Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 8521–8525, November 1988
Cell Biology

Two interferon-induced nuclear factors bind a single promoter element in interferon-stimulated genes

(transcriptional regulation/positive and negative control/interferon action/interferon-stimulated response element mutagenesis)

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Contributed by James E. Darnell, Jr., August 15, 1988

ABSTRACT **Nuclear proteins induced by interferon (IFN) treatment of human cells are capable of forming two specific complexes with DNA fragments containing the IFN-stimulated response element (ISRE). These two complexes, designated B2 and B3, are distinguished by differential migration in gel retardation assays. The factor that forms the B3 complex, termed IFN-stimulated gene factor 3 (ISGF-3), preexists in cells, is activated upon IFN treatment, and appears with kinetics paralleling those for transcriptional activation of IFN-stimulated genes. The factor that forms the B2 complex (ISGF-2) appears following a time lag after IFN treatment during which protein synthesis must occur. By extensive point mutagenesis of the ISREs from two IFN-stimulated promoters (ISG54 and ISG15), we demonstrate that the B2 and B3 complexes are formed by factors binding to the same DNA sequence. Mutations at this site decrease or eliminate transcriptional activation and impair binding of both ISGF-2 and ISGF-3. This analysis has shown that the ISGF-3 binding site is slightly broader than the ISGF-2 binding site, which is completely contained within the sequence necessary both for ISGF-3 binding and for transcriptional activation. The evidence strongly implicates ISGF-3 as the positive transcriptional regulator of IFN-stimulated genes.**

Transcriptional activation of gene expression underlies the primary response of cultured human cells to interferon (IFN) treatment, resulting in accumulation of a number of new proteins (1–3) and subsequent long-term changes in cellular physiology. In a study of mechanisms responsible for this immediate transcriptional response elicited by IFN binding to specific cell-surface receptors, we identified cDNA clones representing IFN-induced mRNA species (1) and have isolated and characterized genomic DNA sequences for two such IFN-stimulated genes, termed ISG54 (4, 5) and ISG15 (6) to indicate that polypeptides of 54 and 15 kDa, respectively, are encoded by these genes. Analysis of progressive deletions of 5′ flanking DNA sequences indicated that ≈120 nucleotides upstream of each mRNA start site was necessary to direct the IFN transcriptional response (4–6). A 77-base-pair fragment from this region of ISG15 has been shown to function as a position- and orientation-independent IFN-stimulated enhancer (6). Sequence comparison of these functional regions revealed a 12- of 15-base-pair sequence similarity in opposite orientations in the ISG54 and ISG15 promoters (5, 6). Similar sequences have been found in promoter regions of all other IFN-α/β-stimulated genes described so far (5–10). This conserved sequence, which constitutes an IFN-stimulated response element (ISRE), was protected in DNase I footprint experiments with crude extracts from IFN-responsive cells. Introduction of two different point mutations into the ISG54 ISRE abolished transcriptional activity of the DNA in transfection experiments (5).

Nuclear proteins that specifically recognize the ISRE have been characterized further by gel retardation assays and by methylation interference footprints (5). At least three protein–DNA complexes, which were distinguished by differential mobilities in gel retardation assays, can be formed between factors from IFN-treated cells and DNA fragments containing the ISRE (5, 8–10). One complex (termed B1) is formed by proteins found in untreated or IFN-treated cells that do not vary in abundance or binding ability during the IFN response. Two additional complexes (termed B2 and B3) are formed by factors present only in IFN-treated cells. All three of these DNA–protein complexes involve recognition of functional ISRE sequences (5).

