Interferon-α (IFNα) and platelet-derived growth factor (PDGF) each rapidly stimulate binding of nuclear factors from Balb/c 3T3 fibroblasts, to a 29-base pair regulatory sequence derived from the 5’ upstream region of the murine 2-5A synthetase gene. This regulatory sequence contains a functional interferon-stimulated response element (ISRE) and also functions as a PDGF-responsive sequence. We show that IFNα induces binding of a protein of molecular mass 65 kDa to the ISRE. Constitutively expressed ISRE-binding proteins of 98 and 150 kDa are also demonstrated. Binding of inducible factors to the ISRE increases significantly within 15 min of IFNα or PDGF treatment. PDGF-induced binding is not mediated by IFNβ. The protein kinase inhibitors, staurosporine and K252a, block PDGF-induced ISRE binding and 2-5A synthetase gene expression. IFNα-induced ISRE binding and gene activation are not blocked by these inhibitors. Treatment of cells with 12-O-tetradecanoyl-13-acetate or dibutyryl cyclic AMP does not activate ISRE binding factors or 2-5A synthetase gene expression. PDGF responsiveness of the ISRE in vivo is also sensitive to staurosporine, indicating that inhibition of a protein kinase activity blocks the PDGF-specific transcriptional signal. Our data indicate the signal transduction pathway for IFNα-induced, ISRE-dependent transcription is distinct from the PDGF-induced ISRE response and is likely independent of cyclic AMP-dependent protein kinase and protein kinase C activities.

Induction of gene expression is thought to be necessary for the antiviral and antigrowth properties of interferon α (IFNα) (1, 2). However, transmembrane signaling pathways mediating the early activation of gene transcription by IFNα-

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Receptor interactions are unknown.

2-5A synthetase is a double-stranded RNA (dsRNA)-dependent enzyme whose expression is transcriptionally induced by IFNα (3). 2-5A synthetase catalyzes polymerization of adenylate residues into a series of 2′,5′-linked oligomers. 2-5A oligomers transiently activate a latent cellular endoribonuclease which acts as a translational regulator of gene expression (reviewed in Ref. 2). 2-5A synthetase activity is also induced by epidermal growth factor (4) or dsRNA, and platelet-derived growth factor (PDGF) (5) in fibroblast cells and in rat PC12 cells by nerve growth factor (6). The induction of 2-5A synthetase mRNA by PDGF occurs in the absence of new protein synthesis, suggesting that it is a direct response to this growth factor (5, 7).

2-5A synthetase activity in the liver decreases dramatically shortly after partial hepatectomy in rats (8) and has been shown to accumulate to high levels late in S phase in synchronized mouse embryo fibroblasts (9). These results have led to speculation that 2-5A synthetase activity is involved in regulating fundamental aspects of cellular metabolism such as growth and differentiation. In direct support of this we have shown that plasmid-directed overexpression of 2-5A synthetase activity results in a marked reduction in both growth rate and colony size of a human glioblastoma cell line (10).

Recently, a number of groups have demonstrated the binding of IFNα-modulated nuclear factors to 5′ cis-regulatory sequences of Type I IFN-inducible genes. We and others have identified one such regulatory sequence, residing in the 5′ upstream region of the 2-5A synthetase gene (11-13), which is highly conserved in a number of IFNα-regulated genes of both mouse and human origin. This sequence functions as an IFN-stimulated response element (ISRE) (14) and specifically binds IFN-modulated nuclear factors in a manner which correlates with transcriptional activation of these genes. Factor binding, contact point analyses, and transcriptional studies have led to the elucidation of a consensus ISRE sequence, (A/G)GAAA(A/G)(N)GAAACT (where N is any nucleotide, Refs. 12–17). Furthermore, an IFNα-inducible DNase I-hypersensitive site lies approximately 50 base pairs upstream of the ISRE in the IFNα-inducible ISG-15 gene, implying that ISRE binding factors induce a transcriptionally open chromatin conformation immediately upstream of ISRE-containing genes (18).

We have also demonstrated that the 2-5A synthetase ISRE sequence is PDGF-responsive in Balb/c 3T3 cells (7). Since the 2-5A synthetase ISRE represents an early target of both mitogenic (PDGF) and growth-inhibitory (IFNα) signals, we have sought to characterize ISRE-specific binding proteins, as well as the intracellular signaling pathway(s) by which activation of ISRE binding occurs. Although protein kinase
Cell Lines and Growth Conditions—Balb/c 3T3 fibroblasts (clone A31) were originally obtained from Dr. C. Dale, Dana Farber Institute, and grown as previously described (7).

