*Proc. Natl. Acad. Sci. USA*
Vol. 85, pp. 6701–6705, September 1988
Biochemistry

# Activation of the human "$\beta_2$-interferon/hepatocyte-stimulating factor/interleukin 6" promoter by cytokines, viruses, and second messenger agonists

(interleukin 1/tumor necrosis factor/epidermal growth factor/serum/protein kinase A and C activators)

ANURADHA RAY, STEPHEN B. TATTER, LESTER T. MAY, AND PRAVINKUMAR B. SEHGAL

The Rockefeller University, 1230 York Avenue, New York, NY 10021

*Communicated by Igor Tamm, June 10, 1988 (received for review April 19, 1988)*

**ABSTRACT** **The hallmark of "$\beta_2$-interferon (IFN-$\beta_2$)/hepatocyte-stimulating factor/interleukin 6" gene expression is its inducibility in different types of human cells (fibroblasts, monocytes, epithelial cells, and endothelial cells) by different stimuli, which include cytokines such as tumor necrosis factor, interleukin 1 (IL-1) and platelet-derived growth factor, different viruses, and bacterial products such as endotoxin. The activation by cytokines, viruses, and second messenger agonists of the IFN-$\beta_2$ promoter linked to the bacterial chloramphenicol acetyltransferase (CAT) gene was studied after transfection into HeLa cells. A chimeric gene containing IFN-$\beta_2$ DNA from $-1180$ to $+13$ linked to the CAT gene was inducible ≈10-fold by phorbol 12-myristate 13-acetate (PMA), followed, in decreasing order, by pseudorabies and Sendai viruses (7- to 11-fold each); serum (6- to 9-fold); the cytokines tumor necrosis factor, IL-1, and epidermal growth factor (3- to 5-fold each); the cAMP agonists BrcAMP and forskolin and the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (2- to 6-fold each); poly(I)·poly(C) (2- to 4-fold); 1,2-diacylglycerol and the calcium ionophore A23187 (1.5- to 2-fold each). Bacterial endotoxin did not activate this IFN-$\beta_2$/CAT fusion gene in HeLa cells. Deletion of the 5′ boundary of the IFN-$\beta_2$ DNA from $-1180$ to $-596$ in the fusion gene preserved its activation by IL-1, tumor necrosis factor, epidermal growth factor, serum, pseudorabies, and Sendai viruses and by PMA, BrcAMP, and forskolin; deletion to $-225$ led to a small reduction (by a factor of 1.5–2) in the responsiveness to serum, PMA, and Sendai virus but not to the other inducers; a further deletion to $-112$ greatly reduced all responsiveness. Thus, the region between $-225$ and $-113$ in IFN-$\beta_2$, which contains DNA motifs similar to the regulatory elements in the human c-*fos* gene, appears to contain the major cis-acting regulatory elements responsible for the activation of the IFN-$\beta_2$ promoter by several different cytokines, viruses, and second messenger agonists.**

The human cytokines variously termed $\beta_2$-interferon (IFN-$\beta_2$), hepatocyte-stimulating factor, B-cell differentiation factor 2, hybridoma or plasmacytoma growth factor, T-cell activating factor, colony-stimulating factor 309, and interleukin 6 represent polypeptides derived from the same human gene located on chromosome 7p21 (reviewed in refs. 1–15). Human fibroblasts can be induced to secrete multiple forms of IFN-$\beta_2$ phosphoglycoproteins in the size range 23–30 kDa: a triplet of molecular mass ≈25 kDa and another triplet of molecular mass 30 kDa (7, 8, 11, 13). Induced human monocytes secrete a similar array of IFN-$\beta_2$ proteins except that the 28- to 30-kDa monocytic proteins are poorly phosphorylated (8, 11, 12). Differential N- and O-glycosylation (the 23- to 25-kDa forms are O-glycosylated; the 28- to 30-kDa forms are O- and N-glycosylated) as well as differential phosphorylation appear to contribute to the observed heterogeneity of the secreted IFN-$\beta_2$ proteins.

IFN-$\beta_2$ gene expression is enhanced in several different types of cells (fibroblasts, monocytes, keratinocytes, other epithelial, and mesenchymal cells) in response to a wide range of "noxious" stimuli, such as bacterial lipopolysaccharide (endotoxin), virus infections, and inflammation-associated cytokines like tumor necrosis factor (TNF), interleukin 1 (IL-1), and platelet-derived growth factor (reviewed in refs. 4–11, 16, 17). Transcription of the IFN-$\beta_2$ gene can be enhanced through the diacylglycerol-, $Ca^{2+}$-, and the cAMP-activated pathways (18–20). The mechanisms that govern the regulation of expression of the IFN-$\beta_2$ gene seem remarkably adapted to the primary function of IFN-$\beta_2$, which appears to be communication between damaged tissues and the hepatocyte, as IFN-$\beta_2$ is a key mediator of the plasma protein response to tissue injury (the "acute-phase" response) (21–24).