The IFN-induced factors giving rise to the B2 and B3 complexes can be distinguished by differential kinetics of accumulation (5) and have been separated by biochemical fractionation (R. Pine, D.E.L., and J.E.D., unpublished data). The factor responsible for formation of B3, termed IFN-stimulated gene factor 3 (ISGF-3), appears rapidly after IFN treatment even in the absence of *de novo* protein synthesis, paralleling the kinetics and characteristics of IFN-stimulated transcription (2, 4, 5). In contrast ISGF-2, the protein responsible for the IFN-induced B2 complex, which has been purified to homogeneity, requires ongoing protein synthesis for its induction and is not detectable until ≈90 min after IFN treatment (5). These characteristics suggested that ISGF-3 may be directly involved in ISG transcriptional induction, whereas ISGF-2 cannot be required for gene activation. ISGF-2 may be involved, however, in the regulated protein synthesis-dependent decrease in ISG transcription characteristic of the IFN primary response (2).

To delineate the specific DNA recognition requirements of ISGF-2 and ISGF-3, and in hope of separating genetically the activities of these two DNA binding proteins, we have carried out extensive mutagenesis of the ISRE of ISG54 as well as site-directed mutagenesis of the ISRE region of ISG15. From the analysis of 20 mutations in ISG54 and 4 specific changes within ISG15, we find that the ISGF-2 binding site is entirely encompassed by a slightly larger binding site for ISGF-3. Furthermore, the critical contacts for ISGF-3 binding *in vitro* correlate with sequences necessary for maximal transcriptional activity *in vivo*.

MATERIALS AND METHODS

ISRE Mutagenesis. Mutagenesis of ISG54 was carried out in an expression construct in which promoter sequences spanning from 122 nucleotides upstream of the RNA initiation site to 55 nucleotides into exon 1 were fused to a reporter

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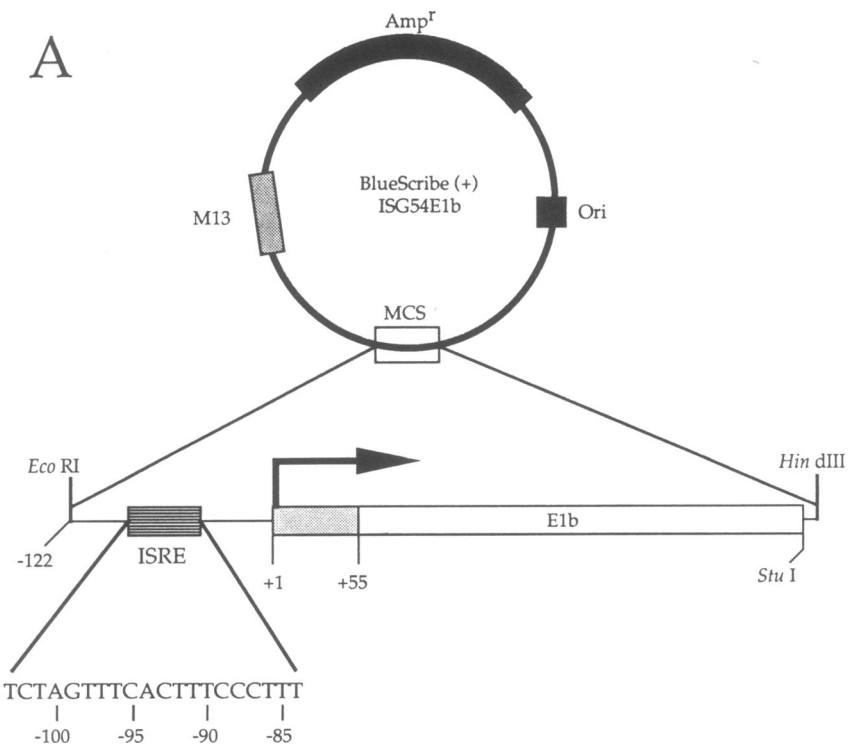
Abbreviations: IFN, interferon; ISG54 and ISG15, IFN-stimulated genes encoding polypeptides of 54 and 15 kDa, respectively; ISRE, IFN-stimulated response element; ISGF, IFN-stimulated gene factor.

