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Regulation of Synthesis and Turnover of an Interferon-Inducible mRNA

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Regulation of synthesis and turnover of an interferon (IFN)-inducible mRNA, mRNA 561, in HeLa monolayer cells was studied. Cytoplasmic levels of this mRNA were estimated by hybridization analyses with a cDNA clone that we have isolated as a probe. IFN-αA induced a high level of this mRNA in a transient fashion, whereas no induction was observed in response to IFN-γ. Surprisingly little mRNA 561 was induced in cells treated simultaneously with IFN-αA and an inhibitor of protein synthesis, suggesting that in addition to IFN-αA, an interferon-inducible protein was needed for induction of this mRNA. Apparently this putative protein could be induced by IFN-γ as well. Thus, although little mRNA 561 was synthesized in cells treated either with IFN-γ alone or with IFN-αA and cycloheximide, a large quantity of this mRNA was induced in cells which had been pretreated with IFN-γ and then treated with IFN-αA and cycloheximide. Once mRNA 561 was induced by IFN-αA, it turned over rapidly. This rapid turnover could be blocked by actinomycin D or cycloheximide indicating that another IFN-inducible protein may mediate this process.

Interferons (IFNs) are proteins with a variety of biological activities, including their ability to render cells resistant to viral infection (9, 17). Two classes of IFN, α and β, interact with the same cell surface receptor, whereas IFN-γ binds to a different one. The mechanisms by which binding of IFN to specific cell surface receptors brings about the various phenotypical changes in an IFN-treated cell are not yet understood fully. However, it is known that the expression of several genes is enhanced in IFN-treated cells. The products of these IFN-inducible genes presumably carry out various actions of IFN.

Recently, partial cDNA clones of several human and murine IFN-inducible mRNAs have been isolated and used for studying the nature of regulation of synthesis and turnover of these mRNAs in IFN-treated cells (1, 6, 8, 10, 13, 15, 16). Enhanced transcription of some IFN-inducible mRNAs can be detected within minutes of IFN coming in contact with cells (3, 6, 8). How long this enhanced transcription continues depends on the nature of the particular IFN-inducible gene (6). The steady-state cytoplasmic level of a particular IFN-inducible mRNA is dependent not only on its rate of transcription but also on its rate of turnover. The turnover rate is again different for different IFN-inducible mRNAs, and the turnover rate for a particular mRNA may vary with time elapsed after IFN treatment has begun (1, 6, 8, 13, 15). All IFN-inducible mRNAs studied so far seem to be the products of primary response to IFN, in the sense that they are induced by IFN in the presence of inhibitors of protein synthesis (1, 6, 8, 10, 13, 15).

One mRNA, mRNA 561, whose synthesis is rapidly induced by IFN in many human cell lines encodes a protein of 56 kilodaltons (1). An mRNA encoding a similar-sized protein is also induced in mouse cell lines in response to mouse IFN (15). The elevated levels of these mRNAs in IFN-treated cells are at least partly due to enhanced transcription of the corresponding gene (3, 8). In the investigations reported here, we studied the characteristics of synthesis and turnover of mRNA 561 in IFN-treated HeLa monolayer cultures. We present experimental evidence suggesting that IFN-inducible proteins are needed for both these processes.

MATERIALS AND METHODS

Materials. Enzymes used for cDNA cloning and restriction mapping were obtained from Bethesda Research Laboratories, Inc., and International Biotechnologies, Inc. For radiolabeling the cDNA probe, a nick translation kit from Bethesda Research Laboratories was used. All the enzymes were used according to the instructions of the manufacturer.

Interferons. Pure recombinant IFN-αA was obtained from Sidney Pestka, Roche Institute of Molecular Biology. Pure recombinant IFN-γ was obtained from Genentech Inc.

Cell culture. All experiments with HeLa monolayer cells were with confluent cultures grown in 100- or 150-mm plates with minimum essential medium containing 10% fetal bovine serum. When the cells were subjected to a series of treatments with different inhibitors or IFN, cells were washed three times with warm culture medium between two consecutive treatments. HeLa suspension culture obtained from Joseph Nevins was grown in Joklik modified minimum essential medium containing 5% fetal bovine serum.

RNA isolation. Cytoplasmic RNA was isolated as described before (18). If desired, poly(A)$^+$ RNA was selected by chromatography on an oligo(dT)-cellulose column (18).

