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# Transcriptional induction of two genes in human cells by β interferon

A. C. LARNER*, G. JONAK†, Y.-S. E. CHENG†, B. KORANT†, E. KNIGHT†, AND J. E. DARNELL, JR.*

*Rockefeller University, 1230 York Avenue, New York, NY 10021; and †Central Research Department, E. I. DuPont, Wilmington, DE 19898

*Contributed by J. E. Darnell, Jr., July 9, 1984*

**ABSTRACT** **The binding of interferons to distinct cell surface receptors leads to the induction of synthesis of several unique polypeptides and their corresponding mRNAs (1–6). We have isolated two cDNAs that are complementary to nuclear RNA whose synthesis is induced from undetectable levels to maximal rates of transcription within 30–60 min after the addition of β interferon to human fibroblasts or to HeLa cells. These results prove that a single polypeptide can, by binding to a specific plasma membrane receptor, promptly activate the transcription of a defined set of genes.**

Treatment of mammalian cells with species-specific type 1 or type 2 interferon (IFN) induces the synthesis of at least 6–12 new polypeptides (1–7) and causes the cells to become refractory to infection by a variety of viruses. The accumulation of these IFN-induced proteins is inhibited by actinomycin D, indicating that their synthesis requires new mRNA formation. An increase in several specific mRNAs has been documented by translation of total cell mRNA of treated and untreated cells and by isolation of two specific cDNA clones complementary to mRNA that is increased in treated cells (8, 9). One of these cDNA clones is complementary to the mRNA for a 56-kDa protein (8), and the other, to the mRNA for 2′,5′-oligo(A) synthetase (9), both of which are prominent IFN-induced protein products (7). Whether this increase in specific mRNA is based on increased transcription and/or increased stabilization of the mRNA in the cytoplasm has not been determined.

In fact, whether transcriptional increases underlie the numerous biologic effects mediated by the binding of peptide hormones, growth factors, and lectins to their specific plasma membrane receptors is at present largely unknown. It is also unclear whether a polypeptide can effect an increase in specific mRNA concentration, particularly by transcriptional control, without entering the cell. Molecules such as cyclic AMP or $Ca^{2+}$ or analogous "second messengers" could mediate the transcriptional stimulation of specific genes after an initial hormone–receptor interaction. Likewise, the effect of intracellular tyrosine kinase activities of membrane receptor proteins is under intense investigation, but no specific transcriptional activations have been documented as a result of these enzyme activities.

A few cases have been analyzed that support the hypothesis of second messengers as transcriptional activators. Thyrotropin-releasing hormone (TRH) and epidermal growth factor (EGF) increase transcription of the prolactin gene in a pituitary cell line (10, 11). The effects of TRH can be mimicked by cyclic AMP treatment of the pituitary tumor cells. EFG-induced prolactin mRNA accumulation requires extracellular $Ca^{2+}$, which implies that increased free intracellular $Ca^{2+}$ is involved in the mediation of this hormone's action (12). In another case, insulin causes a 30–40% inhibition of the transcription of phosphoenolpyruvate carboxykinase (13). One case of a protein hormone effecting an increase in a specific mRNA largely through mRNA stabilization is known. Prolactin increases casein mRNA concentration by a factor of 100 in mammary gland organ cultures (14), but transcription of casein mRNA increases only about 3-fold. Stabilization accounts for the majority of increased cytoplasmic mRNA concentration. Recently, mRNAs have been described that are increased after treatment of cells with platelet-derived growth factor (15) or serum stimulation of resting cells (16). In these cases the level of control of the increased mRNAs has not yet been reported.

The mechanism of IFN action on gene control is an experimentally attractive instance of protein-induced cellular responses because (*i*) there are distinct cell-surface receptors for α, β, and γ IFNs (17), (*ii*) different proteins and mRNAs are induced by human IFNs of type 1 (IFN-α or -β) and type 2 (IFN-γ) (2, 3, 6), (*iii*) the IFNs apparently can induce the antiviral state without entering the cell (18), and (*iv*) the mode of action of IFN is probably not through cyclic AMP (19). Therefore, if IFN stimulates transcription of specific genes, it would represent an excellent system to study transcriptional control mediated from the plasma membrane. For this purpose, we have isolated two cDNA clones that are complementary to mRNA of IFN-β-treated cells but not to mRNA of untreated cells. One of the two clones is complementary to a mRNA of about 1.9 kilobases (kb) that encodes an IFN-induced 56-kDa protein (8). The second clone is complementary to a 2.9-kb mRNA. After treatment with IFN-β but not with IFN-γ, the cytoplasmic mRNA concentration of both these mRNAs increases in both fibroblasts and HeLa cells. This increase results from a rapid increase in transcription of these genes that is maximal within 30–60 min.