sequence, an adenovirus type 5 E1b gene fragment (5), and cloned into a multifunctional plasmid containing an M13 origin of replication (BlueScribe; Stratagene, La Jolla, CA) as diagrammed in Fig. 1*A*. Single-stranded templates containing occasional uridine residues substituted for thymidine (11) were used for oligonucleotide-primed mutagenic repair performed as described (12) with mixed synthesis oligonucleotides (13, 14) produced by using a mixture containing 3% of each of three incorrect nucleotides and 91% of the wild-type nucleotides at each position (obtained from MCRC, Midland, TX). Successfully repaired plasmids were selected for ability to transform wild-type bacteria (11), and each mutation was identified by DNA sequencing (15). Site-directed mutagenesis of ISG15 was carried out by complete synthesis of four sets of 18 nucleotide complementary oligonucleotides spanning nucleotides −111 to −94 of the ISG15 promoter, each containing a single deviation from the wild-type sequence as shown in Fig. 2.

Binding Assays. Protein–DNA binding assays were performed by gel retardation (5, 16, 17). Partially purified preparations of ISGF-3 or ISGF-2 were incubated with 0.1 ng of radiolabeled ISG15 promoter fragment containing the ISRE sequence (−115 to −39) in the presence of nonspecific DNA [4 μg of double-stranded poly(dI-dC)] and a constant amount of total plasmid DNA consisting of varying amounts of wild-type or mutant ISRE sequence. The ability of each mutant DNA sequence to compete with labeled wild-type ISG15 for binding to ISGF-2 and ISGF-3 was determined by comparison to competitions using wild-type or vector DNA. Purification of ISGF-2 and ISGF-3 involved chromatography on heparin-agarose, phosphocellulose, DEAE-cellulose, nonspecific and specific oligonucleotide affinity resins, and Mono Q FPLC and will be reported elsewhere (R. Pine, D.E.L., and J.E.D., unpublished data).

	100	95	90	85	B2	B3
ISG54	T C T A G T T T C A C T T T C C C T T T				+	+
G_{103}	G				+	+
A_{102}	A				+	+/-
C_{101}	C				+	+/-
G_{101}	G				-	-
G_{100}	G				+/-	-
T_{99}	T				+/-	-
$A_{98}G_{97}$	A G				-	-
A_{97}	A				-	-
A_{95}	A				-	-
G_{94}	G				+	+
T_{94}	T				-	-
A_{92}	A				-	-
A_{91}	A				-	-
A_{90}	A				-	-
T_{89}	T				+	+/-
$G_{88}G_{87}$	G G				+	+
$T_{87}G_{86}$	T G				+	+/-
ISG15	C A G T T T C G G T T T C C C T T T				+	+
C_{99}	C				+	+
G_{95}	G				-	-
G_{89}	G				+/-	-
G_{88}	G				+/-	-

FIG. 2. Wild-type and mutant ISRE sequences. Numbered positions indicate nucleotides upstream from the ISG54 RNA initiation site and these numbers are used throughout. The ISG15 ISRE is inverted in its natural setting relative to this representation and corresponds to −111 to −94 from the ISG15 initiation site. Mutations are indicated by the nucleotides that differ from wild type with a subscript denoting the mutated position. Columns B2 and B3 indicate extent of binding of each sequence by partially purified preparations of the IFN-induced nuclear factors ISGF-2 and ISGF-3, respectively. −, <25% competition of wild-type binding; +/−, 25–75% competition; +, equal or nearly equal competition to that of the wild-type sequence (see Figs. 3 and 4).



B

Functional ISREs

T A G T T T C A C T T T C C C	ISG54
C A G T T T C G G T T T C C C	ISG15
G A G T T T C A T T T T C C C	6-16a
C A G T T T C A T T T T C C C	6-16b
T G G T T T C G T T T C C T C	OAS

FIG. 1. Recombinant ISG54 expression plasmid. (*A*) Organization of the ISG54 expression plasmid used for mutagenesis, factor binding studies, and *in vivo* expression experiments. The 20-nucleotide region of the ISRE, which was mutated by random oligonucleotide mutagenesis (see *Materials and Methods*), is indicated, as well as the position of the portion of the ISG54 first exon (+1 to +55) fused to the adenovirus type 5 E1b gene. (*B*) ISRE sequences are shown that have been documented by direct transcriptional analysis and transfection studies to mediate rapid induction by IFN in human cells (4–10).