The regulation of IFN-$\beta_2$ gene expression in human diploid fibroblasts has been studied in our laboratory at the level of protein secretion (7–11), steady-state mRNA expression (5, 6, 9, 10, 16–18), and nuclear transcription (18, 19). We have now carried out experiments in which the activation of the transfected IFN-$\beta_2$ promoter by cytokines, viruses, and second messenger agonists was studied in transient expression assays in HeLa cells by using plasmid constructs containing 5′ flanking sequences of the human IFN-$\beta_2$ gene fused to the transcription unit encoding the bacterial enzyme chloramphenicol acetyltransferase (CAT). We report that IFN-$\beta_2$ DNA between $-225$ and $+13$ mediates the responsiveness of this promoter to different cytokines, viruses, and second messenger agonists; deletion of DNA upstream of $-112$ greatly reduces inducibility of the IFN-$\beta_2$ promoter. These studies indicate that a 113-nucleotide section of the IFN-$\beta_2$ 5′ flanking DNA (between $-225$ and $-113$) is involved in regulating the expression of this cytokine.

## MATERIALS AND METHODS

**Isolation of Human IFN-$\beta_2$ Genomic DNA Clones and Procedure for Nucleotide Sequencing.** IFN-$\beta_2$ genomic DNA clones were isolated by screening a human genomic DNA library in the vector Charon 4A (25) with a 5′ end-labeled 21-mer oligonucleotide probe (3, 5, 16, 17). The subcloning of 5′ flanking sequences was carried out starting from a 2.5-kilobase-pair (kbp) *Xho* I fragment, the 3′ end of which lies 9 base pairs (bp) downstream of the major cap site in human fibroblasts (4). After digestion with *Bam*HI, the resulting 1.2-kbp *Bam*HI/*Xho* I fragment was subcloned between the

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.

Abbreviations: CAT, chloramphenicol acetyltransferase; EGF, epidermal growth factor; IFN, interferon; IL, interleukin; NPT, neomycin phosphotransferase; TNF, tumor necrosis factor; PMA, phorbol 12-myristate 13-acetate; SV40, simian virus 40.

*Bam*HI and *Sal* I sites of pUC18 and M13 vectors. The nucleotide sequence of the 1.2-kbp 5′ flanking sequence (Fig. 1; 1180 bp long) was obtained by subcloning appropriate fragments into M13 vectors and sequencing recombinant single-stranded M13 DNA by the dideoxy chain-termination procedure.

**Construction of DNA Plasmids Containing IFN-$\beta_2$/CAT Fusion Genes.** The plasmid p$P\beta_2$-CAT was constructed (27) by fusing the bacterial CAT transcription unit [containing the CAT coding region, the simian virus 40 (SV40) small T intron, and the SV40 early poly(A) site] from pSV2-CAT (28) downstream from the IFN-$\beta_2$ promoter (from −1180 to +13) (Fig. 1). Briefly, the 1.2-kbp IFN-$\beta_2$ promoter-containing DNA fragment flanked by the restriction sites *Bam*HI and *Xho* I was subcloned into pUC18 between the *Bam*HI and *Sal* I sites, respectively, to give p$P\beta_2$. The CAT transcription unit flanked by *Hin*dIII and *Bam*HI (filled in) was ligated to p$P\beta_2$ digested with *Hin*dIII and *Nde* I (filled in) generating p$P\beta_2$-CAT (≈5.2 kb). Other plasmids containing the IFN-$\beta_2$ 5′ flanking sequence truncated at three different positions were constructed by using the three restriction sites *Bal* I, *Nhe* I, and *Hae* III as the 5′ boundary and the *Xho* I site at +13 as the 3′ boundary (Fig. 1). The resulting truncated constructs contained IFN-$\beta_2$ DNA from −596 to +13 (*Bal* I), from −225 to +13 (*Nhe* I), and from −112 to +13 (*Hae* III) fused to the CAT transcription unit. *Escherichia coli* strain HB101 was used for production of all plasmid DNA preparations. The plasmid pSV2-neo (29) was used as an internal marker to monitor the efficiency of DNA transfection into HeLa cells.

**DNA Transfection Assays.** HeLa S3 cells were routinely cultured as monolayers in Dulbecco's modified Eagle's medium supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum (GIBCO). Although the initial transfection experiments were carried out by maintaining the cells throughout in medium containing 10% serum, the following low serum protocol has been used more extensively. On the day before DNA transfection, $1.2 \times 10^6$ HeLa cells were plated per 10-cm plastic Petri dish in 5% serum-cohtaining medium devoid of antibiotics. [Antibiotics were omitted to avoid interference with the neomycin phosphotransferase (NPT II) assay]. Three hours before DNA transfection, cells were refed with medium containing only 2% serum. Transfections were carried out by the DNA–calcium phosphate coprecipitation technique (30). Cells were transfected with 15 μg of the test plasmid per Petri dish, containing the IFN-$\beta_2$/CAT fusion gene together with pSV2-neo (1 μg per dish) as an internal transfection marker. The HeLa cell cultures were exposed to the DNA–calcium phosphate coprecipitate for 16 hr. The cultures were then washed extensively with serum-free medium and incubated for another 16 hr in serum-free medium containing 0.01% bovine serum albumin (Miles) with or without other reagents as potential inducers. Cytoplasmic extracts containing equal amounts of protein as measured by the Bradford method (31) were assayed for CAT activity (100–150 μg per assay) as described by Gorman *et al.* (28) (except that 0.1 μCi of [$^{14}$C]chloramphenicol was used in each assay; 1 Ci = 37 GBq) and for NPT II activity (20–50 μg per assay) as described by Platt and Yang (32).

