Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 3923–3927, July 1983
Biochemistry

Two levels of regulation of β-interferon gene expression in human cells

(induction/transcription/isolated nuclei/β-interferon mRNA levels)

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Communicated by DeWitt Stetten, Jr., March 28, 1983

ABSTRACT **We cloned α- and β-interferon cDNA and used them as specific probes to determine the relative levels of interferon mRNA in human fibroblast cells induced with poly(rI)·poly(rC) or Newcastle disease virus to synthesize interferon. Both inducers activated only the β-interferon gene; however, the half life of β-interferon mRNA in cells induced with virus was substantially longer than in poly(rI)·poly(rC)-induced cells. The transcription rate of β-interferon RNA sequences was examined in nuclei isolated from poly(rI)·poly(rC)-induced cells; it was found that the induction leads to transcriptional activation of the β-interferon gene and that the active transcription of the β-interferon gene continues during the shutoff period when no interferon synthesis or cytoplasmic βmRNA are detected. Thus, the synthesis of β interferon in poly(rI)·poly(rC)-induced human fibroblasts is controlled both by activation of transcription of the β-interferon gene and by alteration of the β-interferon mRNA stability.**

The type 1 human interferons (IFNs) are represented by a family of multiple genes that code for the synthesis of two antigenically different interferons, IFN-α and IFN-β (1). Whereas the IFN-β gene is present in human DNA in a single copy (2, 3), there have been about 15 different but closely related IFN-α genes identified so far (4–6). Human IFNs can be produced by nearly all differentiated cells upon induction with double-stranded RNA or viral infection; however, only a few cells (generally hematopoietic cell lines) produce type I IFNs constitutively. Although both IFN-α and IFN-β genes are localized on a short arm of chromosome 9 (7–9), the induction of synthesis of IFN-α and IFN-β is not always coordinate and shows specificity, depending on the type of the cell and the inducer. Thus it has been shown that in human fibroblast cells, induction with poly(rI)·poly(rC) leads only to the synthesis of IFN-β (10), whereas the viral infection of human peripheral leukocytes leads mostly to the synthesis of IFN-α proteins. In virus-induced human lymphoblastoid lines, both IFN-α and IFN-β are synthesized (11). Because human IFN-α consists of a mixture of IFN-α proteins, it is possible that the proportion of different proteins synthesized may depend also on the type of cell and inducer.

In human fibroblast cells, the synthesis of IFN induced by poly(rI)·poly(rC) has been shown to be a transient phenomenon. IFN synthesis rapidly decreases in the cells several hours after the induction (shutoff phase), and the cells become temporarily refractory to repeated induction. Treatment of the cells, shortly after the poly(rI)·poly(rC) induction, with inhibitors of RNA and protein synthesis results in a 100-fold increase in IFN production (superinduction) when compared to the amount of IFN synthesized in the same cells induced with poly(rI)·poly(rC) only. The enhancement of IFN synthesis in the presence of metabolic inhibitors has been interpreted in terms of negative control mechanisms at both the transcriptional and post-transcriptional levels (12–14). Tan and Berthold (15) demonstrated that the inhibitors of protein synthesis alone can induce IFN production and postulated the existence of a rapidly turning over repressor. The superinduction process then would involve the removal of this repressor and allow the expression of the IFN gene to proceed. The prolonged synthesis of IFN-β, in which transcription had been terminated by actinomycin D treatment, indicates that the control of IFN synthesis in poly-(rI)·poly(rC)-induced cells may also be at the post-transcriptional level.

We previously have shown that the IFN-β mRNA sequences rapidly disappeared from the cells during the shutoff period and that the fast degradation of IFN-β mRNA sequences occurred only in the presence of ongoing protein synthesis (16). In order to determine whether the shutoff mechanism operates in human fibroblasts on the transcriptional or post-transcriptional level, we compared the rate of IFN-β gene transcription in nuclei isolated from induced cells with the relative levels of IFN-β mRNA present in these cells at different times after induction. The results indicate that the induction of IFN-β synthesis in poly(rI)·poly(rC)-induced cells leads to the transcriptional activation of the IFN-β gene and that the rapid switch off in IFN synthesis during the shutoff period does not involve termination of transcription but occurs at the post-transcriptional level.