Expression Assays. ISG54 mutants were tested functionally for IFN-stimulated transcriptional activity by transient transfection. Plasmid DNA (10 μg of specific DNA) was introduced into HeLa Cl2.2 cells by calcium phosphate precipitation (18), and accumulated RNA levels were measured by RNase protection (5, 19) 72–96 hr later, with or without a final 4-hr treatment with 15 pM IFN-α (500 units/ml). This regimen has been found to circumvent artifactual stimulation of ISGs by DNA calcium phosphate precipitates (20) and allows transient assays for IFN-stimulated transcription (6). RNase protection assays were quantitated by densitometry and normalized for RNA recovery and for transfection efficiency by measuring endogenous γ-actin production (21), endogenous ISG54 expression, and chloramphenicol acetyltransferase activity derived from cotransfected RSVcat (22). HeLa cells were grown in Dulbecco's modified Eagle's medium supplemented with 15% fetal bovine serum and antibiotics. Recombinant bacterial IFN-αA was a generous gift from P. Sorter (Hoffman–La Roche, Nutley, NJ).

RESULTS

ISG54 Mutagenesis. The position and sequence of the ISRE in a recombinant ISG54–adenovirus fusion plasmid (4) is diagrammed in Fig. 1. By modification of the technique of Kunkel (11), mutations were introduced into this plasmid by using a mixture of synthetic oligonucleotides complementary to the ISRE that were synthesized to contain one or two errors in every 20 nucleotides. The oligonucleotide mixtures were hybridized to single-stranded DNA containing the ISRE sequence and enzymatically converted to double strands; mutants were selected by transformation into appropriate bacterial hosts. Identification of each mutation was achieved by DNA sequence analysis. Over 30 individual mutations were studied both by transfection analysis to establish functional effects of mutant sequences and by gel retardation assays to test alterations in DNA–protein interactions. At least one mutation was recovered for each nucleotide between -102 and -86 except for nucleotides at -96 and -93. For gel retardation experiments, we used nuclear proteins that had been partially purified by column chromatography to separate ISGF-2 and ISGF-3. Factors forming the IFN-induced B2 and B3 complexes with ISRE DNA fragments were the only sequence-specific DNA binding activities in these protein fractions (data not shown).

Protein Interactions with Wild-Type ISG54 and ISG15 DNA Sequences. ISRE sequences are not absolutely conserved (5–7), and ISG54 and ISG15 are no exception (see Fig. 1*B*). Their ISREs differ at residues -101, -94, and -93 of the ISG54 sequence. (We have adopted a numbering system based on residue numbers upstream of the ISG54 cap site, as shown in Fig. 2.) The ISG15 ISRE in the ISG15 promoter is inverted relative to the ISG54 orientation; its actual position is from nucleotides -97 to -111 upstream. This natural variation in the consensus sequence of these two genes is compatible with the normal cycle of IFN-induced transcriptional increase and decrease since both wild-type genes respond in such a manner to IFN treatment (23). Likewise, there are differences in ISRE sequences from other IFN-stimulated genes. As shown in Fig. 1*B*, ISREs from 6-16 (8) and 2′–5′-oligo(A) synthetase (OAS; refs. 9 and 10), two other human genes in which ISREs have been demonstrated to be functional by expression *in vivo*, the central two nucleotides show greatest variability. It is interesting to note that the OAS ISRE differs most from ISG54. We have found that the transcriptional induction of this gene is much weaker than either ISG54 or ISG15 (D.S.K., R. Pine, and J.E.D., unpublished data), which may be a direct consequence of lower affinity binding of factors to this divergent ISRE.

