

A gamma-interferon-induced factor that binds the interferon response sequence of the MHC class I gene, H-2K^b

Michael A. Blonar^{1,3,5}, Albert S. Baldwin, Jr.^{2,5},
Richard A. Flavell^{1,4} and Phillip A. Sharp²

¹Biogen Research Corporation, Fourteen Cambridge Center, Cambridge, MA 02142 and ²Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA. Present address: ³Hormone Research Institute, School of Medicine, University of California, San Francisco, San Francisco, CA 94143-0534, USA and ⁴Howard Hughes Medical Institute, Yale University School of Medicine, 310 Cedar Street, FMB 410, New Haven, CT 06510, USA. ⁵The data presented in this communication are the result of an equal contribution by the first two authors; therefore, the order of authorship was determined randomly.

Communicated by R.A. Flavell

Transcription of class I genes of the major histocompatibility complex (MHC) can be induced by interferons. Treatment of HeLa cells with interferon- γ induces a DNA-binding factor, IBP-1, specific for a site within the interferon response sequence (IRS) of the H-2K^b promoter. The mol. wt of IBP-1, as estimated by photoactivated protein-DNA crosslinking analysis, is ~59 kd. Point-mutation of this binding site abolishes IBP-1 interaction and the ability of the MHC promoter to respond to interferon. Induction of this binding activity is rapid and closely parallels the previously reported time course of transcriptional activation of endogenous MHC class I genes. Treatment of cells with cycloheximide, a protein synthesis inhibitor, blocked the induction of the DNA-binding activity. An oligonucleotide derived from the virus- and double-stranded RNA-inducible promoter of the interferon- β_1 gene is able to bind IBP-1. Sequences similar to the IBP-1 binding site are found upstream of many interferon-responsive genes.

Key words: interferon- γ /interferon response element/DNA binding factor/major histocompatibility complex

Introduction

Interferons have been shown to influence a number of cellular processes, including cell growth and differentiation, in addition to stimulating the antiviral response (for review, see Revel and Chebath, 1986). Interferon treatment results in the transcriptional induction of many genes including those of the class I major histocompatibility complex (MHC). These genes encode cell-surface glycoproteins expressed in virtually all adult tissues and have been shown to be required for recognition of virus-infected and neoplastic cells by cytotoxic T lymphocytes (for review, see Flavell *et al.*, 1986).

Stimulation of MHC class I gene expression by treatment with α , β , and γ interferons is controlled by the interferon response sequence (IRS), a region located upstream of the structural gene (Israel *et al.*, 1986; Sugita *et al.*, 1987). We have identified a cellular factor, inducible by interferon- γ

(IFN γ ; type II interferon), that binds to a site within the IRS of the MHC class I gene, H-2K^b. Site-specific mutation of the identified binding site abolishes the capacity of the promoter to respond to IFN γ .

Results

Interferon- γ induces a sequence-specific DNA binding factor, IBP-1

A double-stranded oligonucleotide probe, encompassing a major portion of the H-2K^b IRS, was prepared. This oligonucleotide, which included the sequence from -156 to -135 in the H-2K^b promoter, was end-labeled and used in DNA-binding experiments (Figure 1). One complex was detected in extracts of cells treated with IFN γ that was not present in extracts of untreated cells (Figure 1, lanes b and c). The induced DNA-binding activity was specific, as shown by competition with an ~50-fold molar excess of an unlabeled MHC-derived DNA fragment that includes the IRS region and by the lack of competition by an equivalent excess of an MHC-derived DNA fragment lacking the IRS (data