## METHODS AND MATERIALS

**IFNs.** Human IFN-β ($10^3$ units/mg) was produced and purified to homogeneity as described by Knight *et al.* (20). Human IFN-γ was purchased from Interferon Sciences (New Brunswick, NJ).

**Cell Cultures.** Human diploid fibroblasts were grown in Dulbecco's modified Eagle's medium (DME medium) containing 7% (vol/vol) fetal calf serum in 850-$cm^2$ roller bottles (6). Seven- to 8-day-old confluent monolayers were treated with IFN-β in 50 ml of medium containing 7% fetal calf serum and buffered with 20 mM Hepes (pH 7.2).

**Isolation of mRNA and Labeling of Nascent RNA in Isolated Nuclei.** Cells were rinsed and scraped into ice-cold phosphate-buffered saline, centrifuged, and resuspended on ice in 10 mM Tris, pH 7.4/10 mM NaCl/5 mM $MgCl_2$/1 mM dithiothreitol (RSB) at 3–5 × $10^7$ cells per ml. After swelling for 5 min, the cells were disrupted by Dounce homogenization, and nuclei were removed by centrifugation (3 min at 2000 × *g*). Cytoplasmic RNA was isolated as described by Salditt-Georgieff *et al.* (21). For construction of a cDNA li-

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Abbreviations: IFN-β, β interferon; TRH, thyrotropin-releasing hormone; EGF, epidermal growth factor; kb, kilobases.

brary, cellular RNA was prepared by using guanidium isothiocyanate (22). Poly(A)$^+$ RNA was isolated by two cycles of purification on oligo(dT)-cellulose (23). For transcriptional assay, the nuclei (5–50 × $10^7$ per reaction) were resuspended in 5 ml of 20 mM Hepes, pH 7.9 at 25°C/20% glycerol/140 mM KCl/10 mM $MgCl_2$/1 mM dithiothreitol (nuclear reaction buffer) for 5 min at 4°C, centrifuged, and resuspended in 200–400 μl of nuclear reaction buffer containing 1 mM each of ATP, GTP, and CTP; 3 μM UTP; 0.2–0.5 mCi of [α-$^{32}$P]UTP (3000 Ci/mmol; 1 Ci = 37 GBq). Nuclei were incubated for 15 min at 30°C. DNase buffer (10 mM Tris, pH 7.4/0.5 M NaCl/50 mM $MgCl_2$/2 mM $CaCl_2$/50 μg of DNase per ml) was added to a total of 2 ml, and nuclear RNA was extracted (21) and freed of unincorporated triphosphates by $CCl_3COOH$ precipitation (24).

**Construction and Screening of Recombinant cDNA Clones.** Poly(A)$^+$ RNA (6 μg) was used to produce double-stranded cDNA with C tails. This was hybridized to G-tailed pBR322 cut at the *Pst* I site, ligated, and transfected into *Escherichia coli* (25). Tetracycline-resistant colonies were isolated, and colony screens were carried out with $^{32}$P-labeled cDNA probes made from mRNA of IFN-treated or untreated fibroblasts. The conditions for colony hybridization were as described by Derman *et al.* (26). The plasmid pIFN-IND-2 was selected from the cDNA library by using a 20-base deoxynucleotide probe synthesized from the published cDNA sequence (8). The probe was treated with kinase, and the recombinant clone was selected (27).

