Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 8555–8559, November 1990
Biochemistry

ISGF3, the transcriptional activator induced by interferon α, consists of multiple interacting polypeptide chains

(interferon-dependent transcription factor/factor purification/interferon-stimulated response element)

XIN-YUAN FU*, DANIEL S. KESSLER*, SUSAN A. VEALS†, DAVID E. LEVY†, AND J. E. DARNELL, JR.*‡

*Laboratory of Molecular Cell Biology, The Rockefeller University, New York, NY 10021; and †Department of Pathology and Kaplan Cancer Center, New York University Medical Center, New York, NY 10016

Contributed by J. E. Darnell, Jr., August 20, 1990

ABSTRACT **Interferon-stimulated gene factor 3 (ISGF3) is the ligand-dependent transcriptional activator that, in response to interferon treatment, is assembled in the cell cytoplasm, is translocated to the nucleus, and binds the consensus DNA site, the interferon-stimulated response element. We have purified ISGF3 and identified its constituent proteins: a DNA-binding protein of 48 kDa and three larger polypeptides (84, 91, and 113 kDa), which themselves do not have DNA-binding activity. The multisubunit structure of ISGF3 most likely reflects its participation in receiving a ligand-dependent signal, translocating to the nucleus, and binding to DNA to activate transcription.**

Many genes are subject to immediate transcriptional activation by attachment of specific protein ligands to cell surface receptors. However, different polypeptide ligands activate the transcription of different sets of genes (1–8). How this specificity is achieved remains unknown. We began the study of genes that were transcriptionally responsive to interferon α (IFNα-stimulated genes, or ISGs; refs. 2, 3) with the goal of identifying the nuclear transcriptional regulators and how they might be modified after the IFNα receptor had been occupied. We identified the interferon-stimulated response element (ISRE; refs. 9–13), the required binding site for IFNα activation to which presumed transcription factors bind (11). Then ISRE-binding proteins and the conditions required for their presence in cells were determined (11). One IFNα-induced factor, ISG factor 3 (ISGF3), has the necessary characteristics to be the transcriptional activator of the ISGs: it rises rapidly without ongoing protein synthesis (11, 12), requires the same nucleotides for binding as are required for IFN-dependent gene activation (13, 28), and is absolutely dependent on IFNα treatment (3, 14) and its level in cells under a variety of conditions parallels the rate of ISG transcription (15, 16). For example, IFNγ does not induce ISGF3, but cells pretreated with IFNγ make 10 times more ISGF3 when subsequently treated with IFNα and induced transcription of ISGs is 10 times higher (16).

We showed that ISGF3 activation occurs in the cytoplasm and furthermore can be produced *in vitro* by mixing cytoplasm of IFN-treated cells. Our results accord with the report of Dale *et al.* (17) that an IFN-dependent DNA binding factor that is probably ISGF3 could be formed in cytoplasts (enucleated cells) exposed to IFNα. It is possible that the "waiting" cytoplasmic proteins directly receive a signal generated by receptor occupation and then move to the nucleus to activate transcription. In this report we describe the purification of ISGF3 and identification of the constituent polypeptides of this heteromeric transcriptional activator.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Cell Culture, IFN Induction, and Preparation of Nuclear Extract. HeLa cells (ATCC clone S3) in suspension were treated for 15 hr with IFNγ (1 ng/ml) and for 1 hr with IFNα (500 units/ml). Soluble proteins from isolated nuclei were prepared as described (18) by extraction with 0.38 M NaCl. Extracts were dialyzed in buffer D [100 mM KCl/20 mM Hepes, pH 7.9/0.5 mM EDTA/1 mM dithiothreitol/20% (vol/vol) glycerol] and stored at −70°C. The final protein concentration of extracts was 3–5 mg/ml.

Gel Shift Analysis. Gel retardation analysis was performed essentially as described (12, 19, 20) with ISG15 (24-nucleotide probe) and ISG54 (110-nucleotide probe). *In vitro* complementation was achieved by including an appropriate complementing extract in the standard DNA-binding reaction as described above.

