Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 3964–3968, July 1984
Biochemistry

Inhibition of protein synthesis stimulates the transcription of human β-interferon genes in Chinese hamster ovary cells

(gene regulation/*in vitro* transcription/double-stranded RNA)

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Communicated by I. Robert Lehman, March 5, 1984

ABSTRACT **Using Chinese hamster ovary (CHO) cells transfected with a plasmid carrying the human β-interferon gene, we find that inhibitors of protein synthesis, in the absence of any other inducer, stimulate the production of interferon RNA; this effect is maintained in cells in which the plasmid sequences have been amplified 25- to 50-fold. Nuclear transcription assays show that a major effect of cycloheximide is to increase the rate of transcription of the interferon gene. This contradicts the generally accepted explanation that inhibitors of protein synthesis augment interferon production by stabilizing interferon mRNA. In addition, we have studied the effects of double stranded RNA [poly(rI)·poly(rC)] on the induction of interferon RNA in the presence and absence of cycloheximide. Our results indicate that poly(rI)·poly(rC) by itself causes a transient increase in interferon RNA; however, in the presence of cycloheximide this effect is prolonged. We do not, however, find an increase in transcription of the interferon gene(s) as an early response to poly(rI)·poly(rC). Finally, we have found that cells treated with cycloheximide or infected with Newcastle disease virus induce large amounts of a secreted 11-kDa protein. This cellular protein is not inducible by poly(rI)·poly(rC). We propose that both interferon and this 11-kDa protein belong to a family of proteins in which production is regulated in a coordinate fashion during viral inhibition of cellular protein synthesis.**

Interferons (IFNs) are hormone-like polypeptides with antiviral activity that are secreted by cells under a variety of conditions. Type I IFNs (IFN-α and IFN-β) are induced by viral infection or by treating cells with double-stranded RNA, whereas type II (IFN-γ) is produced by lymphocytes in response to antigenic stimulation (1, 2). The human genes encoding all three classes of interferons have been cloned and their nucleotide sequences have been determined (3–8).

Human IFN-β (also known as fibroblast interferon) is a glycoprotein with an apparent molecular size of 22 kDa and is produced by virus-infected or double-stranded RNA-treated cells (1, 2). The production of IFN-β can be augmented by addition of inhibitors of protein synthesis (e.g., cycloheximide) to cells treated with double-stranded RNA. Such cells become superinduced and, on removal of the inhibitor, secrete levels of IFN (9–11) that are significantly higher than after double-stranded RNA treatment. The prevailing explanation for these phenomena is that normal human fibroblasts must first be producing IFN-β mRNA in order to respond to cycloheximide, because superinduction is thought to be a consequence of mRNA stabilization (9).

We have recently reported that the human IFN-β gene can be expressed in transfected Chinese hamster ovary (CHO) cells (12). Furthermore, the transfected gene retains inducibility in response to virus infection and can be superinduced by poly(rI)·poly(rC) and by cycloheximide. These inducible properties are maintained even when the gene is amplified 25-fold by selection for overexpression of a linked mouse dihydrofolate reductase (dhfr) gene. In contrast, when the IFN-β coding region is fused to a simian virus 40 promoter, the hybrid gene does not exhibit virus or poly(rI)·poly(rC) inducible properties.

In this report we have investigated in greater detail the mechanisms responsible for superinduction of the human IFN-β gene. We have specifically taken advantage of the various cell lines described previously to ascertain the effects of inhibitors of protein synthesis on the production of IFN-β mRNA. Our results indicate that inhibition of protein synthesis causes a rapid increase in the production of IFN-β RNA and that superinduction is due to the synergistic action of double-stranded RNA and inhibitors of protein synthesis on mRNA accumulation and, perhaps, on translation. In contrast to the generally accepted model, a major effect of cycloheximide appears to be on transcription of the human IFN-β gene.