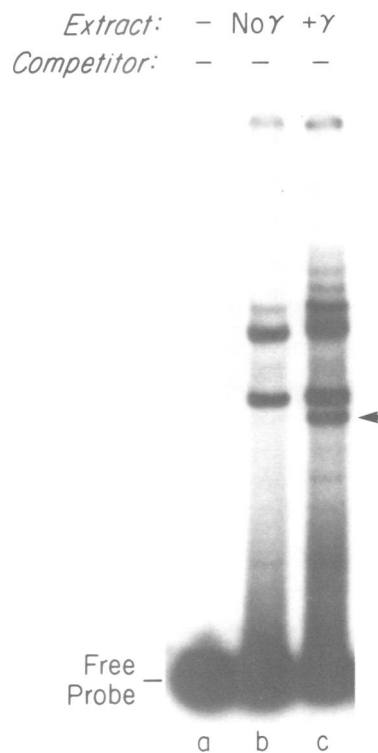


Fig. 1. IFN γ induces a sequence-specific DNA binding activity in HeLa cells. Whole-cell extracts (Manley *et al.*, 1980) of HeLa cells, either untreated or treated with 1000 U/ml of IFN γ (ImmuneronTM, Biogen, Cambridge) for 3 h, were incubated with an oligonucleotide probe and were electrophoresed on Tris/glycine polyacrylamide gels as described in Materials and methods. The arrow indicates the specific complex formed on the DNA probe.

Table I. Potential IBP-1 binding sites in selected genes

Gene		Sequence		IBP-1 Binding
H-2K ^b	-134	AGGTGCAGAAAGT [*] [*] GAAACTGTGGA	-156	+
IFN β	-82	<u>A</u> a a g <u>G</u> g <u>A</u> GAAGT <u>G</u> AAA g <u>T</u> Gg <u>G</u> a <u>A</u>	-60	+
HSP70	-65	t <u>G</u> Gc t <u>C</u> AGAAGg <u>G</u> AAAa g <u>G</u> c <u>G</u> Gg	-43	-
Ig κ		<u>A</u> a <u>G</u> a t <u>C</u> AGAAGT <u>G</u> AAg t c t g c c <u>A</u>		-
OASE	-105	t t c <u>T</u> Ga g <u>G</u> AAa c <u>G</u> AAACc a a c a g	-83	
202	-49	t <u>G</u> c <u>T</u> c a <u>A</u> GAAa <u>T</u> GAAACa a c t c t	-27	
IP-10	-226	g t t <u>T</u> t g g a <u>A</u> AGT <u>G</u> AAACc t a a t t	-204	
6-16	-157	<u>A</u> Ga g <u>G</u> g g a <u>A</u> Aa <u>T</u> GAAACTc <u>T</u> Gc <u>A</u>	-135	
	-116	<u>A</u> Ga g <u>G</u> g g a <u>A</u> Aa <u>T</u> GAAACTGc a <u>G</u> A	-94	
ISG-15	-118	c c t c <u>G</u> g g a <u>A</u> AGg <u>G</u> AAACc <u>G</u> a a a c	-96	
ISG-54	-83	<u>A</u> a a a <u>G</u> g g a <u>A</u> AGT <u>G</u> AAACTa g a a <u>A</u>	-105	
HLA-A2	-412	g <u>G</u> Ga t a <u>A</u> a <u>A</u> AGT <u>G</u> AAA g g a g a <u>G</u> g	-390	
	-165	<u>A</u> Ga g <u>G</u> g <u>A</u> GAAa a <u>G</u> AAACTGc <u>G</u> GA	-187	
HLA-A3	-168	c a <u>G</u> g <u>G</u> g <u>A</u> GAAa a <u>G</u> AAACTGc <u>G</u> GA	-190	
INV(γ)	-80	<u>A</u> Ga <u>T</u> Gt g <u>G</u> AAGT <u>G</u> AAA g c t a c a <u>A</u>	-102	