PDGF, IFN, and Kinase Inhibitors—HuIFNαAD was the gift of Drs. P. Trotta and T. L. Nagabushan, Schering Corp., NJ. Purified MuIFNβ was obtained from Interferon Sciences Inc. (New Brunswick, NJ). Crude PDGF was extracted from clinically outdated human platelets obtained from the Hospital for Sick Children blood bank and processed as previously described (7). HuBB-PDGF was obtained from Genzyme, and highly purified HuAB-PDGF was from R & D Systems. Staurosporine was obtained from Calbiochem, and K252b was obtained from Kamiya Biochemical (Thousand Oaks, CA). These latter were reconstituted in dimethyl sulfoxide at 2 mM and stored at 4 °C. TPA, Br-cAMP, dithiothreitol and NEM were from Sigma. Polyclonal antibody to human IFNβ was a gift from Dr. J. Vilecek, New York University Medical School, and monoclonal antibody against murine IFNβ (7F-D3) was provided by Profs. Y. Kawade and Y. Watanabe, Institute for Virus Research, Kyoto University, Japan.

Northern Blots—Isolation of total cellular RNA was by the guanidinium isothiocyanate method and Northern blot analyses were performed as described previously (19). An RNA probe was transcribed in vitro, with bacterial SP6 polymerase in the presence of [α-32P]UTP, from a cDNA insert representing the 5′ half of the mouse 2-5A synthetase gene (20). Blots were hybridized at 55 °C, and the most stringent wash was 0.1× saline/sodium/phosphate/EDTA at 65 °C. A cDNA representing the chicken actin gene (Oncor, Seattle) was random primer-labeled to a specific activity of >107 cpm/μg. Hybridization and washing was at 42 °C for this probe.

Oligonucleotides—A double-stranded oligonucleotide, representing nucleotides −80/−52 (relative to a putative translational start site) of the murine 2-5A synthetase gene (11) which contains a functional ISRE, was synthesized on an Applied Biosystems 308A DNA synthesizer. An oligonucleotide representing nucleotides −65/−99 of the human IFNβ gene was synthesized for use as a control for ISRE binding reactions. The sequences are, ISRE, 5′-CCCTTCCTC GTGAAATGGAACCTGAAATAC; IFNβ IRE, 5′-CATAGGAAA- CAGTAGAGGAGGAACTGTGAAAATC TCTCTG. Underlined in the ISRE is the binding element recognized by IFN-induced factors. Underlined in the IFNβ IRE sequence are nucleotides involved in recognition of a factor exhibiting characteristics of NF-κB, which is activated by dsRNA (21–23). Oligonucleotides were synthesized with BamHI-compatible linkers at the 5′ terminus (GATC). Gel-purified oligonucleotides were mixed with an equimolar amount of their respective complements, heated to 65 °C for 15 min, and annealed at room temperature for 18 h. These preparations were used directly in labeling reactions.

Extract Preparation and Electrophoretic Mobility Shift Assays—Electrophoretic mobility shift assays (EMSA) were done as previously described, using native 4% polyacrylamide gels run in a Tris/glycine buffer (pH 8.3) (7, 24). Nuclear extracts were prepared according to Dignam et al. (25), or whole cell extracts were prepared as follows. Cell pellets (2–4×106 cells) were extracted with buffer A (25) for 5 min on ice. Cells were then pelleted for 10 s at 10,000 × g, and the supernatant was discarded. Pellets were washed twice more in buffer A and extracted with 100 μl of buffer C (25) for 5 min on ice. Cell debris was pelleted and the supernatant subjected to 15,000 × g for 30 min. This high salt, whole-cell extract (WCE) was dialyzed in 100 volumes of buffer D (25) for 1 h, then 4 h in fresh buffer D. All extraction steps were carried out at 4 °C. Oligonucleotides were end-labeled for use in EMSA by T4 DNA kinase in the presence of [γ-32P]ATP.

UV-induced Cross-linking Assays—For cross-linking experiments, the ISRE synthetic oligonucleotide was random primer-labeled in the presence of [α-32P]dATP, according to standard procedures. TTP was substituted by bromodeoxy-UTP in order to render the labeled binding sites UV-sensitive. After incubation with nuclear or whole cell extracts (40–80 μg) binding reactions were resolved on native 6% polyacrylamide gels. These gels were then exposed to a 302 nm UV light for 60 min (Fotodye UV 300 transilluminator), under an ice pack. The gel was then dried under vacuum and subjected to autoradiography.