**Gel Electrophoresis, Blotting, and Hybridization of RNA.** Poly(A)$^+$ RNA (1–2 μg) or total RNA (40 μg) samples were subjected to electrophoresis in formaldehyde/agarose gels containing borate buffer, transferred to either nitrocellulose (26) or GeneScreen (New England Nuclear), hybridized with nick-translated recombinant plasmid DNA, washed, and autoradiographed (27). Labeled nuclear RNA was hybridized to excess plasmid DNA bound in "dots" to nitrocellulose as described (28, 29). Between 5 × $10^6$ and 20 × $10^6$ cpm in 2 ml was hybridized with filters containing 6–12 dots. After being washed and treated with ribonuclease, the filters were autoradiographed (26).

## RESULTS

Poly(A)-containing RNA was prepared from human fibroblasts that had been treated with IFN-β for 5 hr. Double-stranded cDNA was made and inserted into the *Pst* I site of G-tailed pBR322. Colonies from this cDNA library were plated on duplicate filters, and colony hybridization was carried out with single-stranded [$^{32}$P]cDNA made from poly(A)$^+$ RNA of both IFN-β-treated and untreated cells. Colonies that hybridized only or more strongly with the probe from IFN-treated cells were selected. After a second screening, several plasmids were isolated, nick-translated, and hybridized to RNA blots containing equal concentrations of poly(A)$^+$ RNA from either normal fibroblasts or fibroblasts treated with various concentrations of IFN-β for 5 hr. One plasmid, pIFN-IND-1 (plasmid IFN-induced-1), hybridized to a mRNA of 2.9 kb that is present only in IFN-treated cells (Fig. 1). [On high resolution gels, this large band is split into two, and mRNA selected by this clone translates into two proteins of about 42 and 58 kDa (unpublished observations).] The pIFN-IND-2 clone was originally identified by using a synthetic oligonucleotide probe prepared from the published cDNA sequence of the 56-kDa protein (8). The mRNA that hybridizes to pIFN-IND-2 is 1.8–1.9 kb and is also present only in cells that have been exposed to IFN (Fig. 1). The dose of IFN-β necessary to evoke a maximum induction of these mRNAs was between 25 and 50 units per ml. An example of a mRNA whose mRNA concentration did not change with IFN-β treatment was included in this experiment. This

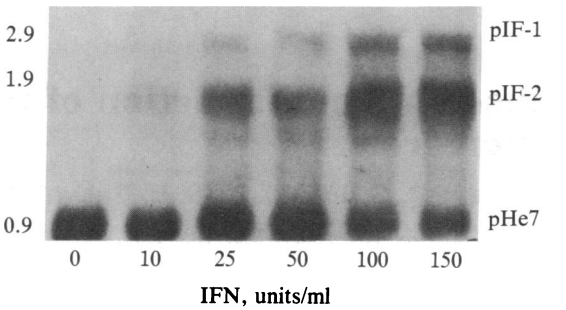


FIG. 1. Increase in mRNA complementary to pIFN-IND-1 (pIF-1) and -2 (pIF-2) RNA in human fibroblasts treated with various concentrations of IFN-β. Human fibroblasts were incubated with varying concentrations of IFN-β for 1 hr at 37°C. Total RNA was extracted, and 40-μg samples were subjected to electrophoresis through a 1.4% formaldehyde/agarose gel and transferred to nitrocellulose. The RNA blot filter was hybridized to $10^7$ cpm of nick-translated pIFN-IND-1 and -2, and 3 × $10^6$ cpm of pHe7 and then autoradiographed. pHe7 is a cDNA clone isolated from HeLa cells (28). In separate experiments, it was determined that pIFN-IND-1 hybridizes to the mRNA of 2.9 kb; pIFN-IND-2, to the mRNA of 1.9 kb; and pHe7, to a mRNA of 0.9 kb.

cDNA, called pHe7, was cloned from growing HeLa cells (28), and the pHe7 mRNA was equally present in all RNA samples.

mRNA was also prepared from HeLa cells after 5 hr of IFN-β treatment and subjected to RNA blot analysis. Again, mRNAs complementary to pIFN-IND-1 and pIF-IND-2 were not present in untreated cells and gave strong signals after treatment (Fig. 2).