MATERIAL AND METHODS

IFN Induction and mRNA Preparation. Human fibroblast cells (HH foreskin cells, obtained from Stanford University) were grown to confluency and induced with poly(rI)·poly(rC) (50 μg/ml) as described (16) or with Newcastle disease virus (NDV) at a multiplicity of infection of 0.3 for 90 min at 37°C. The inducers were removed, and the cells were washed, overlaid with minimal Eagle's medium supplemented with 2% fetal calf serum, and incubated at 37°C for the times indicated. The medium was then collected, and the IFN titer was determined on human cells trisomic for chromosome 21 (GM2504) (17). The IFN levels are expressed in the international standard units. The cells were harvested, RNA was prepared by a guanidine hydrochloride method (18), and poly(A)$^+$ RNA was selected on an oligo(dT) column.

Agarose Gel Electrophoresis of RNA and Identification of IFN-α and IFN-β mRNAs. For the blot hybridization, poly(A)$^+$ RNA (4 μg as indicated) was denatured in glyoxal and dimethyl sulfoxide, electrophoresed in a 1.2% agarose gel, and transferred to nitrocellulose paper as described (19). The papers were pretreated for 24 hr in 5× NaCl/Cit (1× NaCl/Cit is 0.15 M

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Abbreviations: NDV, Newcastle disease virus; IFN, interferon; NaCl/Cit, 0.15 M NaCl/0.015 M Na citrate, pH 7.

NaCl/0.015 M sodium citrate, pH 7.0), 5× Denhardt's solution (1× Denhardt's solution is 0.02% Ficoll/0.02% polyvinylpyrrolidine), 0.1% $NaDodSO_4$, 250 μg of herring sperm DNA per ml, 10 μg of poly(A) per ml, and 50% formamide and then were hybridized with ^{32}P-labeled probe in the same solution containing 10% dextran sulfate for another 2–3 days. The papers were washed stepwise in decreasing concentrations of NaCl/Cit (2× and 0.5×) containing 0.1% $NaDodSO_4$ at 60°C. The filters were then exposed at −70°C to Kodak XAR film with a Quanta III intensifying screen.

Recombinant Plasmids (IFN-α and IFN-β cDNAs). The preparation of IFN-β cDNA (HF-βcDNA-pBR) has been described (16). The IFN-α cDNA clone was identified in the pool of cDNA clones reverse-transcribed from poly(A)$^+$ mRNA isolated from Namalva cells induced with Sendai virus to produce IFN-α and -β and will be described in detail elsewhere. The IFN-α cDNA clone used here was identified as IFN-α_2 or -αA (20, 21) by restriction analysis and sequence determination. This clone is an incomplete transcript of IFN-α_2 mRNA and is missing ≈250 nucleotides from the 5′ end. The IFN-α_2 cDNA and IFN-β cDNA were labeled by nick-translation with [α-^{32}P]dCTP (400 μCi/mmol; 1 Ci = 3.7×10^{10} Bq) to an initial specific activity of 10^8 cpm/μg.

***In Vitro* Transcription with Isolated Nuclei and Isolation of RNA for Hybridization.** Nuclei were isolated from human fibroblast cells induced with poly(rI)·poly(rC) (50 μg/ml) for different lengths of time as described (22). For transcription, nuclei were incubated in a 100-μl reaction mixture containing 20 mM Tris·HCl (pH 8.0), 5 mM $MgCl_2$, 150 mM KCl, 16% glycerol, 0.4 mM each of ATP, GTP, and CTP, and 200 μCi of [α-^{32}P]UTP (3,000 Ci/mmol) for 45 min at room temperature. The reaction was made 1% in $NaDodSO_4$ and was deproteinized by digestion with proteinase K (100 μg/ml); the RNA was extracted by hot phenol and precipitated with ethanol. The nucleic acid was treated with DNase I (10 μg/ml) for 30 min at room temperature, followed by phenol/chloroform extraction. The aqueous phase was then precipitated with cold 10% trichloroacetic acid in the presence of 30 mM sodium pyrophosphate and 1 mM UTP. The precipitate was collected and washed with 5% trichloroacetic acid, dissolved in 0.1 M sodium acetate (pH 7.0), and precipitated by ethanol.