MATERIALS AND METHODS

Cells. MI7.1 are CHO cells transfected with the plasmid pMI7, which contains a mouse dihydrofolate reductase cDNA and a 1.8-kilobase DNA fragment encoding the entire human IFN-β gene (12). R1000 cells were derived from MI7.1 by selection in increasing concentrations of methotrexate (up to 1 μM) and contain 25–50 times as much human IFN-β DNA and RNA as do MI7.1 cells (12).

Analysis of Secreted IFN-β. Confluent R1000 cells were treated with cycloheximide (2 μg/ml), poly(rI)·poly(rC) (20 μg/ml), or both for various times. Newcastle disease virus (NDV) infections were carried out using the Manhattan strain of NDV at 10^8 plaque-forming units/ml; virus was added for 3 hr. After removal of the drugs or NDV, cells were washed 3 times with phosphate-buffered saline and then labeled for 1 hr with 0.5 ml of [^{35}S]methionine at 100 μCi/ml (1164 Ci/mmol; 1 Ci = 37 GBq; New England Nuclear) in serum and methionine-free Dulbecco's modified Eagle's medium. A 50-μl aliquot of the tissue culture supernatant was concentrated by drying under vacuum and then it was suspended in $NaDodSO_4$/polyacrylamide gel sample buffer. After boiling for 5 min, the samples were electrophoresed on a 15% polyacrylamide gel according to the procedure of Laemmli (13). The gel was dried and exposed to x-ray film for 12 hr.

RNA Isolation and Dot Blots. RNA was prepared from cells lysed in guanidine thiocyanate as described by Chirgwin *et al.* (14) and analyzed on nitrocellulose dot blots as described (12). The probe used was the 1.8-kilobase *Eco*RI fragment

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Abbreviations: IFN, interferon; SSPE, 180 mM NaCl/10 mM $NaPO_4$, pH 7.7/1 mM EDTA; NDV, Newcastle disease virus.

encoding the IFN-β_1 nick-translated (15) to a specific activity of 1×10^8 cpm/μg. The filters were exposed to Kodak XAR-5 film in the presence of a Cronex intensifying screen at −70°C for the indicated periods of time.

Nuclear Transcription Assays. R1000 cells were grown to confluence in methotrexate-free medium and incubated with cycloheximide (10 μg/ml) and/or poly(rI)·poly(rC) (20 μg/ml) for the indicated lengths of time prior to isolation of nuclei. Preparation of nuclei for RNA polymerase elongation reactions was essentially as described by Stallcup and Washington (16) and nuclear RNA was prepared as described by Smith *et al.* (17).

The ^{32}P-labeled products (2×10^6 dpm) from each reaction were hybridized to nitrocellulose filters containing 1 μg of denatured IFN-β DNA (prepared as the *Eco*RI fragment from plasmid pMI7), human actin cDNAs (gifts of P. Gunning, P. Ponte, and L. Kedes), and rat tubulin cDNAs (gift of S. Feinstein) in 200 μl of 50% formamide/5× SSPE (1× SSPE = 180 mM NaCl/10 mM $NaPO_4$, pH 7.7/1 mM EDTA)/2× Denhardt's solution (18)/yeast RNA (200 μg/ml) at 37°C for 4 days with gentle mixing. Filters were washed once with 2× SSPE/0.1% (wt/vol) $NaDodSO_4$ at room temperature for 15 min, and then with 0.1× SSPE/0.1% $NaDodSO_4$ twice at 50°C for 15 min and twice at 60°C for 15 min. The filters were subsequently treated with pancreatic RNase (10 μg/ml) in 10 mM Tris·HCl, pH 7.4/300 mM NaCl for 1 hr at 37°C, washed briefly with the same buffer, and exposed to film at −70°C with a Cronex intensifying screen.