**Activation of the Endogenous IFN-$\beta_2$ Gene in HeLa Cells.** The activation of the endogenous IFN-$\beta_2$ gene in HeLa cells by the different inducers was monitored by immunoblot analyses of the secreted IFN-$\beta_2$ species with a rabbit antiserum to *E. coli*-derived recombinant IFN-$\beta_2$ as a probe (8) and by measuring the ability of the secreted HeLa-cell-derived IFN-$\beta_2$ to enhance $\alpha_1$-antichymotrypsin synthesis and secretion in human hepatoma Hep3B2 cells (American Type Culture Collection; catalogue no. 8064) as described elsewhere (8, 9).

**Other Reagents.** Recombinant *E. coli*-derived human IL-1α (specific activity, $3 \times 10^7$ units/mg) was a gift from Hoffmann–LaRoche and *E. coli*-derived recombinant human TNF (specific activity, $4.8 \times 10^7$ units/mg) was a gift from the Suntory Institute for Biomedical Research (Osaka, Japan). Murine epidermal growth factor (EGF) (catalogue no. 40010) was purchased from Collaborative Research (Waltham, MA). Sendai and pseudorabies virus stocks have been described (10). 1,2-Dioctanoylglycerol was purchased from Avanti Polar Lipids and dissolved in dimethyl sulfoxide (Fisher-Scientific, Springfield, NJ) before use. The calcium ionophore A23187, phorbol 12-myristate 13-acetate (PMA), bacterial lipopolysaccharide (endotoxin), forskolin, 8-BrcAMP, and 3-isobutyl-1-methyl xanthine were purchased from Sigma. A concentrated stock solution of PMA was prepared in dimethyl sulfoxide. [$^{14}$C]Chloramphenicol, [$^{35}$S]methionine, and [γ-$^{32}$P]ATP were purchased from Dupont–New England Nuclear. Autoradiography was carried out with Kodak XAR-5 film. Immunoblot analyses of IFN-$\beta_2$ (8) were carried out with the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA).

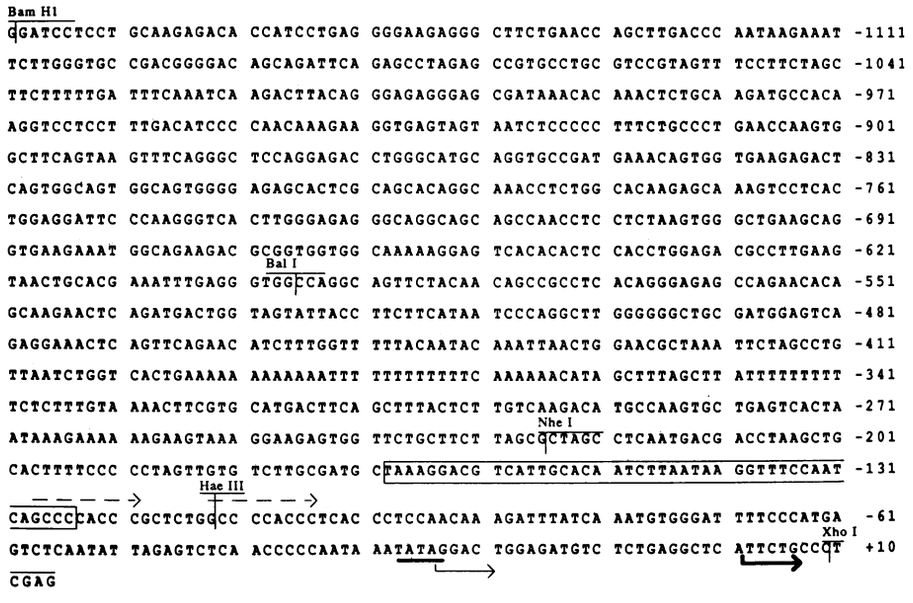


FIG. 1. Nucleotide sequence of the 1.2-kilobase (kb) 5′ flanking region of the IFN-$\beta_2$ gene. Solid arrows, major (thick) and minor (thin) inducible transcription start sites (4). Sequence enclosed in a box is ≈70% identical to the c-*fos* serum-responsive enhancer sequence (18). Broken arrows are the direct repeats similar to those in c-*fos* that have been shown to be important for basal expression (26).