The proposed IBP-1 binding site of H-2K^b was compared to functionally important elements of other genes. A number of identical and highly similar sequences were detected. Nucleotides are numbered relative to the CAP site, except for HLA-A2 and HLA-A3, which are numbered relative to the first nucleotide of exon 1, and 202, which is numbered relative to the first nucleotide of cDNA clone 922. IFN β ₁, interferon- β ₁ gene (Degraeve *et al.*, 1981; Goodbourn *et al.*, 1986; Zinn and Maniatis, 1986); HLA-A2, human MHC class I gene A2 (Koller and Orr, 1985); HLA-A3, human MHC class I gene A3 (Strachan *et al.*, 1984); INV(γ), human MHC class I-associated invariant (γ) chain gene (O'Sullivan *et al.*, 1986); ISG-54, IFN α -stimulated gene 54 (Reich *et al.*, 1987); ISG-15, IFN α -stimulated gene 15 (Reich *et al.*, 1987); IP-10, an IFN γ -inducible gene containing homology to platelet proteins (Luster and Ravetch, 1987); OASE, human 2',5'-oligo(A) synthetase E gene (Benech *et al.*, 1987); 6-16, human IFN α / β -inducible gene (Porter *et al.*, 1988); 202, an IFN β -inducible gene (Samanta *et al.*, 1986); Ig κ , immunoglobulin κ enhancer (Max *et al.*, 1981; Picard and Schaffner, 1984); HSP70, 70-kd heat shock protein (Wu *et al.*, 1986). As shown in Figure 3, + indicates binding of the oligonucleotide to IBP-1; - indicates that the oligonucleotide does not bind to IBP-1. The other sequences listed have not been tested directly for binding.

the level does not change significantly over time, up to 24 h (see Figures 2B and 3). As shown in Figure 3, treatment with CHX significantly reduced the level of IBP-1 binding activity as compared to extracts made from IFN γ -treated control cells. The residual binding activity observed can be attributed to the small amount of protein synthesis not blocked by CHX (~5%; data not shown).

The mol. wt of IBP-1 was estimated by photoactivated protein-DNA crosslinking analysis (Figure 5). The IBP-1-specific complex was isolated and analyzed by SDS-PAGE. This analysis has revealed IBP-1 to be 59 kd in size.

Mutation of the IBP-1 binding site abolishes interferon- γ response

To demonstrate that the response to treatment with IFN γ is mediated through the identified IBP-1 binding site *in vivo*, the activity of a mutant H-2K^b promoter was compared to that of a wild-type promoter. The deletion construct p141H2KCAT, in which sequences from -141 to +5 of the H-2K^b promoter have been inserted upstream of the chloramphenicol acetyltransferase (CAT) gene (Baldwin and Sharp, 1987), was used to construct the mutant and wild-type promoters. Synthetic oligonucleotides containing sequences from -141 to -190 were inserted into the parental vector. One double-stranded oligonucleotide contained the wild-type sequence while the other contained a mutant sequence where G₋₁₄₄ and G₋₁₄₆ were changed

to thymines. A schematic representation of the various constructs is shown in Figure 6.

Plasmid DNAs were introduced with a human growth hormone reference plasmid (Selden *et al.*, 1986) into HeLa cells by electroporation and tested, in transient expression assays, for their ability to respond to treatment with IFN γ as measured by the stimulation of CAT production (Figure 6 and Table II). The wild-type H-2K^b reconstruction responded to treatment with IFN γ with a specific enhancement of 3.1-fold above the uninduced, basal level of expression (Table II). This level of induction of H-2K^b gene expression in HeLa cells by IFN γ is in good agreement with that reported by others (Israel *et al.*, 1986) and is similar to, but reproducibly slightly less than, that observed with p190H2KCAT (Figure 6 and Table II). This difference in induction is not due to an altered transcription start site utilized by the wild-type reconstruction in the presence of IFN γ , since the wild-type reconstruction and p190H2KCAT start transcription at the same nucleotide (data not shown). This difference may be an effect of the *Xho*I linker insertion. In contrast, the promoter reconstruction bearing the two point mutations was not stimulated upon exposure of cells to IFN γ . The basal level of expression from the mutant promoter is similar to that observed from the wild-type H-2K^b promoter. It appears, therefore, that the IBP-1 binding site is essential for stimulation of the MHC class I promoter by IFN γ , but plays little or no role in the basal activity of the H-2K^b promoter.

Discussion

We have identified an IFN γ -inducible, sequence-specific, 59-kd DNA binding factor, IBP-1. The binding site of this factor is required for IFN γ -stimulated expression of the MHC class I gene, H-2K^b. Specifically, mutation of two guanine residues in the binding site prevents the induction of H-2K^b expression by IFN γ ; the same mutation also prevents binding of IBP-1 to this site. Presumably, therefore, binding of IBP-1 to this site is necessary for response of the gene to IFN γ . Interestingly, preliminary experiments utilizing the oligonucleotide containing the H-2K^b sequence from -156 to -135 demonstrate that this sequence by itself is unable to confer IFN γ responsiveness to a thymidine kinase promoter-CAT construction (data not shown), suggesting that IBP-1 binding alone is not sufficient for induction. IBP-1 binding activity is rapidly induced in HeLa cells by treatment with IFN γ and maximal levels of binding activity are observed within 45 min following lymphokine treatment. Induction of IBP-1 binding activity was blocked by treatment with CHX, suggesting a requirement for protein synthesis.