RESULTS

The effects of double-stranded RNA [poly(rI)·poly(rC)] and cycloheximide on production of human IFN-β were studied in CHO cells transformed with the plasmid pMI7 (Fig. 1). This recombinant contains the entire human IFN-β gene linked to a mouse dihydrofolate reductase cDNA. We have recently demonstrated that the entire plasmid can be amplified by selection of cells capable of growing in high concentrations of the dhfr inhibitor, methotrexate (12). Furthermore, the production of interferon is inducible by treatment with poly(rI)·poly(rC) and cycloheximide in both amplified and unamplified cells.

The secretion of biologically active IFN is the final step in the complex process of expressing the IFN-β gene. To assess the role of cycloheximide and poly(rI)·poly(rC) in greater detail, we first determined the effects of these agents on the rate of synthesis of IFN-β. To facilitate the analysis we have used R1000 cells in which the transfected human IFN-β gene has been amplified 25- to 50-fold. As seen in Fig. 2, the secretion of newly synthesized IFN is increased ≈5-fold by a 3-hr treatment with poly(rI)·poly(rC). Cycloheximide alone produces an increase of similar if not greater magnitude after a 3-hr treatment followed by a 1-hr labeling in the absence of drug. As shown in Fig. 2 (lanes A–E), cycloheximide also induces large amounts of an 11-kDa secreted protein; this protein of unknown function, is also inducible in the parental CHO cells (unpublished observation). Lastly, poly(rI)·poly(rC) and cycloheximide together (i.e., superinduction conditions) have synergistic effects on IFN production, resulting in a >50-fold increase in the secretion of newly synthesized IFN. Similar results are obtained after infection with NDV. These data are consistent with analysis of IFN titers as assayed by an antiviral assay (data not shown). Thus, we conclude that each of the inducing agents acts independently on increasing the production of IFN, and together they act synergistically.

Analysis of IFN RNA Levels. The mechanisms of IFN induction by double-stranded RNA and cycloheximide have not been thoroughly investigated. We have therefore analyzed the effects of these agents on the accumulation of IFN-β RNA in MI7.1 and R1000 cells. Total cellular RNA was prepared from control and drug-treated cultures, and various amounts of each were analyzed by hybridization with radioactively labeled IFN-β DNA. The data depicted in Fig. 3 demonstrate that after 12 hr in the presence of cycloheximide (lanes b), IFN RNA levels increase 5- to 10-fold in both

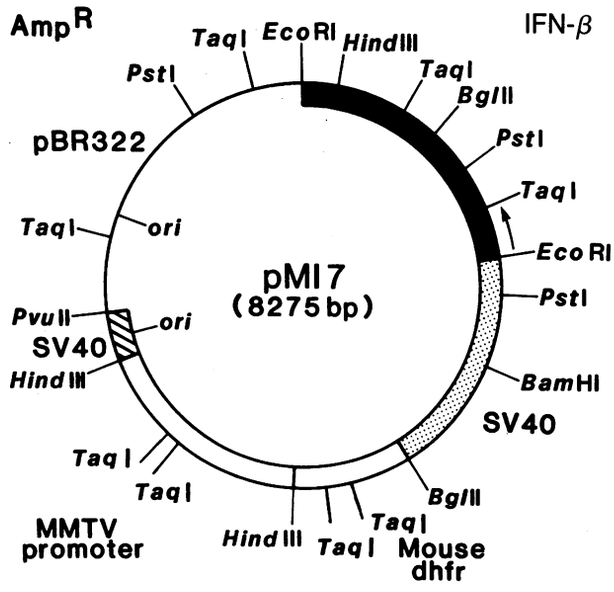

FIG. 1. Structure of the plasmid pMI7. This molecule was constructed by inserting a 1.8-kilobase *Eco*RI fragment containing the entire human IFN-β gene into the plasmid pSVM *dhfr* as described (12). CHO dhfr$^-$ cells transfected with this plasmid express both *dhfr* directed by the mouse mammary tumor virus (MMTV) promoter and human IFN-β under the control of its own promoter (12). Amp^R, ampicillin resistance; SV40, simian virus 40; bp, base pairs.