We have noted consistently that oligonucleotide probes derived from ISG54 gave a much stronger B2 than B3 gel shift pattern, while ISG15 probes gave gel shift complexes that were approximately equal in intensity for both B2 and B3. This difference in binding properties is illustrated in Fig. 3, in which a labeled ISG15 probe was mixed with partially purified preparations of either ISGF-2 or ISGF-3. To compare relative binding affinities of ISG15 and ISG54 for these two different proteins, plasmids carrying ISRE sequences from each gene were used as unlabeled competitors in gel retardation assays. Titration of increasing amounts of unlabeled competitor DNA against constant amounts of labeled probe, protein, and total DNA showed that the ISG54 version of the ISRE preferentially competed for B2 complex formation (Fig. 3*a*, lane 3 versus lane 5), while the ISG15 sequence competed better for B3 (Fig. 3*b*, lane 5 versus lane 3). These results illustrate the stronger interaction of proteins forming the B3 complex (ISGF-3) with ISG15, whereas ISGF-2 interacts more strongly with ISG54. Since we were particularly interested in how mutations would affect binding of ISGF-3 (i.e., the putative positive-acting factor), the gel retardation experiments testing mutant sequences shown in Fig. 4 used labeled ISG15 probes so that a strong B3 as well as a strong B2 band could be clearly discerned in noncompeted samples. However, under these conditions wild-type ISG54 does not compete completely with ISG15 for B3 complex formation. Therefore, subtle effects of mutations on ISGF-3 binding may not be readily apparent.

Protein Interactions with Mutant ISG54 ISREs. Fig. 4 shows a representative sample of gel shift experiments in which a labeled ISG15 DNA fragment (-115 to -39) was incubated with partially purified ISGF-3 (Fig. 4*a*) or ISGF-2 (Fig. 4*b*) preparations in the presence of competitor DNA plasmids. Plasmids containing vector alone, wild-type ISG54, or each of a series of different plasmids containing mutant ISG54 sequences were tested at three concentrations of competing DNA equivalent to 25-, 75-, and 225-fold molar excess. Wild-type competitor (lanes WT) reduced binding to the probe by $\approx$95% at the highest concentration of competitor. In contrast, several mutant DNAs—e.g., M99–M95 and M92–M90—showed little or no ability to compete for formation of the B3 complex. This and other similar results in four additional competition experiments established that sequences flanking and including the two sets of three thymidine residues are crucial for ISGF-3 binding to the ISRE. Note that mutations in residues 102, 89, and 87 also have significantly less competitive ability than wild-type DNA for

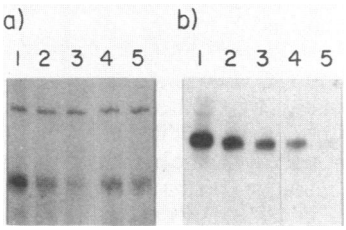


FIG. 3. ISGF-2 and ISGF-3 bind to the ISRE sequences of ISG54 and ISG15 with different affinities. Radiolabeled ISG15 ISRE probe (-115 to -39) was used in gel retardation competition experiments with partially purified preparations of ISGF-2 (*a*) and ISGF-3 (*b*) as described in *Materials and Methods*. Lanes 1 show competition with vector and nonspecific DNA only; binding reactions for lanes 2 contained 25-fold molar excess of the ISG54 ISRE sequence; lanes 3 represent a 75-fold molar excess. Lanes 4 show competition with a 25-fold molar excess of the ISG15 ISRE, and lanes 5 represent competition with a 75-fold excess of ISG15 sequences. In *a* the top band is nonspecific as indicated by its lack of competition by wild-type DNA sequences. The ISGF-2-specific complex is the bottom band. In *b*, the only gel band shown is the ISGF-3-specific complex.