Exposures of autoradiographs in Figs. 1 and 2, which would permit detection of a mRNA present in as low abundancy as 0.005%, revealed no evidence of mRNAs corresponding to pIFN-IND-1 or pIFN-IND-2 in either human fibroblast or HeLa cells not exposed to IFN-β. This result indicated that IFN-β might act to stimulate the transcription of these genes. Nascent chain elongation of RNA from isolated nuclei in the presence of [α-$^{32}$P]UTP is a valid assay to quantitate the relative rates of transcription of individual genes (26, 29). Therefore, nuclei were isolated from human fibroblasts and HeLa cells after IFN-β treatment, and nascent labeled nuclear RNA was prepared and hybridized to DNA bound to nitrocellulose filters (Fig. 3). The cDNAs used in the hybridization analysis included pIFN-IND-1 and pIFN-IND-2 as well as "common" cDNAs—those known to be complementary to mRNAs in a variety of cells. The transcription of sequences complementary to pIFN-IND-1 and pIFN-IND-2 were greatly increased in the nuclei of IFN-β-

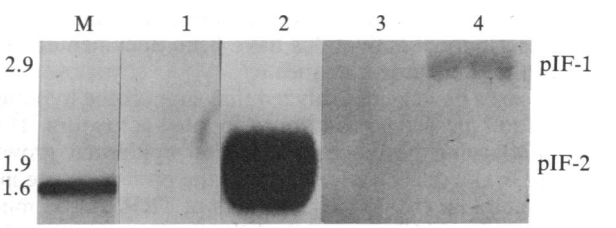


FIG. 2. pIFN-IND-1 (pIF-1) and pIFN-IND-2 (pIF-2) mRNAs are not detected before treatment of HeLa cells with IFN-β. Suspension cultures of HeLa cells ($10^6$ cells per ml) were incubated with 1000 units of IFN-β per ml for 3 hr. RNA was extracted, and poly(A)$^+$ RNA was subjected to electrophoresis, blotted, and probed as described in Fig. 1. Lanes: M, molecular weight markers in kb; 1 and 3, poly(A)$^+$ RNA from untreated cells probed with pIFN-IND-2 and pIFN-IND-1, respectively; 2 and 4, RNA from IFN-treated cells. Exposure was for 1 wk to highlight the absence of the mRNAs in uninduced cells.

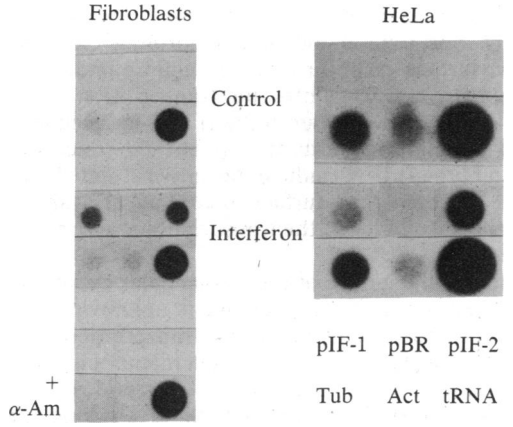


FIG. 3. Dot hybridizations of $^{32}$P-labeled RNA isolated from nuclei of human fibroblasts or HeLa cells. Cells were incubated 3 hr in the presence of IFN-$\beta$ (200 units/ml for fibroblasts or 1000 units/ml for HeLa cells). Nuclei were isolated and assayed for transcription by labeling nascent RNA with [$\alpha$-$^{32}$P]UTP and hybridizing equal amounts of labeled RNA to dots of DNA containing pIF-IND-1 (pIF-1), pBR322 (pBR) (background), pIF-IND-2 (pIF-2), $\beta$-tubulin (Tub), actin (Act), and arginine tRNA (tRNA). The filters were washed, digested with RNase, and autoradiographed as described (26). (*Bottom Left*) Addition of a low dose of $\alpha$-amanitin ($\alpha$-Am) selectively inhibited the activity of RNA polymerase; it abolished IFN-induced transcription of RNA to pIF-1 and -2 actin and $\beta$-tubulin but transcription of arginine tRNA was not affected.