Recently, Porter *et al.* (1988) and Rutherford *et al.* (1988) have described IFN type I-inducible DNA binding activities specific for regulatory sequences upstream of the 6-16 and the 2',5'-oligo(A) synthetase (OAS) genes, respectively. Treatment with CHX apparently has no effect on the induction of the type I IFN-inducible factor (Rutherford *et al.*, 1988). This contrasts with the ability of CHX to inhibit

the induction of IBP-1 by IFN γ , suggesting that IBP-1 and the type I IFN-inducible factor may be different. Of course, it is possible that both type I and type II IFNs induce the same factor, in one case by a pathway that is protein synthesis sensitive and in the other which is insensitive. Similarly, since our experiments and those of Rutherford *et al.* used different cell lines, we cannot exclude the possibility that the pathways of induction are cell-type specific. Consistent with this, IBP-1 binding activity is induced by IFN γ (type II), but preliminary experiments indicate that IFN α (type I) is unable to stimulate IBP-1 binding in HeLa cells (data not shown).

Similar sequences to the H-2K^b IBP-1 binding site are found in the promoter regions of many genes inducible either by IFN α/β or by IFN γ (Table I). In all instances, where it has been tested, the potential IBP-1 binding site is located within regions of DNA required for response of the promoter to treatment with either type I or type II interferons. It is not clear if IBP-1 plays a role in type I IFN-regulated gene expression.

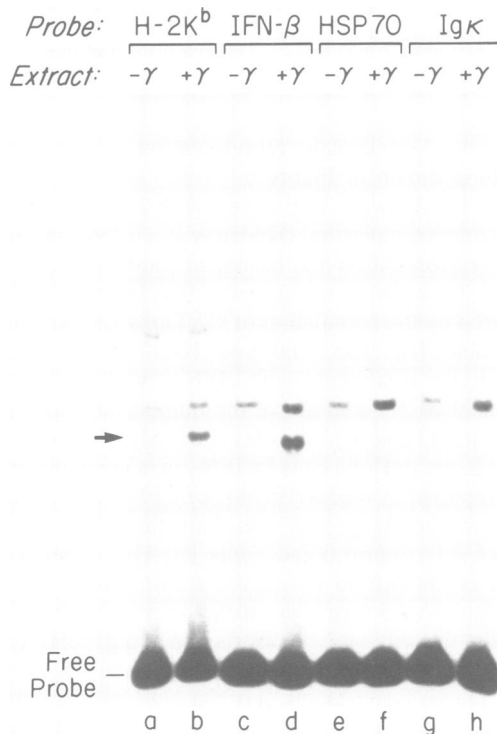


Fig. 3. Binding specificity of IBP-1. Whole-cell extracts of HeLa cells, either untreated (- γ) or treated with 1000 U/ml of IFN γ (+ γ), were incubated with end-labeled, oligonucleotide probes specific to the IBP-1-like binding sequences of the IFN β_1 , HSP70 and Ig κ genes (see Table I) and were electrophoresed as described in Materials and methods. The arrow indicates the specific complex formed on the DNA probe. Binding to the H-2K^b oligonucleotide was performed for comparison.