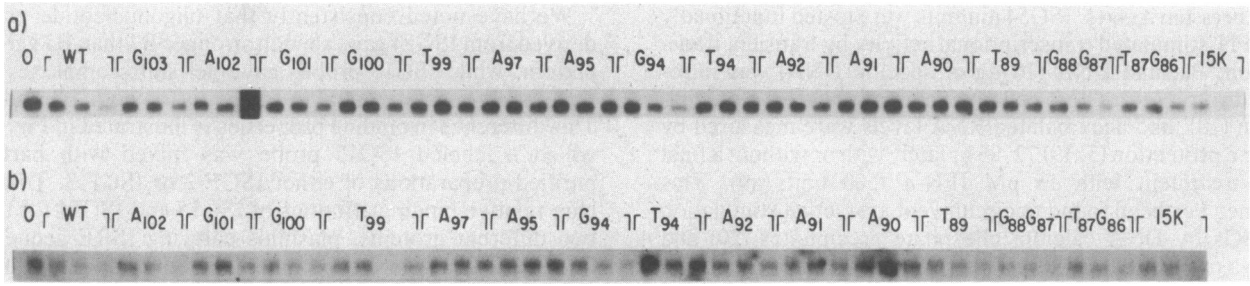


FIG. 4. Protein binding abilities of ISG54 point mutants. Gel retardation competition assays were performed with a radiolabeled ISG15 ISRE probe and different ISRE sequences. Mutated sequences are indicated above lanes by nucleotide and position of the substituted base. Lanes 0, competition with nonspecific DNA only; lanes WT and 15K, competition with wild-type ISG54 and ISG15 ISREs, respectively. Competitor DNA was used in 25-, 75-, and 225-fold molar excess. (*a*) Partially purified ISGF-3. (*b*) Partially purified ISGF-2.

the B3 complex, indicating that ISGF-3 contact points include these residues as well.

Not all changes at a particular position in the ISRE produced the same effect. One particularly dramatic example involves residue 94. Guanine is present at this site in the ISG15 sequence, whereas ISG54 has an adenine at this position. Transition of adenine to guanine in ISG54 did not affect the ability of this mutant ISG54 DNA to compete. However, a transversion of adenine to thymine at this site greatly reduced the competitive ability of the mutant sequence. Thus, a thymine at position 94 abolished ISGF-3 binding to the ISG54 ISRE while a guanine at the same position allows normal interaction with ISGF-3. The cytosine at position 101 also showed greater effect of transversions than transitions, similar to the finding at position 94.

The series of lanes in Fig. 4*b* shows mutant DNA competition for formation of the B2 complex. All mutations that impair B2 complex formation (nucleotides 101–95 and residues 92–90) are included among mutations that also affected competition for the B3 complex. Likewise, mutation of residue 94 to thymine abolished competition for ISGF-2 as it did for ISGF-3, whereas mutation of the same residue to guanine had no effect. There were no cases in which a mutation affected ISGF-2 binding without also disrupting ISGF-3 binding. However, mutations at residues 102 or 89–87 that do impair ISGF-3 binding had little or no effect on the ability of these sequences to compete for ISGF-2 binding. Thus, it appears that the region of the ISRE important for ISGF-2 recognition is contained within, but slightly smaller than, the ISGF-3 binding site (nucleotides 102–87), but that the sequence requirements of most importance (residues 100–90) are exactly the same for the two factors. The effect of mutations on the ability to compete for either ISGF-2 or ISGF-3 is summarized in Fig. 2.

Protein Interactions with Mutant ISG15 Sequences. Since ISGF-3 binding to ISG54 contacts nucleotides between −102 and −87, mutations were introduced into an ISG15 ISRE oligonucleotide at four sites within the equivalent region (actually −111 to −94 in the ISG15 sequence) as shown in Fig. 2. The effects of these mutations on the ability to compete for B3 and B2 complex formation are shown in Fig. 5 *a* and *b*, respectively. A significant loss of competition for ISGF-3 was evident for mutations at positions 88, 89, and 95 (by the ISG54 numbering system); only the mutation at position 99 retained complete competitive ability. In contrast, the pattern of ISGF-2 competition for these mutants was quite different. Not only was the mutation at position 99 essentially wild type for ISGF-2 competition as was the case for ISGF-3 binding, but also mutations at positions 88 and 89 were almost equal to wild-type oligonucleotide in competitive ability. Only the mutation at position 95 (in the middle of the ISRE) abolished competition for both ISGF-2 and ISGF-3 binding to the ISG15 oligonucleotide. These results strongly support the binding data with ISG54 mutants: central nucleotides from positions 90–100 are crucial for both ISGF-2 and ISGF-3 binding. However, residues flanking the core ISRE at positions 88, 89, and 102 play no role in ISGF-2 binding while still being involved in ISGF-3 recognition. Again, the broader ISGF-3 binding site encompasses the ISGF-2 binding site.