RESULTS

Photochemical Cross-linking of IFNα-induced Proteins to the ISRE—We and others have identified both constitutive and IFNα-induced factors, which bind specifically to ISRE sequences found in the upstream of IFNα-inducible genes (12–17). We sought to characterize the DNA-binding protein components of these factors in Balb/c 3T3 cells (clone A31) by covalent, UV-induced cross-linking of IFNα-induced extracts to a labeled ISRE oligonucleotide. The molecular masses of these ISRE-binding proteins were estimated by resolution of cross-linked complexes on denaturing polyacrylamide gels (Fig. 1). IFNα induced two ISRE-specific complexes (A and B, Fig. 1a) which were the slowest migrating complexes seen on native PAGE analysis. In addition there was a constitutive complex of higher mobility (C, Fig. 1a).

![Fig. 1. Photoaffinity labeling of ISRE-binding proteins regulated by IFNα. High-salt extracts (60–70 μg) of quiescent A31 cells treated with rHuIFNαAD (1000 IU/ml) for 15 min were incubated with 106 cpm of bromodeoxyuridine-substituted, random hexamer-labeled ISRE oligonucleotide under standard conditions. a, reaction products from IFNα-treated extracts were run on 6% native polyacrylamide gels and UV-irradiated (300 nm) for 1 h. Complexes forming in IFNα-induced, and C represents a constitutive binding complex (see Fig. 2). b, cross-linked complexes (A–C) were resolved in small scale binding reactions (10 μg/lane), and slices of native gel corresponding to each complex were then excised from the native gel. Slices corresponding to each complex were collected (5–61 lane) and analyzed on a 10% sodium dodecyl sulfate-PAGE system, for molecular mass estimation. Kd, kilodaltons. As expected, no cross-linked bands were obtained from regions of the UV-exposed native gel which did not correspond to A, B, or C (not shown). Autoradiography was for 3 days with an intensifying screen.](image-url)
We isolated each of these complexes from native band mobility shift gels, for cross-linking analysis. Fig. 1b illustrates the results of such an experiment. Both IFN-induced complexes, A and B, contained a DNA-binding species of approximate molecular mass of 65 kDa. Constitutive complex C, seen in A31 nuclear and whole-cell extracts regardless of IFN treatment, contained 98- and 150-kDa DNA-binding species (Fig. 1b). A and B migrated more slowly than C in native gels but contained a smaller DNA binding component than C, suggesting the presence of additional, non-DNA-binding proteins in the activated A and B complexes.

**PK Inhibitors Block PDGF-induced, but Not IFNα-induced, Gene Expression**—To determine the nature of signaling pathways mediating ISRE-dependent gene activation by IFNα and PDGF, we have used the protein kinase inhibitors, staurosporine and K252a (28, 29). 2-5A synthetase mRNA is induced by PDGF or IFNα/β, with similar kinetics (7). This activation has been shown, in both cases, to be mediated by the ISRE-containing sequence represented by the 29-base pair oligonucleotide used in the above analysis (7).

When confluent A31 cells were pretreated with 10 nM staurosporine there was marked inhibition of 2-5A synthetase mRNA induction by PDGF, and no detectable induction with 100 nM staurosporine (Fig. 2). In contrast there was no inhibition of mRNA induction by IFNα at 10 nM staurosporine, with a slight inhibition seen at 100 nM. Similar results were obtained with a structurally related inhibitor, K252a (28) (Fig. 2b). Direct activation of Ca²⁺/phospholipid-depend-
ISRE binding factor(s) through different receptor-coupled pathways. To examine more closely this apparent separation in signal transduction pathways, we employed the EMSA on extracts made from cells pretreated with the kinase inhibitors, staurosporine and K252a, prior to IFNα or PDGF treatment (Fig. 4a).