Preparation of Sequence-Specific DNA Affinity Column. By using established methods (21, 22), the following multimerized double-stranded ISG54 ISRE oligonucleotide (11) was coupled to Sepharose.

```
5′-AATTTCACTTTCTAGTTTCACTTTCCCTTTTGT-3′
    3′-AGTGAAAGATCAAAGTGAAAGGGAAAACATTAA-5′
```

For the mutant DNA affinity column the following doubled-stranded oligonucleotide was used.

```
5′-GGATCCAAAGGGAAACCCAAACTG-3′
    3′-GGTTTCCCTTTGGGTTTGACTAGG-5′
```

Purification of ISGF3. Typically, extracts of 2–4 × 10^{10} cells were loaded in buffer D with 0.1 M KCl at 4°C onto a 100-ml phosphocellulose (Whatman P-11) column and adsorbed proteins were successively eluted in steps with buffer D containing 0.4 M, 0.6 M, or 0.8 M KCl. Fractions with peak gel-shift activity (the 0.6 M eluate) were dialyzed against buffer D containing 100 mM KCl and then applied to and eluted from a 60-ml heparin-agarose column. Peak activity fractions (0.6 M) were pooled, dialyzed against buffer D (with Tris·HCl instead of Hepes), and loaded onto and eluted from a DEAE-Sephacel column. The peak activity fractions (0.4–0.5 M KCl) were dialyzed against HG200 (200 mM KCl/20 mM Hepes, pH 7.9/0.5 mM EDTA/0.1 mM EGTA/0.05% Nonidet P-40/2 mM dithiothreitol) and loaded onto a nonspecific thymus DNA-cellulose column (Sigma) to which ISGF3 did not bind. The flow-through fraction was dialyzed against HG100 buffer (containing 100 mM KCl) and subjected to two rounds of DNA-affinity chromatography with the wild-type ISRE. Peak activity was eluted between 0.4 and 0.6 M KCl, and dialyzed fractions (HG200) were passed through a mutant ISRE affinity column before a third wild-type DNA affinity column. Fractions from the 0.4–0.6 M KCl eluate

Abbreviations: IFN, interferon; ISG, IFN-stimulated gene; ISRE, IFN-stimulated response element; ISGF*n*, ISG factor *n*.

‡To whom reprint requests should be addressed.

were dialyzed against TS100 (100 mM NaCl/20 mM Tris·HCl, pH 7.9/0.5 mM EDTA/20% glycerol/0.1 mM EGTA/2 mM dithiothreitol), loaded onto a 1-ml FPLC Mono Q anion-exchange column, and eluted with a 20-ml gradient from 0.1 M to 1 M NaCl; peak fractions were dialyzed against buffer D. Renaturation of ISGF3 binding activity after SDS/PAGE was carried out essentially as described (22), with minor modifications.

***In Vitro* Transcription.** *In vitro* transcription reactions were conducted in a volume of 50 μl containing 100 ng of pE40 or pE40-7 template DNA (see Fig. 1), 50 ng of adenovirus pMLP template DNA, and 400 ng of nonspecific carrier DNA (pGEM-4; Promega) in transcription buffer (20 mM Hepes, pH 7.9/50 mM KCl/7 mM $MgCl_2$/12% glycerol/1 mM dithiothreitol/0.1 mM EDTA); 0.6 μM ATP, GTP, and CTP; 30 μM UTP (10 μCi of [α-^{32}P]UTP, NEN; 1 μCi = 37 kBq) and 15 μl of nuclear extract (≈5 mg/ml) from cells that had not been treated with IFN. The reaction mixture was supplemented with 100 ng of ISGF3 purified by three rounds of DNA affinity chromatography. After 45 min at 30°C, 100 μl of "stop" buffer (50 mM Hepes, pH 7.9/1% SDS/10 mM EDTA with tRNA at 25 mg/ml) was added, and RNA was isolated, subjected to denaturing PAGE, visualized by autoradiography, and quantitated by laser densitometry.

Preparative Gel Shift Analysis. ISRE complexes were formed with purified fractions of ISGF3 in reaction mixtures containing a total of 175 ng of ISRE or unrelated oligonucleotide and were resolved by the gel shift protocol (12). Complexes were excised following autoradiography, and polypeptides were directly electroeluted onto an SDS/8% polyacrylamide gel for electrophoretic separation and detection by silver stain.

RESULTS

Purification of ISGF3. The purification scheme is shown in Table 1. Nuclear extracts from suspension cultures of HeLa cells that had been treated for 15 hr with IFNγ and 1 hr with IFNα were monitored for ISGF3 activity by electrophoretic mobility-shift assays (12, 19, 20). Complete separation of ISGF3 from the IFN-induced protein factor ISGF2 and the constitutive factor ISGF1 (11, 12) was achieved by phosphocellulose and heparin-agarose chromatography. The most significant step in purification was oligonucleotide affinity chromatography (21, 22), which was carried out by stepwise increases in KCl, with most of the ISGF3 DNA-binding activity resisting washes up to 0.4 M KCl but eluted at 0.6 M KCl. The mutant oligonucleotide column bound about half the protein in the partially purified sample but very little ISGF3 bound. The final step in the overall 20,000-fold purification was chromatography by FPLC on Mono Q.