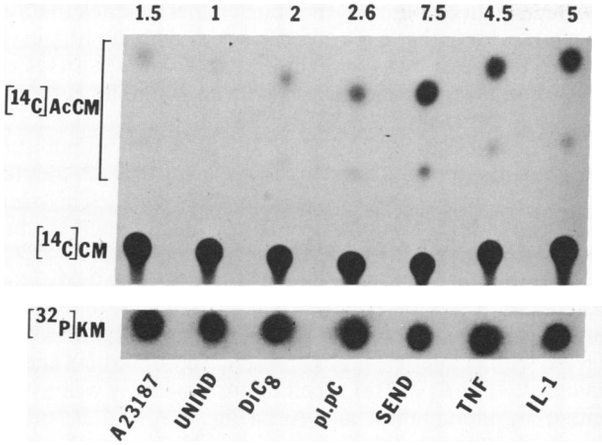


FIG. 2. Activation of the −1180 IFN-$\beta_2$/CAT chimeric gene in the continuous presence of fetal bovine serum (10%). HeLa cells were transfected with 15 $\mu$g of pP$\beta_2$-CAT (containing 1.18 kb of IFN-$\beta_2$ 5′ flanking sequence) and 1 $\mu$g of pSV2-neo. The cells were cultivated throughout in medium containing 10% fetal bovine serum and were induced with various reagents 16 hr after transfection. CAT and NPT II enzyme activities were assayed in cytoplasmic extracts prepared after a 16-hr incubation with the appropriate inducers. 1-Acetyl and 3-acetyl chloramphenicol derivatives (AcCM) formed in the CAT assays were resolved from unreacted chloramphenicol (CM) by TLC and detected by autoradiography (*Upper*). (*Lower*) Autoradiogram showing the kanamycin phosphate (Km) reaction product of the NPT II assay as measured in a dot assay. Numbers denote -fold inductions of CAT expression relative to that in uninduced cells (UNIND) normalized to the NPT II activity in each extract. The inducers used were A23187 (2 $\mu$M), dioctanoylglycerol ($DiC_8$) (50 $\mu$g/ml, which corresponds to 150 $\mu$M), poly(I)·poly(C) (pI·pC) (100 $\mu$g/ml), Sendai virus (SEND) ($4 \times 10^7$ plaque-forming units/ml, which corresponds to a multiplicity of infection of 10), TNF (50 ng/ml), and IL-1$\alpha$ (1 ng/ml).

## RESULTS

**Activation of the −1180 IFN-$\beta_2$/CAT Fusion Gene.** A fusion gene was constructed by linking IFN-$\beta_2$ 5′ flanking DNA from −1180 to +13 to the CAT/SV40 transcription unit and the activation of this chimeric gene was tested by measuring CAT activity in transient expression assays with HeLa cells induced with different reagents. Fig. 2 illustrates an experiment in which the inducibility of the −1180 IFN-$\beta_2$/CAT fusion gene in response to IL-1, TNF, Sendai virus, poly(I)·poly(C), the protein kinase C activator 1,2-dioctanoylglycerol, and the calcium ionophore A23187 were examined. All of the different inducers tested enhanced expression of CAT activity; the greatest enhancement (7.5-fold) was observed in the Sendai virus-induced culture. The low level of enhancement seen in response to 1,2-dioctanoylglycerol and A23187 (1.5- to 2-fold; Fig. 2) was reproducible in additional experiments.

Our initial experiments (Fig. 2) were carried out in HeLa cell cultures that were maintained throughout in medium supplemented with 10% fetal bovine serum. These experimental conditions resulted in fairly high basal levels of CAT activity in "uninduced," transfected HeLa cells. Because bovine serum and platelet-derived growth factor enhance IFN-$\beta_2$ gene expression in fibroblasts (17, 19), the protocol for the transient expression experiment was modified to exclude serum during the induction. Fig. 3 illustrates an experiment in which the transfected HeLa cells were induced with different reagents in medium supplemented with bovine serum albumin. Under these experimental conditions, the basal level of CAT activity in the uninduced cells was low. The inclusion of 10% serum during the induction resulted in a 6-fold increase in CAT expression. Sendai and pseudorabies viruses and the protein kinase C activator PMA caused the greatest increase in CAT expression (7- to 12-fold); poly(I)·poly(C) and the cytokines IL-1, TNF, and EGF enhanced CAT expression 3- to 4-fold; a combination of forskolin, which stimulates adenylate cyclase, and the phosphodiesterase inhibitor isobutylmethylxanthine also produced a clear increase in CAT expression (2.7-fold in this experiment). In additional experiments, forskolin, isobutylmethylxanthine, and BrcAMP alone or in appropriate combinations enhanced CAT expression 2- to 6-fold. CAT expression was reproducibly enhanced by 1,2-dioctanoylglycerol (1.5- to 2-fold); however, under these serum-free conditions A23187 appeared unable to activate the IFN-$\beta_2$/CAT fusion gene.

Bacterial lipopolysaccharide (endotoxin) failed to activate the −1180 IFN-$\beta_2$/CAT chimeric gene in transient expression experiments in HeLa cells (data not shown).

**Activation of Deletion Mutants of the IFN-$\beta_2$/CAT Chimeric Gene.** Chimeric genes containing different lengths of the 5′ flanking IFN-$\beta_2$ DNA linked to the CAT transcription unit were constructed in an attempt to determine the 5′ boundary of the IFN-$\beta_2$ regulatory region sufficient to mediate activation in response to different inducers. The restriction sites *Bal* I, *Nhe* I, and *Hae* III in the IFN-$\beta_2$ 5′ flanking sequence (Fig. 1) were used to construct additional