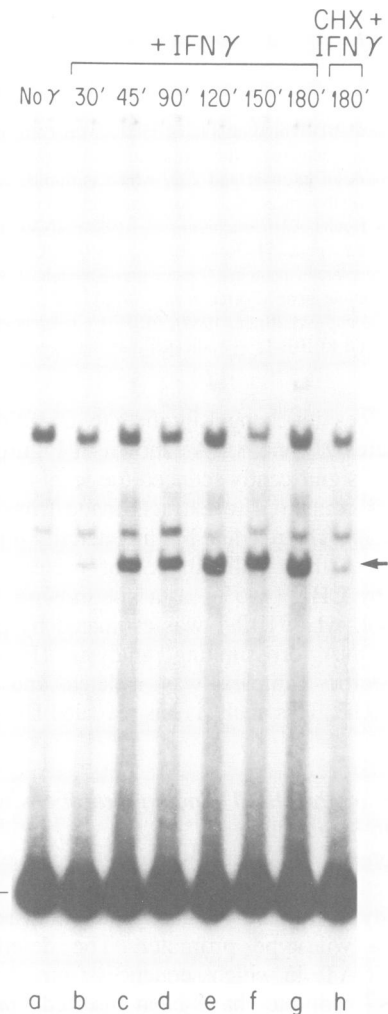


Fig. 4. Time-course of IBP-1 induction by IFN γ and effect of CHX. HeLa cells were treated with 1000 U/ml of IFN γ for the indicated times, after which whole-cell extracts were prepared. CHX-treated cells were pre-treated for 60 min with 10 μ g/ml CHX prior to addition of IFN γ . Extracts were incubated with a wild-type H-2K^b oligonucleotide probe and electrophoresed as described in Materials and methods. The arrow indicates the specific complex formed on the DNA probe.

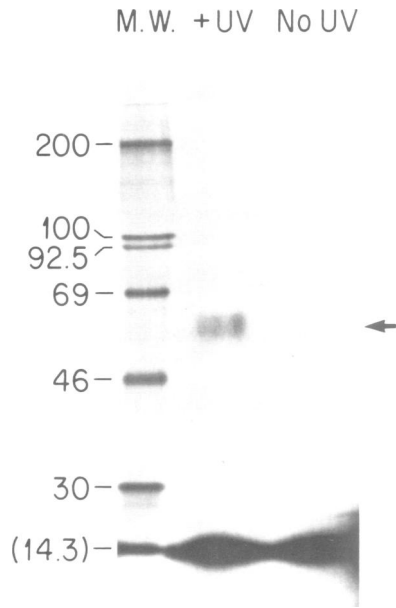


Fig. 5. Identification of IBP-1 by UV crosslinking. Binding reactions and UV irradiation were done as described in Materials and methods. M.W., protein mol. wt markers; +UV, UV-treated; No UV, UV irradiation omitted. Arrow indicates position of the UV crosslinked IBP-1 specific complex.

Table II. IFN γ induction of wild-type and mutant H-2K^b promoter constructions

Construction	IFN γ induction
p190H2KCAT	4.1 \pm 0.2
p141H2KCAT	1.2 \pm 0.1
Wild-type reconstruction	3.1 \pm 0.3
Mutant reconstruction	1.2 \pm 0.1

The ratio of the CAT activity of transfected HeLa cells treated with IFN γ to that of control cells is shown as induction. CAT activities were normalized for transfection efficiency, as determined by hGH assay, and the values shown are the mean of at least three independent experiments.

IBP-1 binds a DNA fragment derived from the interferon- β_1 promoter region (Figure 3, lane d). This fragment contains a sequence which is very similar to the H-2K^b IBP-1 binding site (see Table I). DNase I footprinting of genomic DNA following treatment of cells with double-stranded RNA has identified a region of protection in the interferon- β_1 promoter which centers on this site in the interferon gene response element (IRE; Zinn and Maniatis, 1986). A double-stranded RNA-inducible factor that binds this site, IRF-1, has been identified and found to be \sim 37 kd in size (Miyamoto *et al.*, 1988). This is appreciably different from the mol. wt of IBP-1, shown to be 59 kd. Thus, double-stranded RNA and IFN γ apparently induce distinct DNA-binding activities with similar, or identical, sequence specificity.

MHC class I genes are transcriptionally induced by interferons within 2–3 h (Rosa *et al.*, 1983; Luster *et al.*, 1985; Blonar *et al.*, 1988). This correlates well with the induction of IBP-1 binding activity of HeLa cells: relatively high within 1 h of IFN γ treatment, but barely detectable after only 30 min (Figure 4). Since class I genes are expressed in most cell types, IBP-1 probably functions to stimulate existent gene expression. Analysis of the expression of IBP-1 is likely to be important to our understanding of the process of IFN-stimulated gene expression. Purification of this factor and isolation of its gene will facilitate the unravelling of the complex pleiotropic effects mediated by interferons.