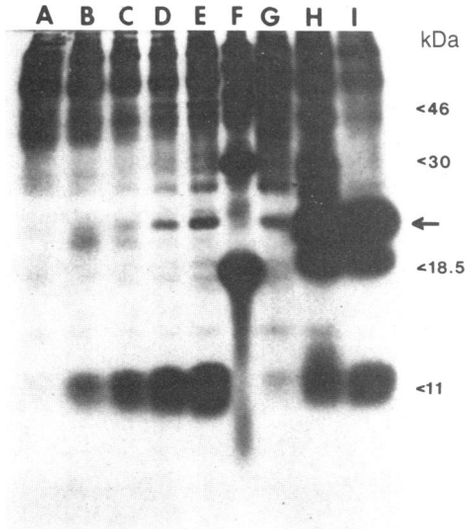

FIG. 2. Effects of cycloheximide, poly(rI)·poly(rC), and NDV on secretion of newly synthesized IFN-β from R1000 cells. Confluent R1000 cells were exposed to various inducing protocols as indicated below and then labeled with [^{35}S]methionine for 1 hr after removal of inducer(s). Aliquots of the supernatants obtained from approximately equal numbers of cells were prepared for electrophoresis and analyzed. Lanes: A, control; B–E, 1, 2, 3, and 4 hr of cycloheximide (2 μg/ml), respectively; F, size markers; G, poly(rI)·poly(rC) (20 μg/ml) for 3 hr; H, NDV for 3 hr; I, cycloheximide (2 μg/ml) and poly(rI)·poly(rC) (20 μg/ml) for 3 hr. Arrow indicates the position of native IFN; the additional induced band in lanes H and I of ≈18.5 kDa represents nonglycosylated IFN (see ref. 12).

MI7.1 and in the amplified R1000 cells. In contrast, the levels of RNA are not altered in cells treated with poly(rI)·poly(rC) for 12 hr (lanes c). However, poly(rI)·poly(rC) does stimulate the accumulation of IFN RNA in cells treated continuously for 12 hr with cycloheximide (Fig. 3, lanes d; Fig. 4).

A time course of induction in response to poly(rI)·poly(rC) indicates that there is in fact a transient stimulation of IFN RNA in R1000 cells (Fig. 4); the effect is seen maximally at ≈3 hr after drug treatment, with a rapid decrease thereafter. The effect of cycloheximide is considerably more protracted with IFN RNA levels peaking after ≈5 hr and remaining high throughout the course of exposure to the drug. As previously noted, the combined action of these drugs results in RNA inductions that exceed those obtained with either alone. Furthermore, the effect of poly(rI)·poly(rC) is retained after long-term exposure in the presence of cycloheximide (e.g., at 12 or 14 hr) despite the fact that the stimulation of RNA by poly(rI)·poly(rC) alone has already decayed. This may be due to inhibition of metabolism of poly(rI)·poly(rC) in cycloheximide-treated cells.

To test whether the induction of IFN RNA by cycloheximide is specific to this drug, we tested the ability of two other inhibitors of protein synthesis (emetine and puromycin) to act as inducers (Table 1). At concentrations that inhibited protein synthesis by >95% (data not shown) both of these drugs are as effective as cycloheximide in stimulating the production of IFN RNA. In this regard, it is noteworthy that puromycin, an analog of aminoacyl-tRNA, inhibits protein synthesis by effecting the separation of the growing peptide chain from the tRNA–mRNA–ribosome complex, whereas cycloheximide and emetine act by immobilizing ribosomes and thereby blocking chain elongation. Thus the induction of IFN RNA appears to be due to inhibition of protein synthesis rather than to an anomalous effect of cycloheximide.