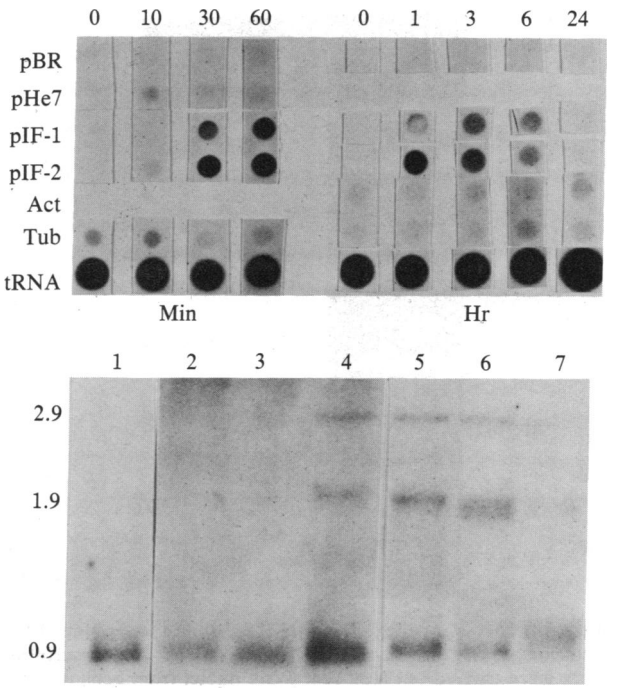


FIG. 4. (*Upper*) Time course of the transcriptional induction of pIF-IND-1 and -2 by IFN-$\beta$. Human fibroblasts were incubated with IFN-$\beta$ for the indicated times, and a transcription assay was performed with nascent labeled RNA obtained from isolated nuclei (Fig. 3). Two separate time courses are shown, one from 0 to 60 min and one from 0 to 24 hr. (*Lower*) Time course of IFN-$\beta$-induced appearance of cytoplasmic mRNA corresponding to pIF-IND-1 and -2. Cytoplasmic poly(A)$^+$ RNA was isolated from the same human fibroblast cultures used for the transcription studies in *Upper*. Samples were subjected to electrophoresis through 1.4% formaldehyde/agarose gels and transferred to GeneScreen. The RNA blot filters were hybridized to pIFN-IND-1, pIFN-IND-2, and pHe7 and autoradiographed. Lanes: 1, no IFN; 2–7, IFN treatment for 10 min, 30 min, 1 hr, 3 hr, 6 hr, or 24 hr, respectively. pBR, pBR322; pIF-1, pIF-IND-1; pIF-2, pIF-IND-2; Tub, $\beta$-tubulin; Act, actin; tRNA, arginine tRNA.

treated cells compared to the nuclei of untreated cells. The signal in untreated cells was not above the background of RNA bound to pBR322 DNA (Fig. 3), and the signal from induced cells was at least 50-fold higher. The transcription rates were not changed for arginine tRNA, actin, $\beta$-tubulin, and a number of other genes whose mRNA concentrations did not change after IFN-$\beta$ treatment. By autoradiographic analysis, the transcription rate of RNA complementary to pIFN-IND-2 appeared to be somewhat greater than that to pIFN-IND-1. However, the insert size of the cDNA for pIFN-IND-2 is about 610 base pairs compared to about 200 base pairs for pIFN-IND-1. Thus, the two genes are induced to a similar degree by IFN-$\beta$ treatment. HeLa cells also increased transcription of both genes when treated with IFN-$\beta$. In the transcriptional analysis of HeLa cells shown in Fig. 3, the pIFN-IND-2 cDNA hybridized considerably more RNA, but transcription of both genes was induced. Also in HeLa cell nuclei, synthesis of RNA complementary to $\beta$-tubulin and several other genes was unchanged by IFN treatment.

A low dose of $\alpha$-amanitin (0.5–5 $\mu$g/ml) selectively inhibits the activity of RNA polymerase II (30). The IFN-induced transcription of RNA to pIF-IND-1 and pIF-IND-2 were completely abolished, while the transcription of arginine tRNA (a polymerase III product) was unaffected (Fig. 3 *Bottom Left*). Thus, the transcription of the IFN-induced genes occurs by RNA polymerase II.