Table 1. Purification of ISGF3

Fraction	Total protein, mg	Specific activity,* units/μg	Fold purification	Cumulative yield
Nuclear extract	400	0.6	1	1
P-11	88	3.2	5	1.2
Heparin-agarose	24	9	14	0.9
DEAE-Sephacel	8	19	30	0.62
Nonspecific DNA-cellulose	5	25	40	0.48
Wild-type DNA-Sepharose (two passes)	0.032	2,266	3,625	0.28
Mutant DNA-Sepharose	0.018	2,834	4,534	0.20
Wild-type DNA-Sepharose	0.006	6,377	10,200	0.14
FPLC Mono Q	0.0025	10,866	>20,000	0.10

*One unit of binding activity is defined as the amount of ISGF3 required to bind 1 fmol of ISG15 ISRE probe.

Functional Characterization of Purified ISGF3. The purified ISGF3 bound to and protected from DNase digestion both strands of the ISG54 ISRE over a 25- to 30-base-pair region (data not shown) that contained all the bases whose sequence integrity is required for IFN stimulation (11, 13).

In addition, the highly purified material had functional activity as an ISRE-dependent activator of an ISG promoter during *in vitro* transcription (Fig. 1). A template with seven copies of the ISRE (E40-7) was transcribed about 4-fold better (normalized to the signal from the control adenovirus major late template) when the purified ISGF3 was added, whereas the transcription of a template lacking the ISRE sequences (E40) was unchanged.

An ≈45-kDa ISRE-Binding Protein Corresponds to ISGF3γ. To determine which polypeptide(s) in the ISGF3 preparation contacted the ISRE, a photoaffinity UV-crosslinking experiment (23) showed that purified ISGF3 plus an internally labeled 5-bromo-2'-deoxyuridine-substituted double-stranded oligonucleotide formed a single 55-kDa complex. This complex, which was specific (i.e., blocked by excess unlabeled ISRE), corresponded to a polypeptide of ≈45 kDa after adjustment for crosslinked DNA (data not shown).

We have shown (12) that ISGF3 can be formed upon mixing the cytoplasm of IFNα-treated cells (containing small amounts of ISGF3) and the cytoplasm of IFNγ-treated cells (having no ISGF3). We refer to the necessary protein(s) in the cytoplasm of IFNα-treated cells as ISGF3α and the necessary protein(s) in the cytoplasm of IFNγ-treated cells as ISGF3γ. Cytoplasmic fractions containing ISGF3γ or ISGF3α activity have been separated so that partially purified complementary preparations were available (24).

To further characterize the polypeptides required to form ISGF3, an affinity-purified ISGF3 sample was denatured and polypeptides were separated by SDS/PAGE. Before denaturation and electrophoresis, this starting material produced

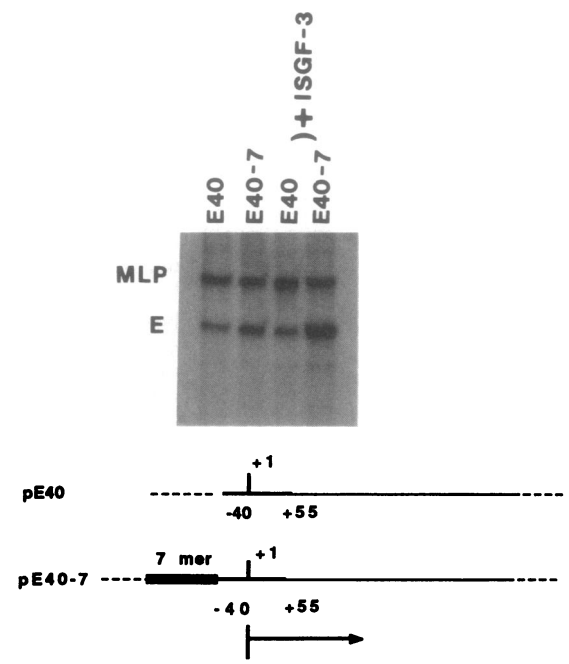

FIG. 1. ISGF3 stimulates ISRE-dependent transcription *in vitro*. ISGF3 supplemented *in vitro* transcription reaction mixtures with pE40 (ISG54 promoter; −40 to +54) and pE40-7 (pE40 plus seven copies of the ISG15 ISRE; see *Materials and Methods* and ref. 28). Transcription from the adenovirus major late promoter (MLP) provided an internal control.