Effects of Cycloheximide on IFN Transcription Rates. Although it is clear that inhibitors of protein synthesis and double-stranded RNA induce the accumulation of IFN RNA in MI7.1 and R1000 cells, this could be due to either an increase in the synthetic rate of the RNA or, as suggested by others for the cycloheximide effect, a decrease in its rate of degradation (9). To test this issue directly, we have analyzed the synthesis of IFN RNA by using a nuclear transcription assay. Briefly, nuclei prepared from cells grown in the absence of inducer or after 1 hr in poly(rI)·poly(rC) and/or cycloheximide were incubated with [^{32}P]UTP and nonradioactive ribonucleoside triphosphates to allow nascent RNA chains to be elongated. The labeled RNAs were hybridized to filters con-

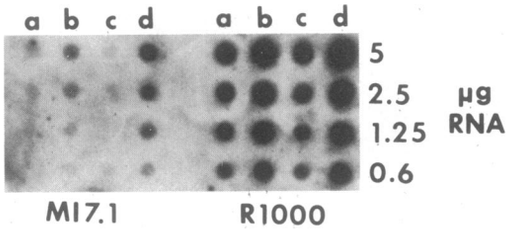

FIG. 3. Effects of cycloheximide and poly(rI)·poly(rC) on IFN-β RNA accumulation. Confluent MI7.1 and R1000 cells were treated continuously with cycloheximide (10 μg/ml), poly(rI)·poly(rC) (20 μg/ml), or both for 12 hr. Total RNA was prepared and the indicated amounts of RNA were loaded onto a nitrocellulose filter using a Plexiglas dot-blot matrix. The filter was hybridized with ≈5 × 10^6 cpm of nick-translated IFN-β DNA, washed, and exposed to x-ray film. Lanes: a, control; b, cycloheximide; c, poly(rI)·poly(rC); d, cycloheximide and poly(rI)·poly(rC). The R1000 dots are overly exposed to show the relative increase in RNA expression in the amplified cells. Quantitation of RNA (see text) came from films exposed for shorter times.

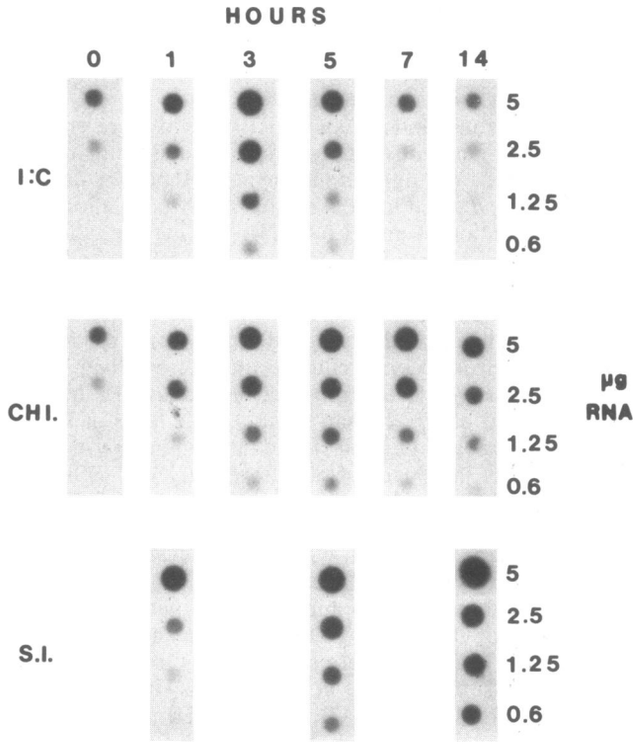

FIG. 4. Time course of the effects of cycloheximide (10 μg/ml) and poly(rI)·poly(rC) (20 μg/ml) on IFN-β RNA levels in R1000 cells. Cells were exposed continuously to either poly(rI)·poly(rC) (I:C), cycloheximide (CHI.), or both (S.I.) for the indicated times. Total RNA was harvested and analyzed as described in *Materials and Methods* and the legend to Fig. 3. The control for the superinduced (S.I.) samples is the same as the 0 time point for either the cycloheximide or poly(rI)·poly(rC) samples.