Isolation of p561 clone. Double-stranded cDNA was prepared with poly(A)$^+$ RNA isolated from HeLa cells treated with 500 U of IFN-αA per ml for 12 h (7). cDNA was tailed with dC, annealed to dG-tailed *Pst*I-cut-pBR322, and used for transforming *Escherichia coli* RRI. Transformed bacteria were plated on nitrocellulose papers and grown in a medium containing tetracycline. Replicas of the filters were processed for screening by hybridization with a labeled oligonucleotide. The oligonucleotide used for screening had the sequence 5'TGCCTAAGGACCTTGTCTCACAGAGT TCTC3'. This sequence corresponds to the sequence at the 3' end of the coding region of C56 clone isolated by Chebath et al. (1). The oligonucleotide was labeled at the 5' end with ^{32}P (12) and used as a hybridization probe.

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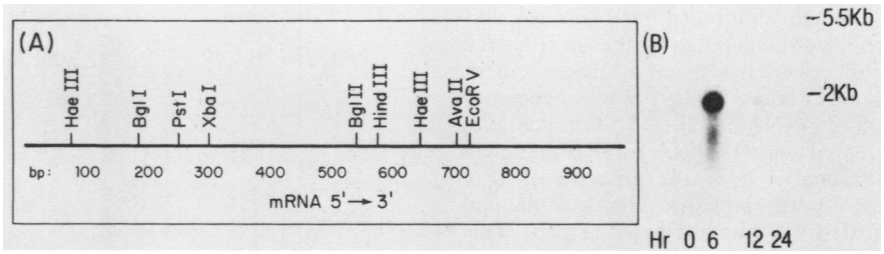

FIG. 1. Restriction map of the insert in p561 and Northern blot analysis of mRNA 561. (A) The partial restriction map was constructed with single, double, and triple restriction enzyme digestions. Lengths of the restriction fragments were determined by agarose gel electrophoresis with a 123-bp DNA ladder as a size marker. Restriction enzymes that did not cut within the insert were *Eco*RI, *Bcl*I, *Sac*I, *Sma*I, *Sac*II, *Hpa*I, *Xho*I, *Ava*I, *Sal*I, *Bam*HI, *Hin*cII, *Pvu*II, and *Kpn*I. (B) Cytoplasmic poly(A)$^+$ RNAs were isolated from HeLa cells that had been treated with 500 U of IFN-αA per ml for 0, 6, 12, and 24 h. Samples of 2 μg of each RNA were analyzed by agarose gel electrophoresis after denaturation in the presence of formaldehyde and blotted on a nitrocellulose paper (20). The blot was processed and hybridized to nick-translated p561. Human rRNAs were included as size markers and their positions are marked.

One of the positive colonies was purified by repeated plating at low density and grown in a large quantity. The plasmid harboring in this bacterium was purified by CsCl banding and designated p561. It contained an insert of about 980 base pairs (bp) at the *Pst*I site of pBR322. The insert contained an internal *Pst*I site and other restriction sites which were identified by standard procedures (11). For all dot blot analyses, the large 730-bp *Pst*I fragment of the insert in p561 was used as the probe. It was purified by gel electrophoresis and nick translated in the presence of [α-^{32}P]dCTP for this purpose (14).

Dot blot analysis. Total cytoplasmic RNA was analyzed by dot hybridization as described by White and Bancroft (21). All RNA samples were analyzed at four serial twofold dilutions containing 16, 8, 4, and 2 μg of total RNA. The autoradiograms were scanned with a densitometer equipped with an integrator. Only those readings which increased linearly with linear increase in the concentrations of total RNA were used for calculations. To obtain such linearity for samples in the same experiment having very different intensities of signal, it was sometimes necessary to scan several autoradiograms with different lengths of exposure.

RESULTS

Isolation of a cDNA clone corresponding to mRNA 561. We prepared a cDNA library with mRNA isolated from HeLa cells that had been treated with IFN-αA for 12 h. This library was screened by use of a synthetic 30-base oligonucleotide as the hybridization probe. The sequence of the oligonucleotide corresponded to a sequence present in the IFN-inducible cDNA clone described by Chebath et al. (1). We isolated several cDNA clones that hybridized with this oligonucleotide. One such clone, p561, was used for the studies described here. Clone p561 had an insert of about 980 bp. A partial restriction map was recorded (Fig. 1A). It hybridized with an IFN-induced mRNA of about 1,800 bases (Fig. 1B). The kinetics of induction of this mRNA, mRNA 561, in HeLa monolayer cells were similar to what has been reported for other cell lines (1, 4). A high level of this mRNA accumulated after 6 h of IFN-αA treatment; the level then decreased rapidly as treatment was continued for 12 or 24 h (Fig. 1B). IFN-γ did not induce a detectable level of mRNA 561 in these cells (data not shown).