We confirmed that authentic murine IFNα/β induced the same complex pattern as seen with the human hybrid rIFNaAD, used in the experiments shown in Fig. 3 (Fig. 4a, lanes 1 and 2). The induction of ISRE binding activity by IFNα was not blocked by either staurosporine or K252a (Fig. 4a, lanes 1–4), although staurosporine did inhibit this response to some degree. PDGF-induced ISRE binding, in contrast, was almost completely inhibited by either K252a or staurosporine treatment (Fig. 4a, lanes 5–9). It is possible the partial inhibition of IFN-induced binding by staurosporine is reflected in the inhibition of PDGF-induced binding, a much weaker response. The differential sensitivity of PDGF- and IFN-induced binding to K252a (compare lanes 4 and 9, Fig. 4a) makes this unlikely. rPDGF-BB homodimer and purified human PDGF-AB heterodimer preparations induced ISRE binding identical to crude PDGF, which was predominantly of the AB heterodimer type (Fig. 4a, lanes 5–7). These results indicate that IFNα- and PDGF-receptor interactions trigger different signaling pathways in the activation of ISRE-binding factors. This result is in accordance with the effects of these kinase inhibitors on PDGF-induced, ISRE-dependent transcription (Table I), and induction of 2-5A synthetase gene expression by these two agents (Fig. 2a).

Since staurosporine and K252a did not block IFN-induced ISRE binding, we tested for the effect of activators of kinase-dependent signal transduction on induction of ISRE binding. Neither TPA (Ca2+/phospholipid-dependent protein kinase C activator) or Bt2cAMP (cAMP-dependent protein kinase A activator) treatment of intact A31 cells activated ISRE bind-

Fig. 3. Differential signaling ISRE binding factors by IFNα and PDGF. a, high salt extracts of quiescent A31 cells were probed with a body-labeled ISRE oligonucleotide. Cells were treated with crude PDGF (200 units/ml) or IFNα (1000 IU/ml) for 2 min or 15 min before extraction. The induced complexes (unmarked arrows) are those labeled A and B in Fig. 1. b, The same extracts as in a were probed in standard binding reactions with an oligonucleotide probe representing IRE sequences (nucleotides −99/−55) from the 5′ upstream of the human IFNβ gene. This probe includes sequences recently shown to bind an inducible factor, indistinguishable from NF-κB (see "Results"). Unmarked arrows indicate bound IRE probe. In a and b, F indicates free probe.

Fig. 4. PDGF-induced, but not IFN-induced, signaling of ISRE binding factors is sensitive to protein kinase inhibitors. a, WCE of A31 cells were incubated (5 μg/reaction) with a body-labeled ISRE oligonucleotide, with binding and gel conditions as in Fig. 3. Lanes represent cells treated (15 min) as follows: 0, untreated; 1 and 2, 1000 IU/ml rHuIFNaAD or murine IFNα/β, respectively; 3 and 4, as in 1, with 15-min pretreatment of 100 nM staurosporine or K252a, respectively, 5, 200 units/ml crude AB-PDGF; 6, 10 ng/ml rBB-PDGF; 7, 20 ng/ml highly purified AB-PDGF; 8 and 9, as in 5, with 15-min pretreatment at 100 nM staurosporine or K252a, respectively; 10 and 11, as in 5, including 200 neutralizing units/ml 7F-D3 anti-MuIFNα/β or polyclonal anti-HuIFNα, respectively. Induced complexes (A/B, according to Fig. 1) are indicated. The major constitutive band is labeled C, as in Fig. 1. The minor, nonspecific band (starred) is not seen consistently in these experiments. The autoradiogram shown represents a 20-h film exposure. b, confluent cultures of A31 cells were untreated, or treated with 1000 IU/ml IFNα, 100 ng/ml TPA, or 1 mM Bt2cAMP for 15 min, then extracted as in a. WCE (5 μg) from each treatment was analyzed by EMSA using labeled ISRE as probe. TPA treatment of parallel cultures resulted in a marked stimulation of c-fos transcription, as determined by nuclear runoff analysis (not shown). Bt2cAMP activity is routinely assayed in our laboratory by induction of neurite outgrowth in a human neuroblastoma cell line. c, WCE of IFNα-induced A31 cultures were pretreated at room temperature for 10 min prior to analysis of ISRE binding activity by EMSA with the following: no compound (0), 10 mM NEM, 10 mM dithiothreitol (DDT), or 10 mM each of NEM and dithiothreitol. Subsequent incubation with end-labeled ISRE was under otherwise standard EMSA conditions (4, a–c). Complexes are marked A/B as in Fig. 1.
might be a component of murine ISGF3, we treated extracts of IFNα-treated A31 cells with NEM in vitro. ISRE binding activity of the complex containing the 65-kDa protein (band A/B, Fig. 4c) was abolished by NEM treatment; thus this protein may be the DNA-binding subunit of murine ISGF3 (NEM would not necessarily have to work directly on p65). Identical results were obtained with the PDGF-induced ISRE complex (data not shown).