taining denatured human IFN-β DNA in order to detect newly synthesized IFN RNAs. The results in Fig. 5 and Table 2 indicate that cycloheximide alone is capable of increasing the transcription rate of the IFN-β gene, whereas poly(rI)·poly(rC) treatment for either 15 or 60 min is not. When the drugs are added together, the rate of transcription is equivalent to that seen in the presence of cycloheximide alone. Thus, it appears that a major effect of inhibiting protein synthesis is to stimulate the transcription of the IFN-β gene. As controls in this assay, we initially used a human actin cDNA that detects both β- and γ-actin RNAs (19). However, as shown in Fig. 5*A*, actin gene transcription is also increased ≈2.5-fold in cycloheximide-treated cells. Using DNAs specific for β- and γ-actins individually (20), we find that both genes are induced by cycloheximide (Fig. 5*B*). In contrast, when nuclear transcription products are hybrid-

Table 1. Induction of IFN RNA by inhibitors of protein synthesis

	Treatment	-fold induction
Exp. 1	Cycloheximide	5
	Cycloheximide and poly(rI)·poly(rC)	15
	Emetine	5
	Emetine and poly(rI)·poly(rC)	20
Exp. 2	Cycloheximide	10
	Cycloheximide and poly(rI)·poly(rC)	60
	Puromycin	8
	Puromycin and poly(rI)·poly(rC)	55

Cytoplasmic RNA was prepared 6 hr after treatment of R1000 cells with the indicated drugs. Cycloheximide was used at 10 μg/ml, emetine at 10 μg/ml, puromycin at 50 μg/ml, and poly(rI)·poly(rC) at 20 μg/ml. RNAs were analyzed by the dot blot procedure using 2-fold serial dilutions of each sample as described (12).

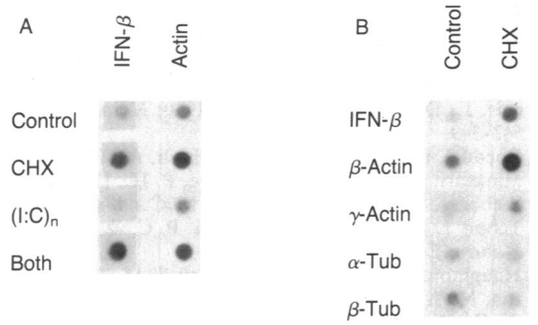

FIG. 5. Effect of cycloheximide (10 μg/ml) and/or poly(rI)·poly(rC) (100 μg/ml) on transcription rates of IFN-β, actin, and tubulin genes in R1000 cells. Nuclei from untreated control cells or cells treated for 1 hr with poly(rI)·poly(rC) [(I:C)$_n$], cycloheximide (CHX), or both drugs were prepared and used in nuclear transcription assays. ^{32}P-labeled RNA (2×10^6 dpm) from each transcription reaction was hybridized to cDNAs immobilized on nitrocellulose filters. The DNAs used in *A* were gel-isolated *Eco*RI fragment from pMI7 (IFN-β) and *Bam*HI fragment of the plasmid pHFβA-1 (19) containing full-length β-actin cDNA, which detects both β- and γ-actins. (*B*) The transcription rates of β- and γ-actins were determined by hybridizing against unique 3'-untranslated cDNA fragments isolated from plasmids pHFβA-3'ut and pHFγA-3'ut described by Ponte *et al.* (20); α- and β-tubulin (α-Tub and β-Tub) cDNA clones in λgt10 were obtained from S. Feinstein (Stanford University). After washing and RNase treatment, the filters were exposed to x-ray film with an intensifying screen at −70°C. Exposure times were as follows: (*A*) IFN-β (2 hr) and actin (4 hr); (*B*) IFN-β (2 hr), β- and γ-actin (20 hr), and α- and β-tubulins (3 days). To assess whether differences in hybridization could be ascribed to differences in length of the [^{32}P]RNAs, the nuclear transcription products were analyzed by polyacrylamide gel electrophoresis. All were found to be of equal average length (400–500 nucleotides). Furthermore, no significant differences were observed in total incorporation among the nuclear transcription products from cells treated with cycloheximide or poly(rI)·poly(rC).

ized to rat α- and β-tubulin cDNAs (gift of S. Feinstein), no stimulation (or perhaps a slight decrease) of tubulin gene transcription rates is observed in the cycloheximide-treated cells (Fig. 5*B*).