Hybridization of RNA to Immobilized DNA. Plasmid DNA containing IFN-β cDNA (1 μg) was denatured in 0.2 M NaOH by boiling for 1 min and neutralized. The DNA was diluted in 10× NaCl/Cit, spotted on a nitrocellulose paper, and allowed to air dry. The filters were then baked at 80°C for 2 hr. Hybridization with the ^{32}P-labeled RNA was performed in the same solution as that used for RNA blotting in a final volume of 20 μl with mineral oil on the top. After 3 days at 37°C, the filters were washed several times with 0.3 M NaCl and 0.1% $NaDodSO_4$. The filters then were washed in the same buffer without $NaDodSO_4$, digested with RNase A (10 μg/ml) at 37°C for 1 hr, and then washed for another 1–2 hr. The filters were treated with 0.04 M NaOH to release the RNA, which was neutralized, and the radioactivity was determined.

RESULTS

Accumulation of IFN mRNA in Induced Cells. The induction of human fibroblast cells with poly(rI)·poly(rC) leads to a short outburst of IFN production; the IFN synthesis is maximal at 4–6 hr after the induction and falls to undetectable levels by 8–10 hr (23). The IFN synthesized in these cells has been characterized by neutralization with appropriate antisera as IFN-β, and the analysis of the induced IFN-β mRNA indicated that only one species of translatable IFN-β mRNA, identical to the cloned IFN-β cDNA, was present in the induced cells (16). In the same cells induced with NDV, synthesis of IFN starts several hours after infection, attains the maximal rate between 9 and 12 hr, and then slowly decreases between 12 and 24 hr (24).

Since it has been shown by others that, in some human fibroblasts, NDV induces the synthesis of both IFN-α and IFN-β (10), we examined whether the observed difference in kinetics of IFN synthesis in virus- and poly(rI)·poly(rC)-induced cells could be due to the consecutive activation of both IFN-α and IFN-β genes during the time course of IFN synthesis in NDV-induced cells. Poly(A)$^+$ RNA was isolated from the induced cells at different times after induction and was analyzed by blot hybridization with a probe of either cloned IFN-α_2 cDNA or IFN-β cDNA (Fig. 1). Because the homology on the DNA level between IFN-α and -β genes is only about 45% (25), these two probes do not cross-hybridize under the stringent conditions of hybridization used; however, there is a cross-hybridization between IFN-α_2 cDNA and the other IFN-α genes and RNAs (5). The results show that in the human fibroblast cells used, induction with both poly(rI)·poly(rC) and NDV activated the expression of IFN-β gene only (or predominantly). The IFN-β mRNA detected both in poly(rI)·poly(rC)- and NDV-induced fibroblast cells was about 1,100 nucleotides long; IFN-β mRNA of similar size also was identified in Namalva cells induced with Sendai virus to produce IFN (unpublished data). Thus, the size of the translatable IFN-β mRNA seems to be independent of the type of the cell and the inducer.

When the same amount of poly(A)$^+$ RNA isolated from induced [poly(rI)·poly(rC) or NDV] cells was sized, transferred to nitrocellulose filters, and then hybridized either with IFN-α_2 or -β cDNA probes of the same specific activity, the IFN-β mRNA could be detected as early as 6 hr of exposure (Fig. 1 *A* and *B*) of the autoradiograph, whereas we failed to detect any hybridization corresponding to the IFN-α mRNA even after a week-long exposure (data not shown). Likewise, we did not de-