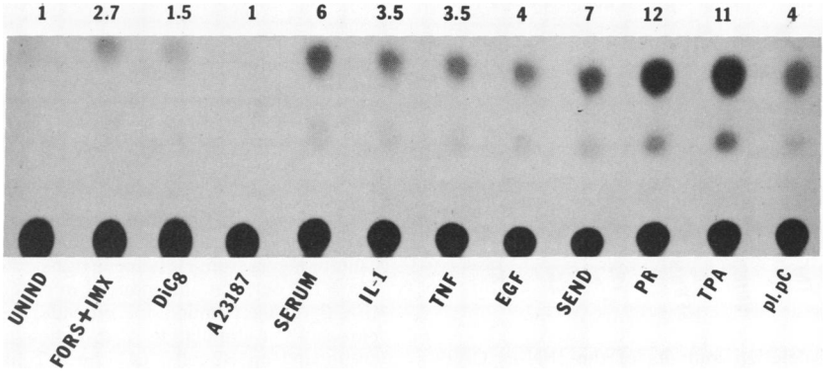


FIG. 3. Activation of the −1180 IFN-$\beta_2$/CAT chimeric gene in serum-free culture conditions. CAT and NPT II enzyme levels were measured in cell extracts of the transfected and induced HeLa cells. The autoradiogram illustrates the CAT assay; results of the NPT II assay are not shown. The -fold induction of CAT expression normalized for NPT II expression is shown above each lane. In this experiment, the transfected HeLa cells were induced with the same reagents and at the same concentrations used in the experiment in Fig. 2 as well as with a combination of forskolin (FORS) (50 $\mu$M) and isobutylmethylxanthine (IMX) (0.5 mM), fetal bovine serum (10%), EGF (100 ng/ml), pseudorabies virus (PR) ($4 \times 10^7$ plaque forming units/ml, which corresponds to a multiplicity of infection of 10), and PMA (TPA) (100 ng/ml, which corresponds to 160 nM).

IFN-$\beta_2$/CAT fusion plasmids containing IFN-$\beta_2$ DNA from $-596$ to $+13$ (*Bal* I), $-225$ to $+13$ (*Nhe* I), and $-112$ to $+13$ (*Hae* III). The four IFN-$\beta_2$/CAT chimeric constructs (designated $-1180$, $-596$, $-225$, and $-112$) were used at the same time in each experiment with different inducers (Fig. 4). Deletion of the 5′ boundary of IFN-$\beta_2$ DNA from $-1180$ to $-596$ preserved activation of the chimeric gene by IL-1, TNF, EGF, serum, pseudorabies and Sendai viruses, and PMA (Fig. 4). Deletion to $-225$ led to a small (1.5- to 2-fold) but reproducible reduction in the responsiveness to serum, PMA, and Sendai virus but not to TNF, IL-1, EGF, or pseudorabies virus (Fig. 4). Further deletion to $-112$ greatly decreased all responsiveness (Fig. 4). Results similar to those illustrated for TNF and IL-1 in Fig. 4 were also obtained in an experiment in which BrcAMP and forskolin were tested for their ability to induce the four chimeric genes (data not shown). Thus, the major decrease in inducibility of the IFN-$\beta_2$ promoter by several different cytokines, viruses, and second messenger agonists is observed when the 5′ flanking DNA is trimmed from $-225$ to $-112$.

In the experiment illustrated in Fig. 4 and in additional experiments, the $-112$ construct retained some (2- to 3-fold) inducibility in response to pseudorabies virus. This shows that the 112-bp 5′ flanking IFN-$\beta_2$ sequence retains the basic elements required for RNA transcription per se.

**Activation of the Endogenous IFN-$\beta_2$ Gene in HeLa Cells.** Enhanced levels of IFN-$\beta_2$ derived from the endogenous HeLa gene were observed in medium from cultures transfected with chimeric $-112$ IFN-$\beta_2$/CAT constructs and then induced. IFN-$\beta_2$ in HeLa cell culture medium was assayed either by immunoblot analyses (Fig. 5 *A* and *B*) or by a bioassay based on the ability of IFN-$\beta_2$ to enhance $\alpha_1$-antichymotrypsin synthesis and secretion in Hep3B2 cells (Fig. 5*C*). Fig. 5 shows a clear enhancement of endogenous IFN-$\beta_2$ production in response to the different inducers tested except in response to lipopolysaccharide and A23187.