A time course for the transcriptional response to IFN-$\beta$ treatment and the time for accumulation of mRNA was established in fibroblasts. The transcription of both the pIFN-IND-1 and pIFN-IND-2 genes increased to a maximum within 30 min (transcription of pIF-IND-2 was detectable even within 10 min after incubation with IFN-$\beta$; Fig. 4 *Upper*). After 6 hr of IFN treatment, the level of transcription had declined and was undetectable after 24 hr. The accumulation of both IFN-induced mRNAs was detectable within 1 hr, lagging about 30 min behind the induction (Fig. 4 *Lower*) of transcription. The maximum concentration of cytoplasmic mRNAs for pIFN-IND-1 and -2 were observed after about 6 hr of IFN treatment. After 24 hr the induced mRNAs were present at <1/10th of their peak concentrations, indicating that synthesis ceased and turnover reduced the mRNA concentration by at least a factor of 10 between 6 and 24 hr. These results suggest that the mRNAs complementary to pIFN-IND-1 and -2 have half-lives considerably less than 8–10 hr and perhaps as short as 2–4 hr.

Both IFN type I (IFN-$\alpha$ and -$\beta$) and type II (IFN-$\gamma$) stimulate the antiviral state in human fibroblasts, and both types of IFN induce some common and some unique polypeptides in these cells (2, 3, 6). However, ligand-binding studies indicate that IFN-$\beta$ and IFN-$\gamma$ bind to different plasma membrane receptors (17). Thus, the intracellular mechanism by which IFN-$\beta$ and -$\gamma$ exert their actions might not be identical. Therefore, we tested whether IFN-$\gamma$ induces the transcription of RNA complementary to pIFN-IND-1 and pIFN-IND-2 in human fibroblasts. The nuclei from cells treated by IFN-$\beta$ but not IFN-$\gamma$ showed a marked simulation of transcription for RNA complementary to pIFN-IND-1 and -2 (Fig. 5). In addition, the cytoplasmic mRNA was examined from cells treated with both IFNs. Although the mRNA complementary to pIFN-IND-1 and pIFN-IND-2 increased in cells treated with IFN-$\beta$, no such mRNA increase was found after treating cells with IFN-$\gamma$ concentrations ranging from 10 units/ml to 2000 units/ml (data not shown). Thus, IFN-$\beta$ and -$\gamma$, which have distinct surface receptors, have separate effects on gene transcription and on the stimulation of the synthesis of different proteins as described (2, 3, 6). An earlier report (8) described an increase in a transformed

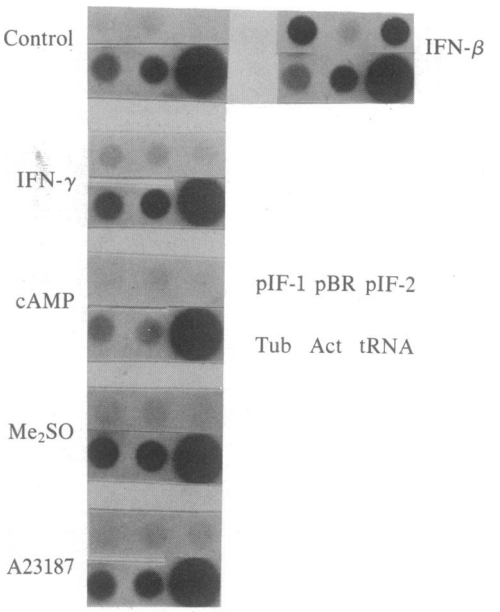


FIG. 5. IFN-γ, cyclic AMP, and the calcium ionophore A23187 do not induce the transcription of pIF-IND-1 or -2. Transcriptional assays were carried out (see Fig. 3) on nuclei from cells incubated with IFN-γ (3 hr; 2000 units/ml), 8-bromo-cAMP (3 mM) or A23187 (2 μM) or IFN-β (200 units/ml). The dimethyl sulfoxide treatment was a control for A23187, which is dissolved in that solvent. The labeled RNA was hybridized to DNA on dots; pIF-1, pIFN-IND-1; pIF-2, pIFN-IND-2; pBR, pBR322; Tub, β-tubulin; Act, actin; tRNA, arginine tRNA.

cell line of the mRNA of the 56-kDa protein by IFN-γ. Perhaps there is a difference between the type of IFN-β and -γ receptors in different cells, since we failed to find such an increase in IFN-γ-treated fibroblasts, although the cells were rendered resistant to virus by the preparation of IFN-γ that was used.