Characteristics of induction of mRNA 561 by IFN-α. In HeLa monolayer cells, accumulation of mRNA 561 increased with increasing doses of IFN-αA up to 1,000 U/ml. There was an almost linear relationship between mRNA 561 levels and the logarithms of the units of IFN-αA per milliliter used between 10 and 1,000 U/ml (Fig. 2). mRNA 561 levels were quantitated in this and other experiments by dot blotting of serial dilutions of total cytoplasmic RNA as described above. Autoradiograms of the dot blots for this experiment were recorded (Fig. 2, inset).

To measure the rate of accumulation of mRNA 561 in cell cytoplasm, cells were treated for different times with IFN-αA, total cytoplasmic RNA was isolated, and mRNA 561 content was determined. The level of mRNA 561 increased linearly between 2 and 5 h after IFN treatment began, and then it leveled off (Fig. 3). Incubation of 5 to 6 h was therefore necessary for maximum induction of this mRNA. To determine whether continuous presence of IFN was necessary during this period or whether an initial exposure to IFN followed by continued incubation in the absence of IFN for up to 6 h was sufficient for maximum induction of mRNA 561, another experiment was done (Fig. 3). Cells

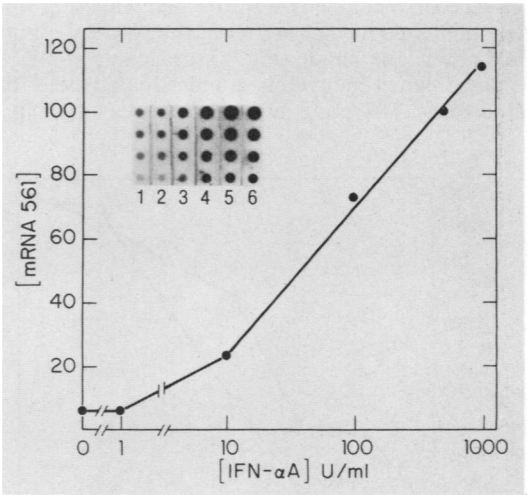

FIG. 2. IFN dose response of mRNA 561 induction. Confluent monolayers of HeLa cells were treated with 0, 1, 10, 100, 500 or 1,000 U of IFN-αA per ml for 6 h. Cytoplasmic RNAs were isolated and used for dot blot analysis. The inset shows one exposure of the autoradiogram. Lanes 1 through 6, RNA from cells treated with 0 through 1,000 U of IFN per ml, respectively. Serial dilutions from top to bottom contained 16, 8, 4, and 2 μg of RNA. The intensities of the dots were quantitated as described in the text and plotted in arbitrary units. For this experiment and for all subsequent experiments, the signal generated by RNA isolated from cells that had been treated with 500 U of IFN-αA per ml for 6 h was assigned a value of 100 U.

were treated with IFN-αA for different times, IFN-αA was washed off, and the cells were incubated further in IFN-free medium for 6 h, when they were harvested for measurement of mRNA 561. Continuous contact with IFN was necessary for maximum induction of mRNA 561 (Fig. 3). For example, cells that had been treated with IFN-αA for 2 h and then incubated for an additional 4 h in the absence of IFN contained only 30% mRNA 561 compared with cells that had been treated continuously for 6 h with IFN-αA (Fig. 3). This observation suggests that cells do not become desensitized to IFN after a short exposure to it, and continuous exposure is necessary for maximum response.