Whether the entire induction of IFN-β RNA by inhibitors of protein synthesis is due to increased transcription is difficult to assess, but the 3- to 4-fold increase in RNA synthesis, as compared with the 5- to 10-fold increase in RNA accumulation typically seen in these cells, suggests that there may be additional effects of inhibitors of protein synthesis on IFN RNA stability. Unlike cycloheximide, it appears that double-stranded RNA does not significantly alter the synthetic rate of IFN RNA within 1 hr of treatment. Similar results have been reported by Raj and Pitha (21). Thus, the transient increase in RNA levels seen with poly(rI)·poly(rC) in R1000 cells may be a consequence of altered processing or turnover of IFN RNA.

DISCUSSION

We have investigated the mechanisms by which double-stranded RNA and inhibitors of protein synthesis regulate the production of human IFN-β in stably transformed CHO cells. Our results strongly suggest that a major site of action of cycloheximide is at the level of transcription. We believe that this effect may be the consequence of inhibiting the production of a labile protein whose action regulates IFN-β gene transcription. An attractive possibility is that a short-lived repressor normally prevents the IFN gene from being transcribed. Alternatively, the inhibition of protein synthesis itself could lead to the production of a small molecule that acts as an inducer of the IFN-β gene. Such a molecule might be analogous to the guanosine tetra- and pentaphosphates

Table 2. Stimulation of IFN gene transcription in cycloheximide-treated cells

Treatment	-fold induction		
	Exp. 1	Exp. 2	Exp. 3
Cycloheximide (10 μg/ml)	3.1	4.3	3.0
poly(rI)·poly(rC) (100 μg/ml)	0.5	0.9	0.6
			0.8*
Cycloheximide (10 μg/ml) and poly(rI)·poly(rC) (100 μg/ml)	4.1	5.4	2.7

Products of nuclear transcription reactions were hybridized to immobilized IFN-β DNA. The -fold induction was determined by densitometric scanning of autoradiograms such as the one shown in Fig. 5 and represents the ratio of each experimental sample to a control sample. Cells were exposed to inducing agents for 60 min prior to isolation of nuclei.

*In this sample, cells were exposed to poly(rI)·poly(rC) for only 15 min.

produced in *Escherichia coli* during amino acid starvation (22) and could act either by removing a repressor from the gene, as in the case of the *E. coli lac* repressor, or by forming an inducing complex, as in the case of steroid-receptor molecules (23).

Since the effect of cycloheximide is maintained in cells containing a 25- to 50-fold amplification of this gene, we also surmise that if a repressor controls IFN-β gene expression, it must be present in excess of what is minimally required to regulate this gene. It is intriguing to speculate that, regardless of the mechanism responsible for the induction of the IFN-β gene, other genes are likely to be regulated in a similar fashion. The 11-kDa protein induced by cycloheximide in wild-type and transfected CHO cells (Fig. 2) may be the product of such a gene. Additional evidence has recently been obtained that early adenovirus gene transcription can be induced by inhibiting protein synthesis with cycloheximide if early gene expression is allowed before treatment (24). Nevins (25) has argued that the product of the early adenovirus gene 1A inactivates a cellular *trans*-acting repressor that normally suppresses transcription of other adenovirus early genes. Strikingly similar data have recently been obtained demonstrating that the gene encoding the polycyclic hydrocarbon-inducible form of cytochrome P-450 is transcriptionally activated by inhibitors of protein synthesis (D. Israel and J. Whitlock, personal communication). Thus, the fact that we have not titrated the cycloheximide-inducible property of the IFN-β gene by a 25- to 50-fold amplification may simply reflect a redistribution of some of the regulatory factor(s) normally associated with a large set of genes that are under similar control.