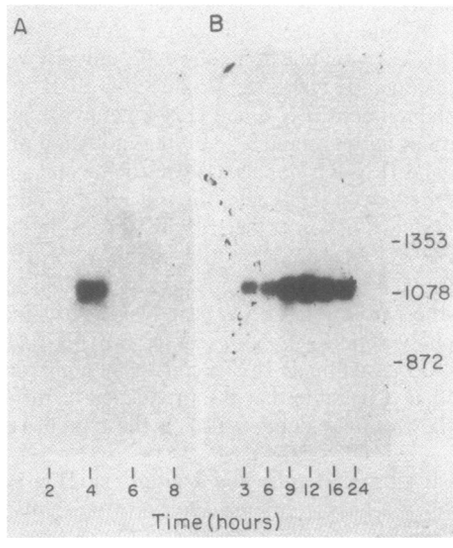

FIG. 1. Kinetics of IFN mRNA expression in induced human fibroblast cells. Human fibroblast cells grown to confluency were induced either with poly(rI)·poly(rC) (50 μg/ml) or NDV (multiplicity of infection, 0.3). At the times indicated, cells were harvested and poly(A)$^+$ RNA was isolated; 4 μg of poly(A)$^+$ RNA was denatured with glyoxal and dimethyl sulfoxide, electrophoresed in 1.2% agarose, and transferred to nitrocellulose filters. The papers were pretreated and then hybridized with ^{32}P-labeled IFN-α_2 cDNA and IFN-β cDNA probes separately on duplicate filters. (*A* and *B*) Poly(A)$^+$ RNA isolated from poly(rI)·poly(rC)- and NDV-induced cells, respectively, and hybridized with the IFN-β cDNA probe. The positions of 1,353-, 1,078-, and 872-nucleotide-long fragments are shown as references.

tect any IFN-α or -β mRNA sequences in the poly(A)$^-$ fraction. These data indicate that under the conditions used, the NDV induction does not lead to substantial (if any) activation of IFN-α genes in the human fibroblast cells used.

IFN synthesis in virus- and poly(rI)·poly(rC)-induced human fibroblasts correlated with the accumulation of IFN-β mRNA in these cells. With both types of inducers, IFN-β mRNA could be detected in the cells as early as 3 hr after induction. In poly(rI)·poly(rC)-induced cells, the maximal accumulation occurred at 3 hr after induction, and then IFN-β mRNA rapidly disappeared. In contrast, in virus-induced cells, the accumulation of IFN-β mRNA continued until 12 hr after infection, and then the levels of IFN-β mRNA gradually decreased over a period of 12–24 hr (Fig. 1). These results show that in virus-induced cells, IFN-β mRNA is present for substantially longer times than in cells induced with poly(rI)·poly(rC). We have shown that the disappearance of IFN-β mRNA from poly(rI)·poly(rC)-induced cells does not occur in the absence of cellular protein synthesis (16). Because viral infection generally leads to inhibition of cellular protein synthesis, it seemed possible that this effect may contribute to the stabilization of IFN-β mRNA in NDV-induced cells. The rate of protein synthesis in the cells infected with NDV was therefore measured at different times after infection and compared to that of uninfected cells (Table 1). The human cells are not fully permissive to NDV, and little viral protein synthesis occurs in these cells (26); thus, the measurements represent cellular protein synthesis predominantly. The data in Table 1 show that the NDV infection of human cells led only to a partial inhibition of cellular protein synthesis and that there was no significant change in the protein content of the cells during the time period studied. This degree of protein inhibition was not sufficient to superinduce IFN synthesis in poly(rI)·poly(rC)-induced cells, where the superinduction occurs when cellular protein synthesis is inhibited by >90% (27). Thus, it seems unlikely that the increased stability of IFN-β mRNA in virus-induced cells could be entirely due to the virus-induced decrease in cellular protein synthesis.

Transcription in Isolated Nuclei. To determine whether the difference in the accumulation of IFN-β mRNA in poly(rI)·poly(rC)- and NDV-induced cells reflects the length of time for which the IFN-β gene is activated by these two inducers, we studied the rate of IFN-β gene transcription. Pulse labeling of the cells for a time period shorter than that required for the synthesis of a complete transcript would enable us to test the transcription rate of IFN-β gene directly. However, the IFN-β mRNA represents only <0.1% of the total poly(A)$^+$ RNA population in the cells, and the short pulse-labeling does not allow enough radioactivity to be incorporated into IFN-β mRNA transcripts. It has been shown by others that comparative transcription rate measurements are best carried out with isolated nuclei, where the already initiated RNA chains are faithfully elongated (28). The isolated nuclei do not reinitiate *in vitro* synthesis of RNA molecules but complete the synthesis of those RNAs that were initiated *in vivo* at the time when the nuclei were isolated.