Turnover of mRNA 561. The cytoplasmic level of mRNA 561 decreased rapidly at between 6 and 12 h of IFN-αA treatment (Fig. 1B). We wondered whether the level would decrease even more rapidly if we stopped continued synthesis of mRNA 561 by treating the cells with an inhibitor of transcription such as actinomycin D. Cells were treated with IFN-αA for 4 h, and then incubated further without IFN and with or without actinomycin D. In cells not treated with actinomycin D, the level of mRNA 561 increased slightly at between 4 and 6 h and then fell rapidly (Fig. 4). In actinomycin D-treated cells, the level of mRNA 561 did not increase at 6 h, but with continued incubation it also did not decrease as rapidly as in untreated cells (Fig. 4). The cytoplasmic level of β-actin mRNA remained the same throughout this experiment in both actinomycin D-treated and untreated cells (data not shown). If the unlikely possibility of actinomycin D directly blocking turnover of mRNA 561 is disregarded, it appears that rapid turnover of mRNA 561 was dependent on the synthesis of another putative mRNA which was also induced by IFN-αA and whose protein product was presumably needed for this process. Confirming this hypothesis, turnover of mRNA 561 was also slowed down by inclusion of an inhibitor of protein synthesis, e.g., cycloheximide or anisomycin in place of actinomycin D. When cells were treated with IFN-α for 4 h and then incubated further in the absence of IFN-α but in the presence of cycloheximide (Fig. 4), the levels of mRNA 561 were much higher in cycloheximide-treated cells than in untreated ones. There are two possible reasons why these

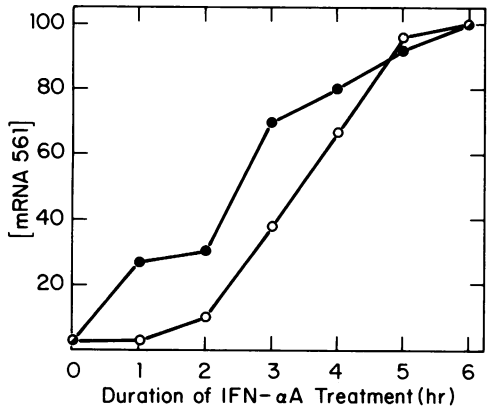

FIG. 3. Kinetics of accumulation of mRNA 561 in response to IFN-A treatment. Cells were treated with 500 U of IFN-αA per ml for the indicated times, cytoplasmic RNAs were isolated, and mRNA 561 content was determined as described above. Cells were treated with IFN-αA for the indicated times and harvested right away (○) or treated with IFN-αA for the indicated times, washed, and further incubated in media without IFN until 6 h, when all cells were harvested (●).

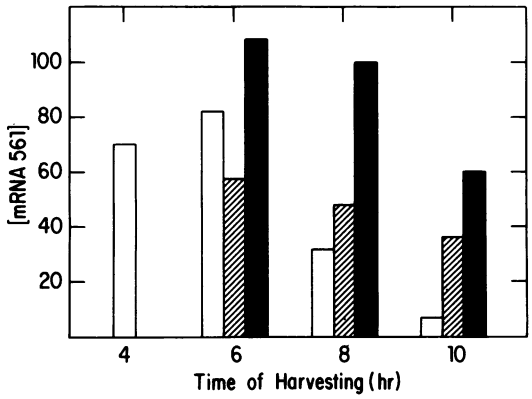

FIG. 4. Turnover of mRNA 561 in cells treated with inhibitors. Cells were treated with 500 U of IFN-αA per ml for 4 h, washed, and incubated further in medium without IFN with no addition (▭), with 1 μg of actinomycin D per ml (▨), or with 50 μg of cycloheximide per ml (■). Cells were harvested at different times, and cytoplasmic levels of mRNA 561 were determined by dot blot analysis.

levels were even higher than those in actinomycin D-treated cells. First, in cycloheximide-treated cells, synthesis of mRNA 561 could have continued after removal of IFN, and second, mRNA for the putative turnover protein could have been synthesized to some degree by 4 h, and this mRNA could have been translated into the active protein in actinomycin D-treated cells but not in cycloheximide-treated cells. In summary, in cells treated with IFN-α for 4 h and then chased for another 6 h without IFN-α, mRNA 561 levels decreased by 91% if no inhibitor was present, by 48% if actinomycin D was present, and by only 14% if cycloheximide was present during the chase (Fig. 4).