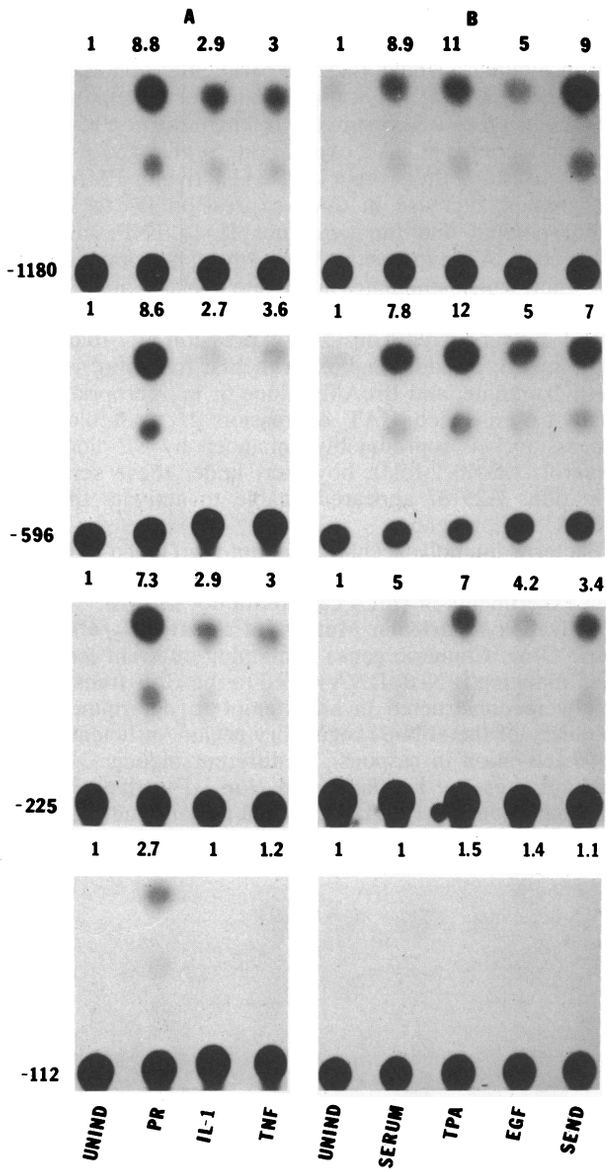


FIG. 4. Activation of deletion mutants of the IFN-$\beta_2$/CAT chimeric gene. Autoradiograms show results of CAT assays on cytoplasmic extracts of HeLa cells transfected with different deletion mutants of the IFN-$\beta_2$/CAT chimeric gene and then induced with different reagents in serum-free conditions. (*A* and *B*) Results of two separate experiments involving sets of different inducers used at concentrations indicated in legends to Figs. 2 and 3. Numbers denote relative induction of CAT expression normalized for NPT II expression.

## DISCUSSION

The role of nucleotide sequences within a 1.2-kbp stretch of DNA immediately upstream of the human IFN-$\beta_2$ gene in mediating the inducible expression of this gene was studied in a HeLa cell transient expression system. The data obtained indicate that activation of an IFN-$\beta_2$/CAT chimeric gene in this experimental system by different cytokines, viruses, and second messengers appears to be mediated primarily through cis-acting regulatory elements located between $-225$ and $-113$ in the IFN-$\beta_2$ DNA.

TNF, IL-1, EGF, bovine serum, poly(I)·poly(C), pseudorabies and Sendai viruses, protein kinase C-activating reagents (dioctanoylglycerol and PMA), the $Ca^{2+}$ ionophore A23187 (under certain experimental conditions), as well as reagents that stimulate the cAMP-activated pathways (BrcAMP, forskolin, isobutylmethylxanthine) were able to activate the $-1180$ IFN-$\beta_2$/CAT chimeric gene (range, 1.5- to 12-fold activation by different inducers). Deletion of the IFN-$\beta_2$ DNA to $-596$ did not affect the responsiveness of the chimeric gene. Deletion of the IFN-$\beta_2$ DNA to $-225$ led to a small but reproducible reduction (by a factor of 1.5–2) in responsiveness to Sendai virus, serum, and PMA. The consensus sequence (TGA$^{G}_{C}$TCA) for the binding of the

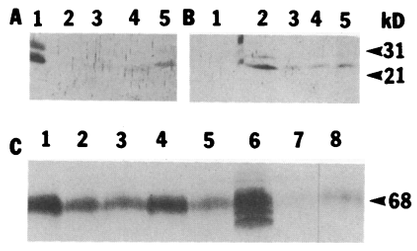


FIG. 5. Activation of the endogenous IFN-$\beta_2$ gene in HeLa cells. Culture media from HeLa cells transfected with plasmids containing the $-112$ deletion and induced in different ways were assayed for IFN-$\beta_2$ using immunoblot procedures (*A* and *B*) or by using a bioassay (*C*; stimulation of $\alpha_1$-antichymotrypsin synthesis and secretion in Hep3B2 cells). (*A*) Lanes: 1, fibroblastic IFN-$\beta_2$ control (7, 8); 2, uninduced HeLa control; 3, lipopolysaccharide; 4, A23187; 5, dioctanoylglycerol. (*B*) Lanes: 1, uninduced; 2, PMA; 3, EGF; 4, Sendai virus; 5, IL-1$\alpha$. (*C*) Assay of medium (1:3 dilution) from HeLa cells treated with IL-1$\alpha$ (lane 1), TNF (lane 2), serum (lane 3), PMA (lane 4), EGF (lane 5), Sendai virus (lane 6), uninduced HeLa cells (lane 7), and IFN-$\beta_2$ added to Hep3B2 cells (lane 8). All the observed stimulation of $\alpha_1$-antichymotrypsin secretion was blocked by anti-recombinant IFN-$\beta_2$ antiserum (data not shown). kD, kDa.

transcription factor AP-1 is considered to mediate some of the responses to PMA (33, 34). This sequence is very similar to the ras responsive element, which also mediates responses to PMA and serum (TGACTAA; ref. 35). An AP-1 consensus sequence (TGAGTCA) is present between nucleotides −280 and −274 in the promoter of the IFN-$\beta_2$ gene.