To determine whether the short appearance of IFN-β mRNA in the cells is due to the transient activation of IFN-β gene, nuclei were isolated from poly(rI)·poly(rC)-induced cells at different times after induction, and the growing RNA molecules previously initiated by RNA polymerase II were elongated in the presence of [α-^{32}P]UTP (Table 2). To measure the relative rate of transcription of the IFN-β gene, the labeled RNA was hybridized to IFN-β cDNA immobilized on nitrocellulose paper. The relative transcription of the IFN-β gene was increased 2-fold within the first 3 hr after induction and reached a 5-fold increase at 9 hr after induction (Table 2). When the nuclei from induced cells (12 hr) were incubated with [α-^{32}P]UTP in the presence of α-amanitine (5 μg/ml), the total transcription was inhibited by ≈50%, and specific transcription of the IFN-β gene dropped from 266 to 62 ppm, indicating that the IFN-β gene is transcribed by RNA polymerase II as are other cellular mRNAs. The total rate of RNA transcription in isolated nuclei increased gradually within the first 3 hr after induction and stayed high for another 6 hr. These results confirm our previous observations that poly(rI)·poly(rC) induction leads to an activation of several cellular genes in addition to IFN (29, 30).

The data show that the accumulation of IFN-β mRNA in human cells in response to poly(rI)·poly(rC) was due to the transcriptional activation of the IFN-β gene. There was a good correlation between the onset of transcription of IFN-β mRNA in induced nuclei and the detection of IFN-β mRNA in the induced cells. However, at times later than 6 hr after the induction, there was no correlation between the rate of tran-

Table 1. Effect of NDV infection on the rate of protein synthesis in human fibroblast cells

Time of labeling, hr	Specific activity of protein, % of control
0–1	100
2–3	92
7–8	70

Human fibroblast cells grown to confluency (35-mm dishes) were infected with NDV for 1 hr. The cultures were washed and further incubated in medium containing 2% fetal calf serum. The cultures were changed to methionine-free medium 30 min prior to labeling, and at indicated times the cells were labeled with [^{35}S]methionine (40 μCi/ml) in methionine-free medium for 60 min. At the end of the labeling period, the cells were washed thoroughly with cold phosphate-buffered saline and solubilized in 0.5% $NaDodSO_4$. Aliquots were precipitated with 10% trichloroacetic acid on Whatman filter discs, boiled for 10 min, washed with cold 5% trichloroacetic acid, washed with ethanol and dried, and the radioactivity was measured. Protein concentration was measured by the Bio-Rad protein assay kit. Results were calculated as the cpm/100 μg of protein; values are shown as a percentage of the control. Control values were calculated from uninfected cells labeled for 1 hr.

Table 2. Relative rate of transcription of IFN-β gene in poly(rI)·poly(rC)-induced human fibroblast cells

Exp.	Time after induction, hr	Input cpm $\times 10^{-6}$	Hybridized cpm	Relative rate of IFN-β gene transcription, ppm	Stimulation rate
1	0	4.24	130	30	—
	3	4.47	157	35	1
	6	3.88	252	65	2
	8	4.65	449	96	3
	12	2.84	412	145	5
2	0	4.52	220	49	—
	3	5.21	740	100	2
	9	5.92	860	145	3
	12	2.92	790	266	5
	12*	2.40	150	62	1

Human fibroblast cells were induced with 50 μg of poly(rI)·poly(rC) for various times, and nuclei were isolated. *In vitro* transcription, RNA isolation, and hybridizations were done as described. Background, which represented hybridization to immobilized pBR322 in experiments 1 and 2, was 25 and 45 ppm, respectively, and the numbers given are not corrected for background. Thus the stimulation rates represent the lowest estimates. ppm, parts per million.