Dual actions of inhibitors of protein synthesis. Since protein

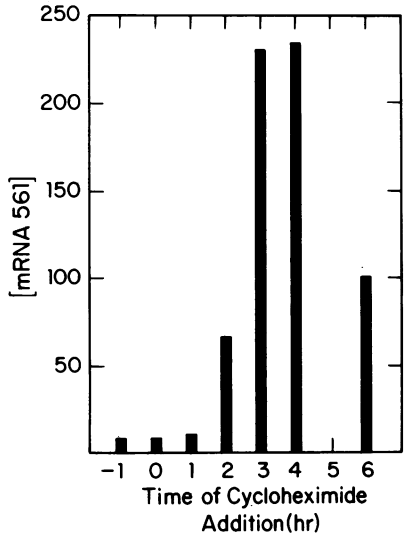

FIG. 5. Dual effects of cycloheximide on induction of mRNA 561. All plates of cells were treated with 500 U of IFN-αA per ml from 0 to 6 h; 50 μg of cycloheximide per ml was added to different plates at the indicated times. Cells were harvested at 6 h, and mRNA 561 content was determined. In separate experiments, it was determined that this dose of cycloheximide inhibited protein synthesis by about 96% and inhibited total poly(A)$^+$ RNA synthesis by about 15%.

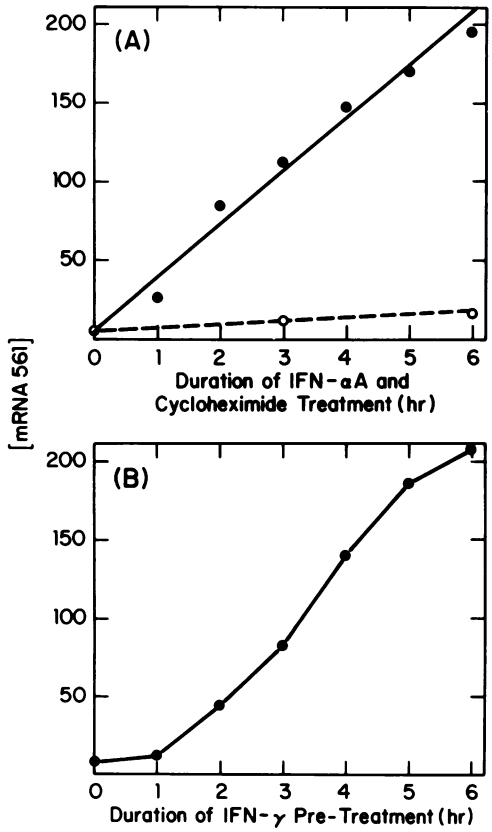

FIG. 6. Effects of IFN-γ pretreatment on mRNA 561 induction by IFN-αA. (A) Cells were treated with 500 U of IFN-αA per ml and 50 μg of cycloheximide per ml for the indicated times and harvested. Before these treatments, one set of plates was treated with 500 U of IFN-γ per ml for 3 h (●), whereas the second set received no pretreatment (○). (B) Cells were first treated with 500 U of IFN-γ per ml for the indicated times and then treated with 500 U of IFN-αA per ml and 50 μg of cycloheximide per ml for 3 h before harvesting.

synthesis inhibitors such as cycloheximide or anisomycin slowed down the rate of turnover of mRNA 561 at between 6 and 12 h of IFN-αA treatment, we argued that inclusion of such an inhibitor in the culture medium from time zero would cause the accumulation of a very high level of mRNA 561 in IFN-treated cells. Results were contrary to our expectations, however (Fig. 5). In cells that had been treated with both cycloheximide from −1 h, time zero, or 1 to 6 h and IFN-αA from 0 to 6 h, little mRNA 561 was induced. On the other hand, cells treated with cycloheximide at between 3 to 6 h or 4 to 6 h of IFN treatment accumulated about 2.5 times as much mRNA 561 as cells did that were treated with IFN-αA alone for 6 h. These data suggest that IFN-induced proteins were involved in both synthesis and turnover of this mRNA. Induction of the protein(s) needed for synthesis of mRNA 561 presumably preceded that of the protein(s) needed for its turnover. By varying the timing of cycloheximide treatment, one could therefore cause accumulation of more or less mRNA 561 compared with the accumulation over the same period of time in cells treated with IFN-αA alone.