Antibodies to p58 IFNα Inhibited PDGF-induced 2-5A Synthetase mRNA Expression but Not ISRE Binding—Previous work on the PDGF-induced 2-5A synthetase response has suggested that it is an indirect result of IFNβ induction (5, 7). To address this possibility, we included a neutralizing antibody, 7F-D3, to p58 IFNα in the culture medium during PDGF treatment of A31 cells. These cells were treated for 6 h (mRNA, Fig. 2a) or 15 min (ISRE binding, Fig. 4a) with PDGF (with and without 200 neutralizing units of 7F-D3) or an equivalent amount of anti-HuIFNα as control. 7F-D3 efficiently inhibited PDGF-induced accumulation of 2-5A synthetase mRNA (Fig. 2a), indicating that IFNα is involved in this response.

We next determined whether 7F-D3 had an inhibitory effect on PDGF induction of ISRE binding factors. This antibody completely inhibits PDGF-induced chloramphenicol acetyltransferase expression from pMulISRECatF (7). Surprisingly, PDGF induced ISRE binding activity regardless of the presence or absence of 7F-D3 antibody (Fig. 4a, compare lanes 5 and 10). Thus, induction of ISRE binding by PDGF did not appear to be mediated by IFNα. This apparent discrepancy with the 2-5A synthetase gene expression data (Fig. 2a) is discussed below.

DISCUSSION

A number of recent studies indicate that ISRE sequences are highly conserved in the 5’ upstream region of a number of IFNα/β-inducible genes. This sequence confers inducibility by IFNγ (32), IFNα (12-17), PDGF (7), and dsRNA (data not shown) on promoters normally unresponsive to these agents. Here we have used photoaffinity labeling experiments to reveal a minimum of three nuclear proteins which specifically contact the ISRE, exhibiting apparent molecular masses of 150, 98, and 65 kDa. Only the 65-kDa protein bound to the ISRE sequence in an IFN-dependent manner. PDGF induced an identical pattern of ISRE-specific complex formation as IFNα (Figs. 3 and 4) on nondenaturing gels, but the abundance of the PDGF-induced complex was much less than was seen with IFN (Fig. 3a). Thus we have not been able to definitively cross-link a 65-kDa (or any other) induced protein in extracts from PDGF-treated cells. Proof of the identity between PDGF- and IFNα-induced ISRE binding factors will require more rigorous biochemical analyses of these induced complexes. We propose the identity of the IFNα-induced 65-kDa ISRE-binding protein as p6558, for 65-kDa IFN-stimulated protein. p6558 is almost certainly a component of the E (early) factor described by Stark, Kerr, and colleagues (15), which binds to the ISRE sequence of the IFNα-inducible 6-16 gene. A similar factor, ISGF3, has been described (31), which mediates activation of the ISRE sequence of ISG-54 and ISG-15 genes (16). Active ISGF3 is a heteromeric complex of two identified protein subunits, ISGF3α and ISGF3γ. IFNα-stimulates the stoichiometric association of ISGF3α and ISGF3γ in the cytoplasm of HeLa cells, followed by nuclear translocation (31). It has not been established which ISGF3 subunit binds to the ISRE. Similarly, active E factor is found in purified cytoplasts of human lymphoblastoid cells after IFNα treatment, in a form that is rapidly translocated to the nucleus in intact cells (15). The 6-16 gene ISRE sequence competes for induced factor binding to the 2-5A synthetase ISRE in vitro. These reports are consistent with identity between ISGF3 and E factor. Formation of HeLa cell ISGF3 in vitro was sensitive to NEM treatment, and NEM treatment of A31 extracts prevented formation of the IFNα-induced p6558 complex in vitro (Fig. 4c). Thus p6558 may represent a DNA-binding subunit of murine ISGF3.