Materials and methods

Cell culture

HeLa cells were maintained as spinner cultures in Dulbecco's modified Eagle's medium supplemented with 5% horse serum. Cells were treated with highly purified recombinant human IFN γ (Biogen ImmuneronTM) at a concentration of 1×10^3 U/ml for the indicated times. CHX was used at a final concentration at 10 μ g/ml.

Whole-cell protein extracts

Whole-cell extracts of HeLa cells were prepared according to Manley *et al.*

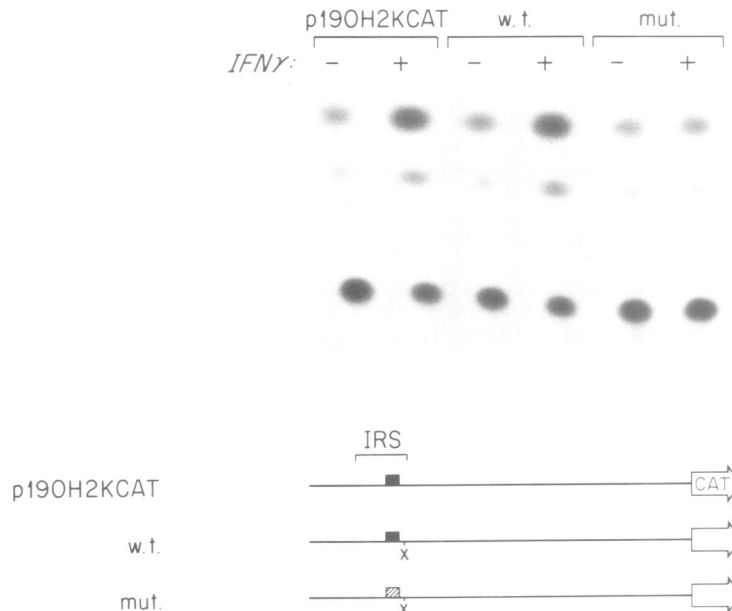


Fig. 6. Functional analysis of wild-type and mutant H-2K^b promoter reconstructions. **Top.** The result of one representative CAT expression experiment is shown (see Materials and methods). 100 μ g of protein extract was used per reaction and the data shown is from a 2-h incubation. **Bottom.** The solid box represents the wild-type IBP-1 binding site and the hatched box represents that of the mutant. x, position of the XhoI site. CAT, chloramphenicol acetyltransferase gene.

(1980). Protein concentrations were determined by the method of Bradford (1976) using bovine γ -globulin as a standard.

Gel electrophoresis DNA-binding assay

DNA binding reactions were performed as described previously (Fried and Crothers, 1981; Singh *et al.*, 1986). Extracts were added to the binding reactions as noted. Reactions were electrophoresed on Tris/glycine polyacrylamide gels as described (Singh *et al.*, 1986). Oligonucleotides corresponding to the wild-type sequence from -156 to -134 in the H-2K^b promoter and to a mutant sequence (see Figure 2B) were synthesized with *Bam*HI ends and were cloned in the *Bam*HI site of the pUC13 polylinker. Oligonucleotides corresponding to the IFN- β_1 , HSP70 and Ig κ sequences, as shown in Table I, similarly were synthesized with *Bam*HI ends.

Methylation interference assay

An end-labeled H-2K^b oligonucleotide probe was partially methylated with dimethyl sulfate (Maxam and Gilbert, 1980) and used in a gel binding assay as described above. Free probe and the bound complex were eluted as described (Baldwin and Sharp, 1987) and cleaved with piperidine. These reactions, along with cleaved unreacted probe, were electrophoresed on 8% polyacrylamide/7.5 M urea gels and were exposed for autoradiography.