The above observations indicate that IFN-αA was not a primary inducer of mRNA 561, since induction of another protein was needed for this process. However, this protein by itself could not have induced the synthesis of mRNA 561; continued presence of IFN-αA was needed for this process. Thus, when cells were treated with IFN-αA and cycloheximide for 3 h, presumably allowing the synthesis of mRNA for this putative protein, and then cultured for a further 3 h in the absence of IFN-αA or cycloheximide, little mRNA 561 was induced. On the other hand, a high level of mRNA 561 was induced when IFN-α was included in the culture medium during the latter 3 h (data not shown). Other experiments described below support the notion that this putative protein by itself could not have induced the synthesis of mRNA 561.

IFN-γ can induce the putative protein needed for induction of mRNA 561 by IFN-αA. IFN-γ was a poor inducer of mRNA 561 in HeLa monolayer cells. Cells that had been treated with 500 U of IFN-γ per ml for 3, 6, 12, or 24 h did not contain any more mRNA 561 than untreated cells (data not shown). IFN-γ also did not seem to induce the synthesis of the putative protein needed for rapid turnover of this mRNA, since 6 h of IFN-αA treatment induced as much mRNA 561 in previously untreated cells as in cells that had been pretreated with IFN-γ for 12 or 24 h (data not shown).

Results (Fig. 6), however, demonstrated that IFN-γ induced synthesis of the putative protein needed for induction of mRNA 561 by IFN-αA. In an experiment (Fig. 6A), one set of cultures was pretreated with IFN-γ for 3 h, while the second set received no such pretreatment. Both sets were then treated with IFN-α and cycloheximide for increasing lengths of time, and induction of mRNA 561 was measured. As expected, there was little induction of mRNA 561 in cells treated with IFN-αA and cycloheximide alone. High levels of mRNA 561 were induced, however, in cells that had been pretreated with IFN-γ. The simplest interpretation of these results is that the putative protein was induced by IFN-γ, but by itself IFN-γ could not induce mRNA 561, and it needed IFN-α for this purpose. IFN-α, on the other hand, could not induce synthesis of this protein in the presence of cycloheximide, but it could cooperate with the presynthesized protein to induce mRNA 561. When this protein had been presynthesized, IFN-α could induce mRNA 561 more quickly (Table 1). For example, 3 h of IFN-αA treatment produced about twice as much mRNA 561 in IFN-γ-pretreated cells than it did in cells which had not been pretreated with IFN-γ (Table 1, plates 3 and 6). Presence of cycloheximide during the treatment with IFN-αA did not make much difference if the cells had been pretreated with IFN-γ (Table 1, plates 6 and 7). If cycloheximide was present during the whole experiment causing a block in the synthesis of this putative protein, the effects of IFN-γ pretreatment were eliminated (Table 1, plates 7 through 9).

TABLE 1. Requirement of protein synthesis for induction by IFN-γ of the putative protein needed for synthesis of mRNA 561

Plate no.	Time (h) of treatment			Harvest	mRNA 561 level
	IFN-α	IFN-γ	Cycloheximide		
1				0	4
2	0–6			6	100
3	0–3			3	45
4	0–3		0–3	3	10
5		0–3		6	4
6	3–6	0–3		6	90
7	3–6	0–3	3–6	6	107
8	3–6	0–3	0–3	6	35
9	3–6	0–3	0–6	6	4
10	6–9	0–3	0–3 6–9	9	127

mRNA 561 was synthesized, however, in cells that had been incubated first with IFN-γ and cycloheximide, allowing synthesis of mRNA for the putative protein, then without any additive, allowing translation of this mRNA, and finally with IFN-αA and cycloheximide (Table 1, plate 10). The fact that the level of mRNA 561 continued to increase over a 6-h period of IFN-α and cycloheximide treatment (Fig. 6A) suggested that once synthesized, the putative protein could maintain its activity for at least 6 h.

We measured the kinetics of synthesis of the putative protein in response to treatment with IFN-γ (Fig. 6B). Cells were first treated with IFN-γ for various times and then treated with IFN-αA and cycloheximide for 3-h periods before mRNA 561 levels in the cells were measured. There was little induction of the mRNA in cells pretreated with IFN-γ for only 1 h, but 2 to 5 h of pretreatment caused a linear increase in the level of accumulated mRNA 561. Beyond 5 h, the rate of increase tapered off. These results suggested that the saturation level of this protein had been synthesized in IFN-γ-treated cells by about 5 to 6 h. They also suggested that the putative protein needed for rapid turnover of mRNA 561 was probably not induced by IFN-γ.