The role of double-stranded RNA in regulating the production of IFN remains a puzzle. The results presented here indicate that only a transient effect is observed at the level of RNA accumulation, and virtually no effect is seen at the level of IFN gene transcription at short times after poly(rI)·poly(rC) treatment. Raj and Pitha (26) have reported very similar results on transient accumulation of IFN-β RNA in poly(rI)·poly(rC) treated human cells that could be prolonged by cycloheximide treatment. They have also recently reported (21) an increase in transcription of the IFN-β gene by poly(rI)·poly(rC). This increase, however, was observed after 8–12 hr of treatment (i.e., at times when IFN mRNA concentrations had already returned to basal levels); thus, the significance of this delayed increase in transcription remains unclear. There is additional evidence, however, that the effect of poly(rI)·poly(rC) on IFN production requires the IFN promoter region (12, 27, 28). When a simian virus 40 promoter is fused to the IFN-β coding region, the hybrid gene is not inducible by either cycloheximide or poly(rI)·poly(rC) (12). Similarly, deletions that remove sequences upstream of the

IFN-β gene's start of transcription abrogate its inducibility by poly(rI)·poly(rC) (27, 28). Thus, although the data presented here suggest a nontranscriptional role for poly(rI)·poly(rC), the hybrid and deleted promoter data as well as the delayed transcriptional response suggest the opposite. Obviously, additional experiments will be required to clarify this paradoxical situation.

Analysis of the effects of cycloheximide and poly(rI)·poly(rC) on synthesis of mature IFN indicate yet another level of control. As shown here and previously (12), the increase in IFN production, measured either by protein synthesis or biological activity, can be 100-fold or greater when cells are treated with both agents (Fig. 2). Similar increases are observed when cells produce IFN-β in response to virus infection. Clearly, the magnitude of the IFN response exceeds the induction of RNA observed under these conditions. Thus, it seems possible that treatment of cells with double-stranded RNA (or, less likely, cycloheximide) increases the translational capacity of IFN mRNA.

In sum, the experiments presented here document that inhibitors of protein synthesis in the absence of other agents induce human IFN-β by stimulating the accumulation of IFN RNA. Similar observations have recently been made by Maroteaux *et al.* (29) using the IFN-β gene cloned into bovine papillomavirus vectors, and by Raj and Pitha (21, 26) studying the induction of the human IFN-β gene in human fibroblasts. In contrast to their interpretation and the generally accepted explanation (1, 2, 9), we have found that this effect is due at least in part to increased transcription of the IFN-β gene. Although the precise mechanisms by which double-stranded RNA stimulates the production of secreted IFN remain to be elucidated, our data suggest a major role for post-transcriptional events in this process. Lastly, these observations indicate that perhaps it is the decrease in host protein synthesis associated with infection of cells by many classes of viruses that acts as the primary signal for induction of IFN gene expression (30). In this view, the translation of IFN and a few other mRNAs would be refractory to the general inhibition of host protein synthesis during virus infection, whereas that of a putative repressor molecule would not be. In support of this idea, we observe a large induction of the (cycloheximide inducible) 11-kDa secreted protein in NDV-infected cells, even though poly(rI)·poly(rC) alone has little or no effect on its production.

We thank Drs. J. Papkoff, H. Schulman, and J. Whitlock for their comments on the manuscript and Karen Benight for typing it. We thank Drs. P. Gunning, P. Ponte, and L. Kedes for actin cDNA clones and Dr. S. Feinstein for tubulin cDNA clones. The work performed at Stanford was supported by a grant from the Cetus Corporation. G.M.R. is the recipient of an Established Investigator Award from the American Heart Association.

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