Functional Effects of ISG54 Mutations. The proposition that ISGF-3 functions in cells as a positive-acting transcription factor responsive to IFN treatment suggests that protein binding to mutated ISRE sequences *in vitro* would correlate with the ability of these sequences to direct IFN-dependent transcription *in vivo*. We have at present no functional test for mutations affecting the regulated decline in transcription occurring late in the response, although we do know from transcriptional analysis of promoter constructs in recombinant adenovirus vectors that repression of the IFN response is mediated through 5′ flanking sequence (4). Therefore, in the present set of experiments we have used transient transfection assays to measure the ability of different ISRE sequences to activate transcription following IFN treatment. The mutations that had the greatest effect on fully induced levels of steady-state mRNA corresponded to the same mutations that had the greatest effect on *in vitro* binding of ISGF-3. Fig. 6 shows representative examples of these assays. A number of mutants at nucleotides 100–90 reduced mRNA accumulation to background levels whether or not IFN was added to the transfected cells. The change at residue 94 to guanine (the ISG15 nucleotide at this position) did not affect function, but the transversion to thymine at this site eliminated transcriptional activity as it did protein binding. The requirements for full inducibility implicate ISGF-3 as the critical factor for transcriptional activity. Bases 89, 101, and 102 play a role in transcriptional activation because expression was decreased ≈50% by mutations at these sites. Formation of the B3 complex by ISGF-3 binding to the ISRE involves the same nucleotides required for full inducibility.

DISCUSSION

The experiments presented here show that, although ISGF-2 and ISGF-3 bind to essentially the same promoter element,

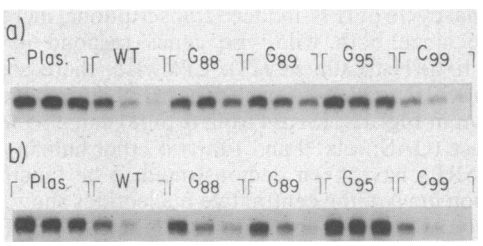


FIG. 5. Protein interactions with mutant ISG15 ISRE oligonucleotides. Radiolabeled ISG15 ISRE probe (−115 to −39) was used in competition gel retardation assays with partially purified ISGF-3 (*a*) or ISGF-2 (*b*) as described in Fig. 4.

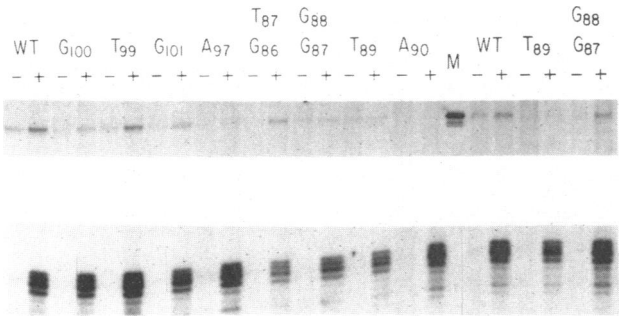


FIG. 6. Transient expression of ISG54 point mutants. Plasmids containing ISG54 wild-type or mutant ISRE sequences were transfected into HeLa cells. Specific RNA expression was measured by RNase protection without (−) or after (+) a 4-hr treatment with IFN. (*Upper*) RNA signal from the expression construct; (*Lower*) expression from endogenous ISG54 used as control (see *Materials and Methods*). Lane M, molecular size marker.