Photoactivated protein - DNA crosslinking analysis

A 41-bp oligonucleotide containing the IBP-1 binding site (CAGGTTAGGTGCAGAAGTGAAGTGTGGAGATGGGGAATCC) was annealed to a complementary 15-bp oligonucleotide (GGATCCCCATCTCC) and body labeled with dATP, dGTP, 5-bromo-2'-deoxyuridine triphosphate and [α -³²P]dCTP as described by Chodosh *et al.* (1986). Binding reactions (50 μ l) contained 1 ng of body labeled probe, 10 μ g of poly(dI-dC)·poly(dI-dC), and 50 μ g of whole-cell protein extract derived from HeLa cells treated for 3 h with 1000 U/ml of IFN γ . UV irradiation was for 60 min at 4°C. Samples were electrophoresed on Tris/glycine polyacrylamide gels as described above and the specific IBP-1 DNA complex was isolated and subsequently analyzed on a discontinuous SDS-10% polyacrylamide gel (Laemmli, 1970).

Transient expression assays

Approximately 1×10^7 cells were electroporated with 100 μ g of the test plasmid, 10 μ g of the human growth hormone reference plasmid, pXGH5 (Selden *et al.*, 1986), and 290 μ g of sonicated salmon sperm DNA in HeBS (290 mM Hepes, pH 7.05, 137 mM NaCl, 5 mM KCl, 0.7 mM Na₂HPO₄, 6 mM dextrose) at 270 V, 960 μ FD for ~10.5 ms (BioRad Gene Pulser™). The cells were allowed to adhere for 12-16 h in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum after which the cells were rinsed once and re-fed with fresh medium, either with or without IFN γ at 1000 U/ml. A cellular protein extract was prepared 24-48 h later and CAT activity measured (Gorman *et al.*, 1982). Protein concentrations were determined by the method of Bradford (1976) using bovine serum albumin as the standard. Human growth hormone concentrations were assayed using a solid-phase immunoradiometric assay (Tandem-R HGH, Hybritech, San Diego, CA). CAT activities were normalized to the HGH transfection control. p190H2KCAT contains the H-2K^b sequence from -190 to +5 and p141H2KCAT contains the H-2K^b sequence from -141 to +5 inserted upstream of the CAT gene (Baldwin and Sharp, 1987). The other constructs were derived by inserting a double-stranded oligonucleotide into the unique *Xho*I site of p141H2KCAT (previously p138H2KCAT, Baldwin and Sharp, 1987). The wild-type IBP-1 oligonucleotide contained the H-2K^b sequence, GGTGGGAAGCCCCAGGGCTGGGGATTCCCATCTCCACAGTTTCACTTCTGCA. Underlined residues are those which have been altered in the mutant from the wild-type sequence (see text). Both these oligonucleotides and their respective complements contained termini suitable for ligation to the *Xho*I site of p141H2KCAT. Oligomer sequences and their orientation in the final constructs were verified by DNA sequence analysis.

Acknowledgements

We thank J. Barsoum, E. Böttger, M. Garcia-Blanco and G. Waneck for critical reading of the manuscript, and J. Tavernier and W. Fiers for providing the interferon- β_1 gene clone. We also thank M. Sifaca for preparation of the manuscript. M.A.B. is an Arthritis Foundation Postdoctoral Fellow and A.S.B. is a Special Fellow of the Leukemia Society of America. This work was supported in part by Biogen NV and in part by grants from NIH P01-CA42063 and NCI P30-CA14051 to P.A.S.