not only the characteristic ISGF3 complexes but also an ISRE-specific complex that migrated more rapidly (open arrow in Fig. 2*A*). This high-mobility activity, like ISGF3 itself, is blocked from complex formation only by the full ISRE sequence and not by the core sequence (11, 13, 24), which is sufficient to block complex formation by ISGF2 or ISGF1 (ref. 12; data not shown). Proteins from a series of gel slices were renatured; protein in the size range 45–50 kDa formed a specific complex with the ISRE that comigrated with the high-mobility complex present in the starting material (open arrow). In no renaturation experiment was the lower mobility ISGF3 recovered. However, when the renatured 45- to 50-kDa protein fraction was mixed with partially purified cytoplasmic ISGF3α and assayed by gel retardation, a complex was formed that comigrated with ISGF3 (Fig. 2*B*), indicating that ISGF3γ is an ≈45-kDa DNA-binding protein. This conclusion is consistent with the UV-crosslinking of an ≈45-kDa component of ISGF3 to the ISRE. No ISGF3α was recovered during renaturation, indicating that it is less stable than ISGF3γ or, alternatively, consists of two or more different-sized polypeptides.

ISGF3 Is Composed of Four Distinct Polypeptides. Since the affinity-purified preparations still contained a number of polypeptides (see silver stains in Fig. 3*B*, lane 2), additional purification was required. An affinity-purified nuclear preparation was subjected to chromatography on the anion-exchange matrix Mono Q; an increasing gradient of NaCl was used for elution and ISGF3 gel shift activity and total protein were monitored (Fig. 3). The majority of ISGF3 activity was eluted within a narrow range of KCl concentration, with the majority of the activity in a single fraction (no. 30; Fig. 3*A*, lane 7). In addition, protein that was eluted earlier (fractions 27–29, lanes 4–6) formed the high-mobility complex consistent with free ISGF3γ activity. These fractions (i.e., nos. 27–29) contained a major protein band of 48 kDa that co-

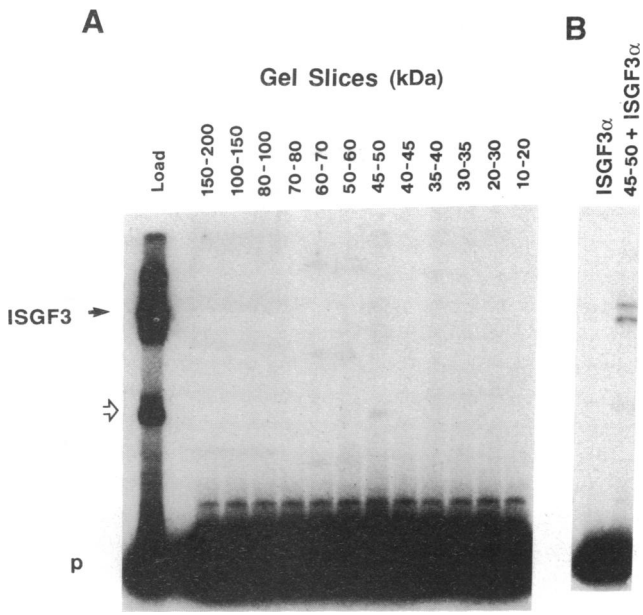

FIG. 2. Renaturation of the DNA-binding subunit of ISGF3, an ≈45-kDa polypeptide corresponding to ISGF3γ. (*A*) Affinity-purified ISGF3 was denatured and subjected to SDS PAGE, and proteins of various sizes were separately eluted and allowed to renature (22). The gel shift pattern produced by the starting material (load lane) included ISGF3 and a high-mobility ISRE-binding activity (open arrow). The high-mobility DNA-binding activity was recovered from the 45- to 50-kDa size range; no complete ISGF3 was recovered. p, Probe. (*B*) Renatured protein fraction (45–50 kDa) was supplemented with partially purified cytoplasmic ISGF3α and assayed for ISGF3 activity.