*Transcription was done in the presence of 5 μg/ml of α amanitine.

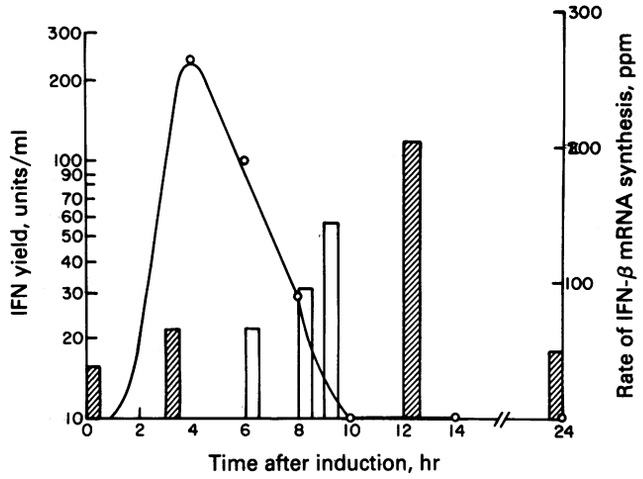

FIG. 2. Time course of IFN-β synthesis and the relative rate of IFN-β mRNA transcription. Human cells were induced with poly(rI)·poly(rC), and the medium was collected at the times indicated and assayed for IFN activity (expressed in international units). Nuclei from the same cells were isolated and transcribed *in vitro* as described. Relative rates of transcription (histograms) are shown in parts per million; hatched histograms represent mean values from two independent experiments, and the empty ones represent values from a single experiment.

scription of the IFN-β gene and accumulation of IFN-β mRNA in the cells. In nuclei isolated from the induced human cells, the IFN-β gene was actively transcribed for at least 12 hr after the induction, whereas, in the induced cells, cytoplasmic IFN-β mRNA and IFN synthesis could not be detected at times later than 7–9 hr after the induction (Fig. 2).

These results show that the IFN-β gene is transcribed during the shutoff period of IFN synthesis when no IFN-β mRNA can be detected in cells. However, if the cells are induced with poly(rI)·poly(rC) in the continuous presence of cycloheximide, there is an accumulation of IFN-β mRNA in the cells up to at least 11 hr after induction (16), and it correlates with the rate of IFN-β mRNA transcription measured in isolated nuclei. It has been suggested that the enhancement of IFN-β synthesis in the presence of cycloheximide occurs both through the stimulation of transcription of IFN-β gene and stabilization of IFN-β mRNA. The rate of transcription (total RNA and IFN-β gene) in nuclei isolated from poly(rI)·poly(rC)-induced cycloheximide-treated cells was only 50% of that measured in nuclei isolated from induced cells in the absence of cycloheximide (data not shown). This indicates that the accumulation of IFN-β mRNA in the induced cells during cycloheximide treatment is not caused by the enhanced transcription (14) of the IFN-β gene but is due to the absence of the degradation of IFN-β mRNA in these cells.

DISCUSSION

It generally has been assumed that the induction of human fibroblast cells with poly(rI)·poly(rC) leads to the synthesis of IFN-β, while viral infection of these cells leads to the synthesis of both IFN-α and IFN-β. The results of this study show that, in the cells studied, both inducers activate the expression of IFN-β only. The production of IFN-α proteins induced by NDV in human fibroblast lines has been reported by others (10); however, the amount of IFN-α induced was low (1–5% of the total IFN synthesized) and depended on the cell line used. Since in these sets of experiments, the amount of IFN-α induced in fibroblast cells was assayed by the ability to protect bovine cells and by neutralization with the anti-IFN-α antiserum (not necessarily monospecific), it is possible that the different results are due to the difference in specificity and sensitivity of the methods used. However, the human fibroblast cells trisomic for chromosome 21 (GM2504) were found to produce reproducibly higher amounts of IFN-α proteins (10–20%) than did other (diploid) lines (10), and, in these cells after NDV induction, we were able to detect trace amounts of IFN-α mRNA by hybridization with the specific probe (data not shown). These results indicate that in human fibroblast cells induced with NDV, the IFN-α genes are poorly inducible, and the induction may be determined by the genetic variability.