Another broadly inducible factor, NF-κB, undergoes nuclear translocation after TPA treatment of intact cells (21). Activation of NF-κB and nuclear translocation can be catalyzed in vitro by addition of the purified subunit of either Ca2+/phospholipid-dependent protein kinase C or cAMP-dependent protein kinase A, directly implicating these two kinases in signal transduction and physiological activation of NF-κB (33). NF-κB has recently been shown to be dsRNA-inducible (22, 23) and binds to the positive regulatory domain II in the dsRNA-inducible IFNβ gene IRE (30, 34). The 2-5A synthetase ISRE is also highly inducible by dsRNA in transient transcription assays, suggesting that NF-κB might be interacting with the ISRE (data not shown), consistent with the proposed overlap in factors mediating inducibility of IFN genes and IFN-responsive genes (35). A non-DNA-binding cytoplasmic protein of 65 kDa has been identified as an integral part of inducible NF-κB (36). However, p6558 is distinct from the 65-kDa NF-κB subunit on the basis of its DNA binding activity. This distinction is further evident from our experiments with a dsRNA-inducible IRE sequence, shown in Fig. 3b. This sequence spans nucleotides 99–55 of the IFNα gene, and contains four GAAANN motifs (35), including one centered in the NF-κB-binding site (23). Two of these GAAANN motifs are also present, as direct repeats, in the core ISRE. Neither IFNα or PDGF induced factor binding to the IFNα IRE site. In addition, the IFNβ IRE sequence did not compete with the ISRE for binding of p6558 in vitro (data not shown). Finally, in contrast to NF-κB activity, which is unmasked by deoxycytidate (21) IFNα-induced ISRE binding (E factor) is abolished by treatment of cytoplasmic extracts with deoxycytidate (15).

We have previously shown that, like staurosporine and K252a, antibodies to murine IFNα block PDGF induction of the ISRE/chloramphenicol acetyltransferase hybrid gene in A31 cells (7). Therefore, since IFNα is active at picomolar concentrations, significant concentrations of the polypeptide may be synthesized in response to PDGF, even in the presence of cycloheximide, which inhibits protein synthesis incompletely (up to 95%; see “Discussion” in Ref. 37). This suggested the possibility that PDGF induces 2-5A synthetase gene expression indirectly, through the kinase-dependent induction of IFNβ. In order to test whether such an indirect mechanism is responsible for PDGF modulation of ISRE-binding factors, we examined the effect of a monoclonal antibody to murine IFNα on the induction of ISRE factors by PDGF (Fig. 4). This is one of the antibodies shown to block PDGF-induced ISRE/chloramphenicol acetyltransferase activity in transient transcription assays (7). Surprisingly, we observed that PDGF stimulated ISRE binding in the presence of this antibody, even though PDGF-induced 2-5A synthetase mRNA accumulation was blocked under these same conditions (Fig. 2a). Similarly, it has recently been shown that the protein kinase inhibitor, H-7, blocks IFNα-induced 2-5A synthetase mRNA accumulation in human lymphoblastoid cells without affecting IFNα-induced transcription (38). H7 does not inhibit IFNα induction of ISRE binding factors (data not shown). Thus, early signals leading to acti-
vation of ISRE binding are distinguished from secondary signals which determine the expression of ISRE-containing genes.

It has recently been shown that binding of heat shock factor to the heat shock element is insufficient for activation of transcription of heat-inducible genes in MEL cells (39). In the case of the PDGF-induced 2-5A synthetase gene, we conclude that activation of ISRE binding factors is a direct, early response to PDGF, but that this activation alone is inadequate for initiation of transcription from the ISRE (our data do not exclude the involvement of non-ISRE sequences in the overall response of 2-5A synthetase to PDGF). This observation may hold true for other ISRE-containing genes.

We have previously shown that small amounts of IFN (detectable only by radioreceptor assay and antibody neutralization) are produced by confluent, arrested cultures of a human glial cell line (3). IFN is likely also present in the culture medium of the confluent 3T3 cells used here.

IFNα has been proposed to signal cells through rapid generation of diacylglycerol and inositol 1,4,5-triphosphate second messengers, suggesting activation of a Ca2+/phospholipid-dependent protein kinase C in IFNα signal transduction (40). In support of this notion, IFNα has been reported to induce c-fos transcription (41) and diacylglycerol production (39, 42) similar to PDGF. These results imply that mitogenic and growth inhibitory factors share early transmembrane signal transduction pathways.

We have shown here that PDGF and IFNα utilize signaling pathways exhibiting distinct kinase dependencies, in the transcriptional activation of a common pattern of ISRE-dependent gene expression in 3T3 cells. Our data leave open the question of whether signaling events upstream of kinase activation might be shared by PDGF and IFNα. Clearly, there is an advantage to the cell in distinguishing transmembrane signal transduction pathways triggered by activation of specific growth stimulatory (PDGF) and growth inhibitory (IFNα) receptors.

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