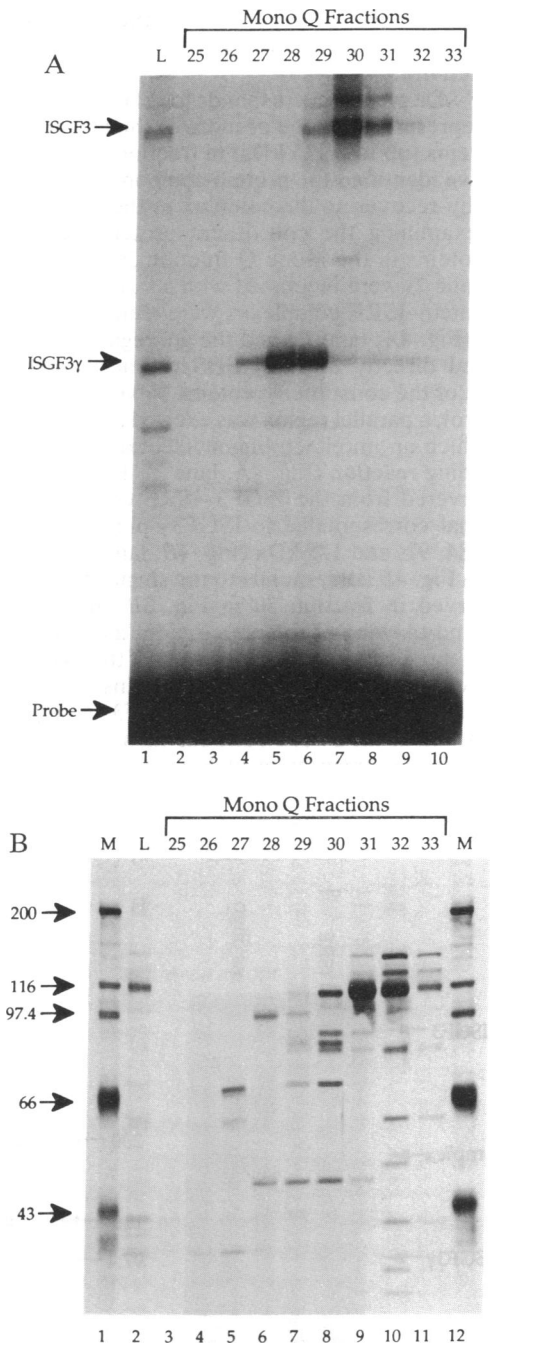

FIG. 3. ISGF3 fractionation on FPLC Mono Q. (*A*) An affinity-purified ISGF3 preparation was applied to an FPLC Mono Q column and adsorbed proteins were eluted with a NaCl gradient (see *Materials and Methods*) and tested for ISRE binding by gel retardation. The starting material contained ISGF3 and the high-mobility ISGF3γ activities (lane 1), which were partially fractionated between fractions 27 and 30. (*B*) Protein composition of Mono Q fractions visualized by SDS/8% PAGE and silver staining. Molecular mass (kDa) markers (M) are indicated at left (lanes 1 and 12).

fractionated with the high-mobility ISRE-binding activity, and (similar to Fig. 2*B*) mixing of fraction 28 with cytoplasmic ISGF3α resulted in the formation of high levels of ISGF3 (data not shown). Separate experiments (24) have shown that sedimentation of ISGF3 in the absence of the ISRE allows resolution of ISGF3α and ISGF3γ, and again the ISGF3γ fraction contains a 48-kDa protein. Thus all experiments using complementation of purified protein fractions with cytoplasmic extracts of IFNα-treated cells support the con-

clusion that the ISGF3γ portion of ISGF3 is a 48-kDa DNA-binding protein that forms ISGF3 in the presence of ISGF3α activity.

If the 48-kDa protein corresponds to ISGF3γ, then ISGF3α must be represented by one or more of the higher molecular mass proteins (up to ≈113 kDa) in fraction 30 (Fig. 3*B*, lane 8). We have identified the protein components of the ISGF3 complex by recovering the complex in the native state and directly examining the constituent proteins (Fig. 4). The ISGF3 proteins in the Mono Q fraction 30 (Fig. 3*B*, lane 8; Fig. 4*B*, lane 2) were incubated with a large excess of ISRE probe, protein–ISRE complexes were identified by autoradiography (Fig. 4*A*, lane 1), and the gel regions were excised and loaded directly onto an SDS/polyacrylamide gel for resolution of the constituent proteins (silver stains, Fig. 4*B*). As a control, a parallel region was excised from a neighboring lane in which an unrelated oligonucleotide was used in the DNA-binding reaction (Fig. 4*A*, lane 2). Five protein bands were recovered from the ISGF3–ISRE complex, a 48-kDa doublet that corresponded to ISGF3γ plus three additional bands of 84, 91, and 113 kDa (Fig. 4*B*, lane 3). (The 48-kDa doublet in Fig. 4*B* corresponds to the single 48-kDa polypeptide observed in fraction 30 in Fig. 3*B* and arose during freezing and thawing of the preparation, as seen in Fig. 4*B*, lane 2.) In three additional experiments of this type (e.g., Fig. 4*C*) we consistently detected four proteins (48, 84, 91, and 113 kDa) as specific components of ISGF3. A single band at 48 kDa as well as the 84-, 91-, and 113-kDa polypeptides were recovered in the experiment of Fig. 4*C*, which used a less pure preparation of ISGF3 than that used for Fig. 4*B*. None of the proteins recovered from the ISGF3 complex was present in the analogous excised region from the control reaction (Fig. 4*B*, lane 5). Proteins that formed the nonspecific complex with either the ISRE or unrelated oligonucleotides corresponded to the two additional major contaminating polypeptides present in fraction 30 (Fig. 4*B*, lanes 4 and 6). Therefore, of the six major polypeptides present in the peak ISGF3 Mono Q fraction, four were specifically retained as components of the ISGF3-ISRE complex (48, 84, 91, and 113 kDa) and two were present as nonspecific DNA-binding activities.