Characteristics of mRNA 561 induction in HeLa cells grown in suspension culture. After the studies described so far had been completed, Faltyneck et al. (4) reported that IFN-α can induce the synthesis of mRNA 561 in HeLa cells grown in suspension culture even in the presence of cycloheximide. To seek an explanation for the apparent discrepancy between our results with HeLa monolayers and their results with HeLa cells grown in suspension, an experiment was performed (Table 2). At 6 h in HeLa cell suspension culture, although cycloheximide inhibited mRNA 561 induction by IFN-αA to some degree, the inhibition was very much less complete than that observed in HeLa monolayers (Fig. 5). Whether there were fundamental differences in the mechanism of mRNA 561 induction by IFN-αA in HeLa cell suspension and monolayer cultures or whether cycloheximide action was more leaky in HeLa cell suspension cultures is not clear at this time. At 12 h (the time point used by Faltyneck et al. [4]) we observed a higher level of mRNA 561 in cycloheximide-treated cells. This is consistent with our model for regulation of synthesis and turnover of mRNA 561. Any mRNA 561 synthesized in cycloheximide-treated cells would be more stable, since the putative protein responsible for its rapid turnover would not be synthesized in these cells.

DISCUSSION

Our present understanding of the processes and components involved in IFN-mediated regulation of synthesis and turnover of mRNA 561 is shown schematically in Fig. 7. IFN-α produced three functionally distinguishable signals. The chemical natures of these signals are unknown, and they

TABLE 2. Effect of cycloheximide on mRNA 561 induction in HeLa cells in suspension culture

Sample no.	Time (h) of treatment:			mRNA 561 level
	IFN-α	Cycloheximide	Harvest	
1			0	2
2	0–6		6	100
3	0–6	0–6	6	44
4	0–12		12	19
5	0–12	0–12	12	52

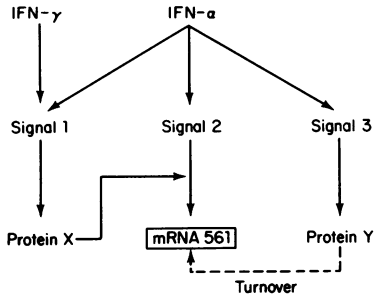

FIG. 7. Schematic diagram of the processes involved in induced synthesis and turnover of mRNA 561.

may share common components. Signal 1, which could be elicited by either IFN-α or IFN-γ, triggered the synthesis of protein x, the putative protein needed for induction of mRNA 561. Signal 2, generated by IFN-α but not by IFN-γ, acted in concert with protein x to induce synthesis of mRNA 561 most probably by enhancing its rate of transcription (8). Signal 3 was generated by IFN-α induced synthesis of protein y, the protein which was needed for rapid turnover of mRNA 561. Enhanced synthesis of protein y was probably a result of induction of its mRNA by signal 3 (Fig. 4). Our data suggest that IFN-γ did not produce signal 3. This is consistent with our earlier observation that syntheses of some IFN-induced proteins continued for longer times in IFN-γ-treated cells than in IFN-α-treated cells, suggesting that the corresponding mRNAs might be stabler in the former cells (19). The temporal order of production of signals 1, 2, and 3 has not been definitively determined, although our data suggest that signal 3 was produced several hours after signals 1 and 2.

Our conclusions regarding the need for protein synthesis for various steps in mRNA 561 metabolism are based on the use of specific inhibitors of protein synthesis such as cycloheximide or anisomycin. The possibility formally exists that these agents also inhibited the synthesis of some other biochemical necessary for mRNA 561 induction. However, if enough of this material, be it a protein or not, were available, mRNA 561 could be synthesized very efficiently even in the presence of cycloheximide (Fig. 6). Thus, the slight possibility of cycloheximide somehow directly and specifically inhibiting the synthesis of mRNA 561 can be safely ruled out.

The need for new protein synthesis for induction of mRNA 561 by IFN-α is not absolute in all cell cultures. In HeLa cell suspension cultures, mRNA 561 could be induced in the presence of cycloheximide (4, 8). Constitutive levels of IFN-inducible mRNAs may be quite different in different cell lines (1, 6, 13). It is conceivable that the putative protein needed for induction of mRNA 561 is a constitutive protein of HeLa cells grown in suspension culture. It is known that the constitutive levels of an IFN-inducible mRNA in mouse L929 or Ehrlich ascites tumor cells vary considerably depending on whether the cells are grown in monolayer or in suspension culture (H. Samanta, personal communication).

