). This protein was able to bind to trimeric ISRE only but not to a monomeric ISRE. IREBF-1 contained an acidic domain, three heptad leucine repeat arrays and an amphipathic C-terminal region, which bears no resemblance to ISGF-3 proteins (Yan and Tamm, 1991). Another ISRE-binding factor, IREBF-2, with sequence homologies to herpes viral proteins has been isolated from mouse cells (Yan and Tamm, 1992). The direct role of these factors in the regulation of ISGs has not been ascertained. However, they may be specific to 2–5A synthetase gene family. More particularly, because of the presence of ISRE and lack of conventional elements such as TATA-box in the basal promoter region, the 2–5AS-ME-12 gene may require the presence of these factors (Yan and Tamm, 1991; Yan and Tamm, 1992). Factors like IREBF-1 may play a role in the regulation of basal but not the inducible expression of these genes. Another ISRE-binding protein identified as IBF-1 has been found to be an inhibitor of IFN-induced gene expression (Haque et al., 1991). This protein, unlike ISGF-3, binds to a simple GAAA motif. Ets-1 oncoprotein also binds to the GAGGAA within the ISRE of 2–5 (A) synthetase (Kumar et al., 1991). Finally, yet another ISRE-binding protein ICSBP has been found to be an inhibitor of ISG expression (Nelson et al., 1993) and appears to interfere with ISRE-binding of ISGF-3 (K. Ozato, personal communication).

Our results provide direct evidences that IFN-activated factors in mouse cells are similar to those identified in human cells. Consistent with this, a murine cDNA of ISGF-3γ (p48) has been isolated (Veals et al., 1992) and the activation of p84/91 by EGF (Ruff-Jamison et al., 1993) in mice has been reported. Similar inhibition of activation this ISGF-3-like factor in the presence of adenoviral E1A and a cellular E1A-like activity both in mouse and in human cells (Kalvakolanu et al., 1991; Kalvakolanu and Sen, 1991) is also consistent with the notion that these factors are very similar. One distinct difference between human and mouse p113 is the antigenic variation between these two proteins. It is unclear whether the C-terminal sequence variation contributes to the species specific action of IFN, because p113 is associated with the receptor polypeptide complex (Colamonici et al., 1993). Isolation of murine p113 cDNA and subsequent mutagenesis should reveal the importance of this divergence.

Acknowledgments

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