subtle but presumably functionally important differences exist in the exact sequence requirements for binding of the two IFN-induced nuclear factors. Thus, the ISGF-2 binding site is contained completely within the broader ISGF-3 site, and ISRE residues critical for ISGF-2 recognition are equally important for ISGF-3 binding. However, these two factors differ in several significant characteristics: the factors form protein–DNA complexes with distinct electrophoretic mobilities; ISGF-2 and ISGF-3 can be separated into discrete fractions by column chromatography; and ISGF-3 preexists in cells in an inactive form prior to IFN treatment, which gains the ability to bind DNA after stimulation by IFN. ISGF-2, in contrast, accumulates slowly and only when *de novo* protein synthesis is allowed after IFN addition (5). Thus, it seems likely that ISGF-2 and ISGF-3 are different proteins that compete *in vivo* for the same DNA binding site.

ISGF-2 binds to the nine-residue central core of the ISRE. Seven of these residues are absolutely conserved in ISREs, which have been demonstrated to be transcriptionally functional (Fig. 1*B*; refs. 5–10). Of the other two residues, position 93 is more variable than 94, which appears to require a purine. The ISG54 mutagenesis reported here reinforces the requirement for a purine at this position for both ISGF binding and ISG expression, suggesting an important protein–DNA contact at this site that is not absolutely nucleotide specific. The sequence requirements for ISGF-3 binding include all those for ISGF-2 and, in addition, nucleotides that flank the core ISRE (positions 102, 101, and 89–87). These extended sequence requirements for ISGF-3 binding correspond to the region necessary for maximal ISG54 expression.

These findings are also consistent with observations of variant cell lines that are resistant to the physiological effects of IFN (24). The resistant cell lines fail to arrest proliferation and cannot develop an antiviral state in response to IFN. We have found a corresponding inability of these cell lines to increase ISG transcription in response to IFN, and they do not activate the ISGF-3 DNA binding factor. Taken together, these results strongly implicate ISGF-3 as the IFN-stimulated gene activator acting through the ISRE DNA sequence. In contrast, the kinetics of accumulation and protein synthesis dependence of ISGF-2 parallel the regulated decline of ISG transcription late after IFN treatment, suggesting a negative effect of ISGF-2 on ISG transcription. If ISGF-2 is a transcriptional repressor opposing already bound ISGF-3, then it must be present in higher concentrations or have a higher avidity for the core ISRE since its binding site is included within that for ISGF-3. With sufficient amounts of these purified proteins, this possible mechanism of action will become testable. We are developing an *in vitro* RNA transcription system in which to test directly the activity of ISGF-3. Such a system may also be required to fully understand the role of ISGF-2 in the IFN response.

It is interesting to contrast ISG transcriptional induction in response to IFN with the induction of IFN genes themselves when activated by virus infection. For example, mutagenesis of the transcriptional regulatory element of human IFN-β has suggested positive and negative control regions within this promoter as well (25). In fact, there is an 8-nucleotide identity between the positive-acting domain of IFN-β (−74 to −67) and the ISRE central core (98 to 91). Indeed, we have recently found that the regulatory domain of the IFN-β gene will effectively compete for ISGF-2 binding, although it does not bind appreciably to ISGF-3 (unpublished results). However, positive and negative control regions of IFN-β are only minimally overlapping unlike those in the ISRE. Furthermore, IFN genes and ISGs respond to distinct physiological signals and act in different arms of the cellular response to viral infection. Further purification and characterization of ISGFs as well as IFN gene factors should define any additional correspondences between these interrelated inducible gene families.

We thank R. Pine for providing purified preparations of ISGF-2, N. Reich for the ISG15 promoter fragment and oligonucleotides, R. Case for assistance in design of random sequence oligonucleotides, and S. Patel for excellent technical assistance. Recombinant IFN was generously provided by Hoffman–La Roche. D.S.K. ws supported by a predoctoral training grant from the National Institutes of Health and D.E.L. was a National Institutes of Health postdoctoral fellow. This work was supported by grants from the National Institutes of Health, the American Cancer Society, and a gift from E. I. duPont de Nemours & Co.

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