DISCUSSION

As the studies of gene activation by IFNα have proceeded, two points of particular interest have emerged. First, the activation of transcription by IFNα absolutely requires specific receptor–ligand interaction: agents such as calcium ionophores, dibutyryl cAMP, or phorbol esters, which increase the intracellular concentration of second messengers, do not stimulate or inhibit the transcriptional response and no other known polypeptide ligand can trigger the response (3, 14). These observations promise the existence of a highly specific intracellular receptor–protein interaction within the cytoplasm that can lead to the demonstrably specific transcriptional inductions. Second, ISGF3 is composed of proteins that are in the cell cytoplasm before IFN treatment (12). The rapidity of ISGF3 activation (within 1 min) suggests that the immediate cytoplasmic targets of the intracellular action by the receptor, or by a protein associated with the receptor, may well be a subunit of the transcription factor ISGF3 itself. Thus, the purification and characterization of the ISGF3 subunits has been a prime goal of our research. The purified ISGF3 binds the ISRE and stimulates transcription of ISRE-

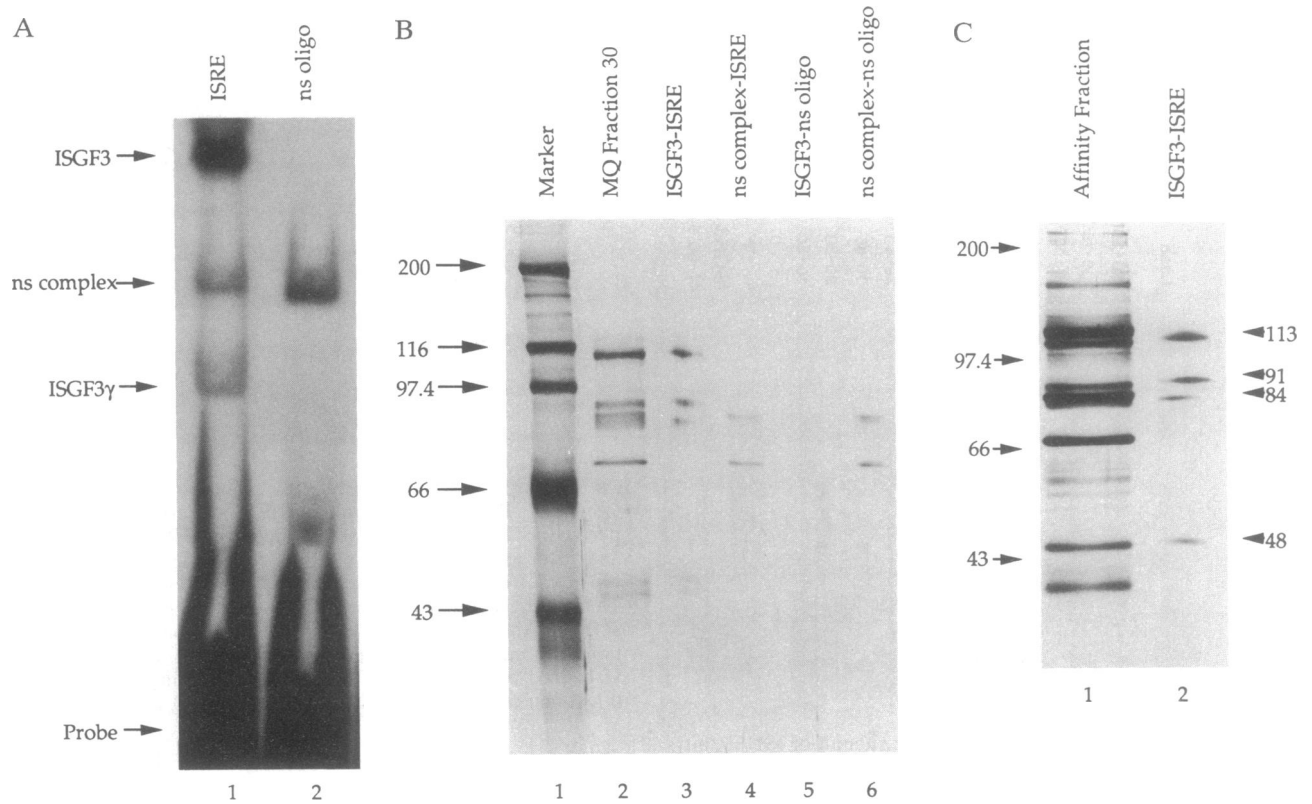

FIG. 4. ISGF3 is composed of four distinct polypeptides. (*A*) Partially purified ISGF3 [Mono Q (MQ) fraction 30] was incubated with a radiolabeled ISRE (lane 1) or unrelated oligonucleotide [nonspecific (ns) oligo; lane 2], and protein–DNA complexes were fractionated by gel retardation. ISGF3 and ISGF3γ complexes were formed with the ISRE and a contaminant nonspecific (ns) complex was formed with both probes as indicated at left. After autoradiography, regions of the preparative gel corresponding to all three complexes were excised from both lanes. (*B*) Polypeptides present in the excised slices were electroeluted onto an SDS/8% polyacrylamide gel for electrophoretic separation and stained with silver. (*C*) An experiment similar to that described in *A* and *B* but using a less pure affinity fraction of ISGF3 (lane 1). Four polypeptides (48, 84, 91, and 113 kDa; lane 2) were specifically recovered from the ISGF3–ISRE complex.

containing templates. Photoaffinity crosslinking, gel renaturation, and chromatography experiments all show that a 48-kDa polypeptide can bind the ISRE and, when complemented with ISGF3α activity, can form the complete ISGF3. Thus, the 48-kDa ISRE-binding activity corresponds to ISGF3γ.