Synergistic interactions between IFN-α and IFN-γ have been reported both for antiviral and anticellular effects of IFN (2, 5). However, the molecular basis of such synergism has not been explored. The observations reported here constitute an example of such synergism at the molecular level. The physiological significance of this particular mode

of interaction between IFN-α and IFN-γ remains to be understood.

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LITERATURE CITED

1. **Chebath, J., G. Merlin, R. Metz, P. Benech, and M. Revel.** 1983. Interferon-induced 56,000 Mr protein and its mRNA in human cells: molecular cloning and partial sequence of the cDNA. Nucleic Acids Res. **11**:1213–1226.
2. **Czarniecki, C. W., C. W. Fennie, D. B. Powers, and D. A. Estell.** 1984. Synergistic antiviral and antiproliferative activities of *Escherichia coli*-derived human alpha, beta, and gamma interferons. J. Virol. **49**:490–496.
3. **Engel, D. A., H. Samanta, M. E. Brawner, and P. Lengyel.** 1985. Interferon actions: transcriptional control of a gene specifying a 56,000-Da protein in Ehrlich ascites tumor cells. Virology **142**:389–397.
4. **Faltynek, C. R., S. MaCandless, J. Chebath, and C. Baglioni.** 1985. Different mechanisms for activation of gene transcription by interferons α and γ. Virology **144**:173–180.
5. **Fleischman, W. R., Jr., J. A. Georgiades, L. C. Osborne, and H. M. Johnson.** 1979. Potentiation of interferon activity by mixed preparations of fibroblast and immune interferon. Infect. Immun. **26**:248–253.
6. **Friedman, R. L., S. P. Manly, M. McMahon, I. M. Kerr, and G. R. Stark.** 1984. Transcriptional and posttranscriptional regulation of interferon-induced gene expression in human cells. Cell **38**:745–755.
7. **Gubler, U., and B. J. Hoffman.** 1983. A simple and very efficient method for generating cDNA libraries. Gene **25**:263–269.
8. **Larner, A. C., G. Jonak, Y.-S. E. Cheng, B. Korant, E. Knight, and J. E. Darnell.** 1984. Transcriptional induction of two genes in human cells by β-interferon. Proc. Natl. Acad. Sci. USA **81**:6733–6737.
9. **Lengyel, P.** 1982. Biochemistry of interferons and their actions. Annu. Rev. Biochem. **51**:251–282.
10. **Luster, A. D., J. C. Unkeless, and J. V. Ravetch.** 1985. γ-Interferon transcriptionally regulates an early response gene containing homology to platelet proteins. Nature (London) **315**:672–676.
11. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
13. **Merlin, G., J. Chebath, P. Benech, R. Metz, and M. Revel.** 1983. Molecular cloning and sequence of partial cDNA for interferon-induced (2′-5′) oligo(A) synthetase mRNA from human cells. Proc. Natl. Acad. Sci. USA **80**:4904–4908.
14. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
15. **Samanta, H., J. P. Dougherty, M. E. Brawner, H. Schmidt, and P. Lengyel.** 1982. Interferon action: cloning of cDNA segments complementary to messenger RNAs induced by interferons. UCLA Symp. Mol. Cell. Biol. **25**:59–72.
16. **Saunders, M. E., D. R. Gewert, M. E. Tugwell, M. McMahon, and B. R. G. Williams.** 1985. Human 2-5A synthetase: characterization of a novel cDNA and corresponding gene structure. EMBO J. **4**:1761–1768.
17. **Sen, G. C.** 1984. Biochemical pathways in interferon action. Pharmacol. Ther. **24**:235–257.
18. **Sen, G. C., J. Racevskis, and N. H. Sarkar.** 1981. Synthesis of murine mammary tumor viral proteins in vitro. J. Virol. **37**:963–975.
19. **Sen, G. C., and B. Y. Rubin.** 1984. Synthesis of interferon-inducible proteins is regulated differentially by interferon-α and interferon-γ. Virology **134**:483–488.
20. **Thomas, P. W.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77**:5201–5205.
21. **White, B. A., and F. C. Bancroft.** 1982. Cytoplasmic dot hybridization. Simple analysis of relative mRNA levels in multiple small cell or tissue samples. J. Biol. Chem. **257**: 8569–8572.