The results show that the accumulation of IFN-β mRNA in the cells in response to poly(rI)·poly(rC) is due to the transcriptional activation of IFN-β gene. It has been shown that treatments that increase IFN synthesis [priming with IFN (16) and pretreatment with butyrate or 5′-bromodeoxyuridine (31, 32)] led to substantial increase of the level of IFN-β mRNA in the cells. Treatment of the cells with cycloheximide alone did not cause an increase in transcription of the IFN-β gene in isolated nuclei, indicating that it is unlikely that in the uninduced cells, in the absence of protein synthesis, the IFN-β gene is transcribed constitutively (15). The transcription of the IFN-β gene in the nuclei isolated from induced cells proceeds during the time period when no IFN-β mRNA can be detected in the cells and the cells do not synthesize IFN-β. These results show that the tightly regulated synthesis of IFN-β in poly(rI)·poly(rC)-induced cells is not due to the shutoff transcription of the IFN-β gene but occurs at the post-transcriptional level. The continuous transcription of the IFN-β gene in the absence of IFN-β synthesis explains the previous observation that IFN synthesis cannot be reinduced in poly(rI)·poly(rC)-induced cells during the shutoff phase by treatment with an additional inducer (refractory state; refs. 24 and 33). Only at later times after induction, when the IFN-β gene is again in an inactive state, can IFN synthesis be reinitiated.

We have shown previously that in cells which were induced with poly(rI)·poly(rC) in the continuous presence of cycloheximide, IFN-β mRNA accumulates for hours beyond the point when it would normally have been destabilized; once the cells were released from the cycloheximide block and protein synthesis was resumed, the IFN-β mRNA degradation proceeded at the same rate and extent as in poly(rI)·poly(rC)-induced cells. These results indicate that the factor regulating IFN synthesis in poly(rI)·poly(rC)-induced cells is a protein. This protein, which is induced within the first few hours after the induction of IFN synthesis, must show a certain degree of specificity for the IFN-β mRNA because the half-life of total poly(A)$^+$ mRNA in the poly(rI)·poly(rC)-induced cells is not greatly affected (16). The fact that we were not able to detect any IFN-β mRNA sequence in cells at later times of poly(rI)·poly(rC) induction, although the IFN-β gene was actively being transcribed, indicates that this protein acts at the post-transcriptional level. We do not know whether the regulatory protein binds to the IFN-β mRNA and targets it for degradation by a cellular nuclease or whether it has a nuclease activity.

IFNs appear to be nucleic acid-binding proteins (34); thus, their synthesis could be self-regulated. Autoregulation has been shown to be a general property of several nucleic acid-binding proteins (35–37), and it was shown recently that the heat shock-induced proteins in *Drosophila* cells (38) are self-regulated both on transcriptional and translational levels. Because the IFNs have definite anticellular activities, self-regulation would ensure that the IFN will not be overproduced and toxic to the induced cells. Our results indicate that the post-transcriptional regulation of IFN-β mRNA stability in poly(rI)·poly(rC)-induced cells is coupled to cellular protein synthesis but probably not to the synthesis of IFN-β (self-regulation) because, in the same cells induced with NDV to produce IFN-β, IFN-β mRNA

persists for a significantly longer time. The lack of shutoff mechanism in the cells induced with NDV explains why in these cells the synthesis of IFN-β cannot be significantly enhanced by superinduction (in the presence of inhibitors of RNA and protein synthesis). The molecular basis of the difference in IFN-β mRNA stability in poly(rI)·poly(rC)- and NDV-induced human fibroblasts remains to be determined. It is possible that viral infection interferes with the induction of the degradation mechanism which occurs in the poly(rI)·poly(rC)-induced cells or that some other factors [e.g., poly(rI)·poly(rC) or poly(rI)·poly(rC)-induced proteins other than IFN] activating the degradation system are not present in the virus-induced cells.

This work was supported by National Institutes of Health Grant 5R01-AI-10944-10.

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