The most purified ISGF3 preparations still contained six proteins. However, use of the most purified preparations to form the ISGF3 complexes allowed us to identify three proteins (84, 91, and 113 kDa) in addition to the 48-kDa DNA-binding protein as constituents of ISGF3. Perhaps it is not surprising that ISGF3 contains several proteins, since we know that at least three functions must be served in fulfilling its role as an activator. The IFNα-activated portion of ISGF3 must (*i*) interact with the IFNα receptor, either directly or via some specific intermediate protein, to conserve the specificity of the ligand–receptor interaction; (*ii*) undergo nuclear translocation; and (*iii*) interact specifically with ISGF3γ to form the complete ISGF3 complex. We assume that the functions other than DNA binding must be carried out by one or more of the larger subunits. Since the same four proteins in the same relative amounts were recovered in several separate experiments, it seems reasonable that each of the observed proteins is distinct and takes part either in ISGF3 formation or in delivery to the nucleus, although only when the genes encoding these proteins are cloned will we be certain. Perhaps first among many important questions that can then be answered is which, if any, of the IFNα-affected portions of ISGF3 undergoes a posttranslational change (e.g., cleavage or phosphorylation) during activation.

A key issue on which all of the work on IFN stimulation of transcription bears is the specificity of responses triggered by different cell surface ligands. The ISRE is distinct for genes stimulated by IFNα, and genes activated by (for example) platelet-derived growth factor, nerve growth factor, or tumor necrosis factor will surely have different consensus response elements. Therefore it seems quite possible, if not in fact likely, that a series of proteins functionally analogous to ISGF3γ (sequence-specific DNA binding) will exist. Obviously, if one of the proteins of ISGF3 specifically contacts the IFNα receptor, an equally specific protein may exist to recognize receptors bound to each of the above-named polypeptide ligands. If so, the transcriptional response in all these cases could depend on two specific intracellular proteins, one that recognizes the receptor and one that is a DNA-binding protein.

Both steroid receptors and NF-κB activation (25–27) are thought to involve cytoplasmic protein–protein interactions that release an active factor from inhibition, a possibly different course of events than IFN-dependent cytoplasmic activation of ISGF3. However, the DNA-binding portion of NF-κB enters the nucleus in association with a second, non-DNA-binding polypeptide. Whether there is a similarity between any of the components of NF-κB and ISGF3 will be of considerable interest.