Deletion of the IFN-$\beta_2$ DNA from −225 to −112 led to a marked decrease in the ability of all of the inducers to activate the chimeric gene. We have previously pointed out that the region from −169 to −124 in IFN-$\beta_2$ is similar to the serum-responsive enhancer in the human c-*fos* gene (19). In addition, the direct repeat sequence present in the human c-*fos* gene between −97 and −76, which has been demonstrated to be important for basal expression (31), is closely similar to the direct repeat sequence GCCCCACC located between −128 and −105 in the IFN-$\beta_2$ sequence. Half of this DNA repeat motif is deleted in the −112 IFN-$\beta_2$/CAT chimeric gene. There are striking similarities between the regulation of expression of the IFN-$\beta_2$ and the c-*fos* genes: both genes are induced by serum, platelet-derived growth factor, EGF, TNF, IL-1, PMA, A23187, cAMP agonists, poly(I)·poly(C), and Sendai virus (reviewed in refs. 19, 26, and 36–38). However, there are important differences in the kinetics of the observed transcriptional activation: the enhancement of c-*fos* transcription is usually very short-lived, whereas that of the IFN-$\beta_2$ gene in response to, for example, IL-1 is sustained for at least 14 hr (19). Thus, there are both striking similarities and differences in the detailed mechanisms by which transcription of the IFN-$\beta_2$ and the c-*fos* genes is regulated.

The chimeric IFN-$\beta_2$/CAT gene containing IFN-$\beta_2$ DNA from −112 to +13 was inducible by pseudorabies virus by 2- to 3-fold. The region between −75 and −66 in IFN-$\beta_2$ (GGGATTTTCC) is very similar to the NF-κB DNA-binding protein motif GGGACTTTCC, which is also found in the cytomegalovirus enhancer, the human immunodeficiency virus enhancer, and the SV40 enhancer (39). It has been previously pointed out that the immediate 5′ flanking region in IFN-$\beta_2$ shares similarity with the 5′ flanking region of the IFN-$\alpha_1$ and -$\beta_1$ genes (40). However, the repeated blocks of purine-rich hexameric sequences present within the 5′ flanking regions of the human IFN-$\alpha_1$ and -$\beta_1$ genes, which have been suggested to be involved in virus inducibility (41), are not detected within the first 596 bp of IFN-$\beta_2$ 5′ flanking DNA, which renders the chimeric IFN-$\beta_2$/CAT gene fully inducible by both Sendai and pseudorabies viruses.

While the transient expression system in HeLa cells used by us to study the function of the transfected IFN-$\beta_2$ promoter largely duplicates the regulation of expression of the endogenous IFN-$\beta_2$ gene in human diploid fibroblasts, several differences are noteworthy. The endogenous IFN-$\beta_2$ gene in human fibroblasts and monocytes is strongly induced by bacterial lipopolysaccharide (endotoxin) (7–9); however, the IFN-$\beta_2$/CAT fusion gene and the endogenous IFN-$\beta_2$ gene in HeLa cells are not responsive to endotoxin. Furthermore, while the endogenous IFN-$\beta_2$ gene in diploid fibroblasts does not appear to respond to EGF (18), the transfected fusion gene and the endogenous IFN-$\beta_2$ gene in HeLa cells respond quite well. The response of the IFN-$\beta_2$ promoter in the HeLa transfection assay to dioctanoylglycerol and A23187 is particularly lower than that observed in human fibroblasts (18). Other investigators have reported that a transfected c-*fos*/CAT chimeric gene responds less well to serum than the endogenous c-*fos* gene (33) and that A23187 was unable to activate a transfected c-*fos* construct while the endogenous gene could be readily activated (34).

In summary, a chimeric CAT gene construct driven by the IFN-$\beta_2$ promoter can be activated by different cytokines, viruses, and second messengers in a transient expression system in HeLa cells. The region between −225 and −113 in the IFN-$\beta_2$ promoter is required for this activation. These data suggest that the different stimuli that turn on the IFN-$\beta_2$ gene utilize biochemical pathways that converge onto this region of the DNA 5′ to the IFN-$\beta_2$ gene.

We thank Dr. Igor Tamm for enthusiastic support and for numerous helpful discussions and Dr. Uma Santhanam for immunoblot analyses in Fig. 5. This work was supported by Research Grant AI-16262 from the National Institutes of Health, a contract from The National Foundation for Cancer Research, and an Established Investigatorship from The American Heart Association (P.B.S.).