References

- Baldwin, A.S. and Sharp, P.A. (1987) *Mol. Cell. Biol.*, **7**, 305-313.
 Benech, P., Vigneron, M., Peretz, D., Revel, M. and Chebath, J. (1987) *Mol. Cell. Biol.*, **7**, 4498-4504.
 Blonar, M.A., Böttger, E.C. and Flavell, R.A. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 4672-4676.
 Bradford, M.M. (1976) *Anal. Biochem.*, **72**, 248-254.
 Carthew, R., Chodosh, L. and Sharp, P.A. (1985) *Cell*, **43**, 439-448.
 Chodosh, L.A., Carthew, R.W. and Sharp, P.A. (1986) *Mol. Cell. Biol.*, **6**, 4723-4733.
 Degrave, W., Derynck, R., Tavernier, J., Haegeman, G. and Fiers, W. (1981) *Gene*, **14**, 137-143.
 Flavell, R.A., Allen, H., Burkly, L.C., Sherman, D.H., Waneck, G.L. and Widera, G. (1986) *Science*, **233**, 437-443.
 Fried, M. and Crothers, D. (1981) *Nucleic Acids Res.*, **9**, 6505-6525.
 Goodbourn, S., Burstein, H. and Maniatis, T. (1986) *Cell*, **45**, 601-610.
 Gorman, C.M., Moffat, L.F. and Howard, B.H. (1982) *Mol. Cell. Biol.*, **2**, 1044-1051.
 Israel, A., Kimura, A., Fournier, A., Fellous, M. and Kourilsky, P. (1986) *Nature*, **322**, 743-746.
 Koller, B.H. and Orr, H.T. (1985) *J. Immunol.*, **134**, 2727-2733.
 Laemmli, U.K. (1970) *Nature*, **227**, 680-685.
 Luster, A., Unkeless, J.C. and Ravetch, J.V. (1985) *Nature*, **315**, 672-676.
 Luster, A. and Ravetch, J. (1987) *Mol. Cell. Biol.*, **7**, 3723-3731.
 Manley, J., Fire, A., Cano, A., Sharp, P.A. and Gefter, M. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 3855-3859.
 Max, E.E., Maizel, J.V., Jr and Leder, P. (1981) *J. Biol. Chem.*, **256**, 5116-5120.
 Maxam, A. and Gilbert, W. (1980) *Methods Enzymol.*, **65**, 499-560.
 Miyamoto, M., Fujita, T., Kimura, Y., Maruyama, M., Harada, H., Sudo, Y., Miyata, T. and Taniguchi, T. (1988) *Cell*, **54**, 903-913.
 O'Sullivan, D.M., Larhammar, D., Wilson, M.C., Peterson, P.A. and Quaranta, V. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 4484-4488.
 Picard, D. and Schaffner, W. (1984) *Nature*, **307**, 80-82.
 Porter, A.C.G., Chernajovsky, Y., Dale, T.C., Gilbert, C.S., Stark, G.R. and Kerr, I.M. (1988) *EMBO J.*, **7**, 85-92.
 Reich, N., Evans, B., Levey, D., Fahey, D., Knight, E. and Darnell, J. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 6394-6398.
 Revel, M. and Chebath, J. (1986) *Trends Biochem. Sci.*, **11**, 166-170.
 Rosa, F., Hatat, D., Abadie, A., Wallach, D., Revel, M. and Fellous, M. (1983) *EMBO J.*, **2**, 1585-1589.
 Rutherford, M.N., Hannigan, G.E. and Williams, B.R.G. (1988) *EMBO J.*, **7**, 751-759.
 Samanta, H., Engle, D., Chao, H., Thakur, A., Garcia-Blanco, M. and Lengyel, P. (1986) *J. Biol. Chem.*, **261**, 11849-11858.
 Selden, R., Howie, K., Rowe, M., Goodman, H. and Moore, D. (1986) *Mol. Cell. Biol.*, **6**, 3173-3179.
 Singh, H., Sen, R., Baltimore, D. and Sharp, P.A. (1986) *Nature*, **319**, 154-158.
 Strachan, T., Sodoyer, R., Damotte, M. and Jordan, B.R. (1984) *EMBO J.*, **3**, 887-894.
 Sugita, K., Miyazaki, J., Appella, E. and Ozato, K. (1987) *Mol. Cell. Biol.*, **7**, 2625-2630.
 Wu, B.J., Kingston, R.E. and Morimoto, R.I. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 629-633.
 Zinn, K. and Maniatis, T. (1986) *Cell*, **45**, 611-618.

Received on April 11, 1988; revised on December 8, 1988

Note added in proof

Since submission of this paper, A. Keller and T. Maniatis (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 3309-3313; Y. Shirayoshi *et al.* (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 5884-5888 and D. Levy *et al.* (1988) *Genes Devel.*, **2**, 383-393, have reported inducible DNA-binding factors that recognize a DNA sequence similar or identical to that for IBP.1.