We thank R. Pine and C. Schindler for advice and help in the purification of ISGF3; N. Reich for plasmid pTK-7; Sushma Patel and Mario Luca for excellent technical assistance; and Drs. P. Sorter (Hoffman–LaRoche) and D. Vapnek (Amgen) for generous gifts of IFNα and IFNγ, respectively. This work was supported by grants from the National Institutes of Health and the American Cancer Society (to D.E.L. and J.E.D.). X.Y.F. is a Fellow of the Cancer Research Institute/Fan Fox and Leslie R. Samuels Foundation (New York). D.S.K. was supported by a predoctoral training grant from the National Institutes of Health (AI07233-15).

1. Murdoch, G. H., Potter, E., Nicolaissen, K., Evans, R. M. & Rosenfeld, M. G. (1982) *Nature (London)* **300,** 192–194.
2. Larner, A. C., Jonak, G., Cheng, Y. S., Korant, B., Knight, E. & Darnell, J. E., Jr. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6733–6737.
3. Larner, A. C., Chaudhuri, A. & Darnell, J. E., Jr. (1986) *J. Biol. Chem.* **261,** 453–459.
4. Greenberg, M. E. & Ziff, E. B. (1984) *Nature (London)* **311,** 433–438.
5. Friedman, R. L., Manly, S. P., McMahon, M., Kerr, I. M. & Stark, G. R. (1984) *Cell* **38,** 745–755.
6. Greenberg, M. E., Greene, L. A. & Ziff, E. B. (1985) *J. Biol. Chem.* **260,** 14101–14110.
7. Lau, L. F. & Nathans, D. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 1182–1186.
8. Lee, T. H., Lee, S. W., Ziff, E. B. & Vilcek, J. (1990) *Mol. Cell. Biol.* **10,** 1982–1988.
9. Levy, D. E., Larner, A. C., Chaudhuri, A., Babiss, L. E. & Darnell, J. E., Jr. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 8929–8933.
10. Reich, N., Evans, B., Levy, D. E., Fahey, D., Knight, E. & Darnell, J. E., Jr. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 6394–6398.
11. Levy, D. E., Kessler, D. S., Pine, R., Reich, N. & Darnell, J. E., Jr. (1988) *Genes Dev.* **2,** 383–393.
12. Levy, D. E., Kessler, D. S., Pine, R. & Darnell, J. E., Jr. (1989) *Genes Dev.* **3,** 1362–1371.
13. Kessler, D. S., Levy, D. E. & Darnell, J. E., Jr. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8521–8525.
14. Lew, D. J., Decker, T. & Darnell, J. E., Jr. (1989) *Mol. Cell. Biol.* **9,** 5404–5411.
15. Kessler, D. S., Pine, R., Pfeffer, L. M., Levy, D. E. & Darnell, J. E., Jr. (1988) *EMBO J.* **7,** 3779–3783.
16. Levy, D. E., Lew, D. J., Decker, T., Kessler, D. S. & Darnell, J. E., Jr. (1990) *EMBO J.* **9,** 1105–1111.
17. Dale, T. C., Imam, A. M. A., Kerr, I. M. & Stark, G. R. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 1203–1207.
18. Dignam, J. D., Martin, P. L., Shastry, B. S. & Roeder, R. G. (1983) *Methods Enzymol.* **101,** 582–598.
19. Fried, A. & Crothers, D. M. (1981) *Nucleic Acids Res.* **9,** 6505–6525.
20. Garner, M. M. & Revzin, A. (1981) *Nucleic Acids Res.* **9,** 3047–3059.
21. Kadonaga, J. T. & Tjian, R. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 5889–5893.
22. Briggs, M. R., Kadonaga, J. T., Bell, S. P. & Tjian, R. (1986) *Science* **234,** 47–52.
23. Chodosh, L. A., Baldwin, A. S., Carthew, R. W. & Sharp, P. A. (1988) *Cell* **53,** 11–24.
24. Kessler, D. S., Veals, S. A., Fu, X.-Y. & Levy, D. E. (1990) *Genes Dev.* **4,** in press.
25. Picard, D. & Yamamoto, K. R. (1987) *EMBO J.* **6,** 3333–3340.
26. Baeuerle, P. A. & Baltimore, D. (1988) *Cell* **53,** 211–217.
27. Baeuerle, P. A & Baltimore, D. (1988) *Science* **242,** 540–546.
28. Reich, N. & Darnell, J. E., Jr. (1989) *Nucleic Acids Res.* **17,** 3415–3424.