1. Sehgal, P. B. & Sagar, A. D. (1980) *Nature (London)* **288**, 95–97.
2. Weissenbach, J., Chernajovsky, Y., Zeevi, M., Shulman, L., Soreq, H., Nir, U., Wallach, D., Perricaudet, M., Tiollais, P. & Revel, M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 7152–7156.
3. Sehgal, P. B., Zilberstein, A., Ruggieri, R., May, L. T., Ferguson-Smith, A., Slate, D. L., Revel, M. & Ruddle, F. H. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 5219–5222.
4. Zilberstein, A., Ruggieri, R., Korn, J. H. & Revel, M. (1986) *EMBO J.* **5**, 2529–2537.
5. May, L. T., Helfgott, D. C. & Sehgal, P. B. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8957–8961.
6. Sehgal, P. B., May, L. T., Tamm, I. & Vilček, J. (1987) *Science* **235**, 731–732.
7. May, L. T., Santhanam, U., Tatter, S. B., Ghrayeb, J. & Sehgal, P. B. (1988) in *Tumor Necrosis Factor/Cachectin, Lymphotoxins and Related Cytokines*, eds. B. Bonavida & H. Kirchner (Karger, Basel), in press.
8. May, L. T., Ghrayeb, J., Santhanam, U., Tatter, S. B., Sthoeger, Z., Helfgott, D. C., Chiorazzi, N., Grieninger, G. & Sehgal, P. B. (1988) *J. Biol. Chem.* **263**, 7760–7766.
9. Helfgott, D. C., May, L. T., Sthoeger, Z., Tamm, I. & Sehgal, P. B. (1987) *J. Exp. Med.* **166**, 1300–1309.
10. Sehgal, P. B., Helfgott, D. C., Santhanam, U., Tatter, S. B., Clarick, R. H., Ghrayeb, J. & May, L. T. (1988) *J. Exp. Med.* **167**, 1951–1956.
11. May, L. T., Santhanam, U., Tatter, S. B., Bhardwaj, N., Ghrayeb, J. & Sehgal, P. B. (1988) *Biochem. Biophys. Res. Commun.* **152**, 1144–1150.
12. Tosato, G., Seamon, K. B., Goldman, N. D., Sehgal, P. B., May, L. T., Washington, G. C., Jones, K. D. & Pike, S. E. (1988) *Science* **239**, 502–504.
13. Gauldie, J., Richards, C., Harnish, D., Lansdorp, P. & Baumann, H. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 7251–7255.
14. Ferguson-Smith, A. C., Chen, Y.-F., Newman, M. S., May, L. T., Sehgal, P. B. & Ruddle, F. H. (1988) *Genomics* **2**, 203–208.
15. Bowcock, A. M., Kidd, J. R., Lathrop, M., Daneshvar, L., May, L. T., Ray, A., Sehgal, P. B., Kidd, K. K. & Cavalli-Sforza, L. L. (1988) *Genomics* **3**, 8–16.
16. Kohase, M., Henriksen-DeStefano, D., May, L. T., Vilček, J. & Sehgal, P. B. (1986) *Cell* **45**, 659–666.
17. Kohase, M., May, L. T., Tamm, I., Vilček, J. & Sehgal, P. B. (1987) *Mol. Cell. Biol.* **7**, 273–280.
18. Sehgal, P. B., Walther, Z. & Tamm, I. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 3663–3667.
19. Walther, Z., May, L. T. & Sehgal, P. B. (1988) *J. Immunol.* **140**, 974–977.
20. Zhang, Y., Lin, J. & Vilček, J. (1988) *J. Biol. Chem.* **263**, 6177–6182.
21. Fåhreus, R. (1921) *Acta Med. Scand.* **55**, 1–228.
22. Tillet, W. S. & Francis, T., Jr. (1930) *J. Exp. Med.* **52**, 561–572.
23. MacLeod, C. M. & Avery, O. T. (1941) *J. Exp. Med.* **73**, 183–190.
24. Kushner, I. (1982) *Ann. N.Y. Acad. Sci.* **389**, 39–48.
25. Lawn, R. M., Fritsch, E. F., Parker, R. C., Blake, G. & Maniatis, T. (1978) *Cell* **15**, 1157–1174.
26. Fisch, T. M., Prywes, R. & Roeder, R. G. (1987) *Mol. Cell. Biol.* **7**, 3490–3502.
27. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
28. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell. Biol.* **2**, 1044–1051.
29. Southern, P. J. & Berg, P. (1982) *Mol. Appl. Genet.* **1**, 227–341.
30. Graham, F. L. & Van der Eb, A. J. (1973) *Virology* **52**, 456–467.
31. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254.
32. Platt, S. G. & Yang, N. S. (1987) *Anal. Biochem.* **162**, 529–535.
33. Angel, P., Imagawa, M., Chiu, R., Stein, B., Imbra, R., Rhamsdorf, H. J., Jonat, C., Herrlich, P. & Karin, M. (1987) *Cell* **49**, 729–739.
34. Lee, W., Mitchell, P. & Tjian, R. (1987) *Cell* **49**, 741–752.
35. Imler, J. L., Schatz, C., Wasylyk, C., Chatton, B. & Wasylyk, B. (1988) *Nature (London)* **332**, 275–278.
36. Treisman, R. (1985) *Cell* **42**, 889–902.
37. Lin, J.-X. & Vilček, J. (1987) *J. Biol. Chem.* **262**, 11908–11911.
38. Zinn, K., Keller, A., Whittemore, L.-A. & Maniatis, T. (1988) *Science* **240**, 210–213.
39. Pierce, J. W., Lenardo, M. & Baltimore, D. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1482–1486.
40. Chernajovsky, Y., Mory, Y., Chen, L., Marks, Z., Novick, D., Rubinstein, M. & Revel, M. (1984) *DNA* **3**, 297–308.
41. Ryals, J., Dierks, P., Ragg, H. & Weissmann, C. (1985) *Cell* **41**, 497–507.