As indicated in the introduction, cyclic AMP can act as a "second messenger" and can induce the transcription of prolactin (10). Also $Ca^{2+}$ may be involved in the ability of EGF and TRH to stimulate prolactin mRNA synthesis (12). We tested whether these second messengers could stimulate the transcription of pIFN-IND-1 or pIFN-IND-2. Transcriptional assays were performed after treating cells with IFN-β, with 8-bromo-cAMP, or with the calcium ionophore A23187, which increases free intracellular $Ca^{2+}$ (31). There was no increase in transcription of either IFN-responsive gene when cells were treated with 8-bromo-cAMP or with the calcium ionophore in contrast to IFN-β treatment (Fig. 5). Actin and β-tubulin, as well as the polymerase III product tRNA arginine, were transcribed similarly under all conditions. In addition, when fibroblasts were incubated with IFN-β in phosphate-buffered saline containing no added $Ca^{2+}$, IFN-β still induced the synthesis of the mRNAs corresponding to IFN-IND-1 and -2 (data not shown). Therefore, it is unlikely that IFN-β action increases the transcription through increased concentrations of cyclic AMP or free intracellular $Ca^{2+}$. We cannot completely eliminate the possibility that IFN-β exerts its effects through these second messengers because we have no cloned human DNA whose transcription is increased by cAMP or increased $Ca^{2+}$ in human cells.

## DISCUSSION

This investigation demonstrates directly that IFN-β induces the transcription rates of specific genes. The increased transcription of the two genes studied rises at least 50-fold above the background level in both human fibroblasts and HeLa cells. The increase occurs within 10 min for pIFN-IND-1 and is maximal by 30–60 min for both genes. Thus, within a remarkably short time, a polypeptide hormone can bind a specific cell-surface receptor and, through an undefined intracellular pathway, regulate the transcription of specific genes. The fact that neither of the IFN-β-induced mRNAs is induced by IFN-γ accords with published conclusions that type I and type II IFNs induce the antiviral state by interacting with different cell-surface receptors (17) and demonstrates the specificity of the transcriptional response reported here.

At present the nature of the intracellular events responsible for the transcriptional increase is unknown. Unlike the steroid hormones that induce the transcription of specific genes by diffusing across the plasma membrane and binding to specific intracellular receptors, polypeptides, such as the IFNs, may exert their effects without entering the cells. However, as discussed, the intracellular effect of IFN is probably not mediated by cyclic AMP or $Ca^{2+}$ influx.

A number of other possible mechanisms may be postulated by which IFN-β stimulates transcription of specific genes. It is known that most polypeptide hormone–receptor complexes are transported into the cell by receptor-mediated endocytosis (32). In human fibroblasts, IFN-α and -γ are known to be internalized and degraded in lysosomes (33, 34). Previous studies suggest that the IFN-induced development of the antiviral state does not require that the polypeptide be internalized (18). However, it is possible that the internalized IFN itself, the IFN receptor, or a degradation product of either could be the transcriptional activating factor. Another possible mechanism of transcriptional stimulation is intracellular protein phosphorylation stimulated by a ligand–receptor interaction at the cell surface (35, 36). In most cases the function of hormonally stimulated phosphorylated proteins is unclear. Murdoch *et al.* (39) recently have suggested that the phosphorylation of a specific nuclear protein is correlated with the induction of prolactin gene expression. Although TRH-induction of prolactin transcription appears to be cyclic AMP-mediated, there are many hormones that do not act via cyclic AMP, which can stimulate the phosphorylation of intracellular proteins (36). It would be worthwhile, therefore, to examine changes in the phosphorylated nuclear proteins of IFN-treated cells. Finally, it is becoming apparent that hormone–cell-surface receptor interactions can trigger the phosphorylation of tyrosine residues on the hormone receptor itself (37, 38). This phosphorylation may be the initial signal for the action of such hormones as insulin or EGF.

The first goal of these studies has been accomplished with the characterization of two genes whose transcription is rapidly induced by IFN-β. It is now possible to begin to dissect the mechanism of transcriptional activation initiated by a hormone binding to a plasma membrane receptor.

While preparing our manuscript, we learned from R. L. Friedman, S. P. Manly, and G. R. Stark (personal communication) that they have selected human cDNAs complementary to seven mRNAs induced by IFN in neuroblastoma cells. At least one of these mRNA sequences is increased at the transcriptional level.

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