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Correlation Between the Antiviral Effect of Interferon Treatment and the Inhibition of In Vitro mRNA Translation in Noninfected L cells

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When noninfected L-cell suspension cultures are treated with interferon (specific activities superior to 10^6 reference units per mg of protein), the cell-free cytoplasmic extracts obtained are inactive for the translation of exogenous natural mRNAs. The dose-response curve shows that comparable amounts of interferon are required to produce a 50% reduction of Mengo virus multiplication in vivo and Mengo RNA translation in vitro. With higher doses of interferon, Mengo RNA translation is completely abolished, while poly U translation and endogenous protein synthesis are only slightly affected. The inactivation of Mengo RNA translation is reversible; after removal of interferon, normal translation activity is regained together with the ability to support Mengo virus multiplication. Fractionation of the cell-free extracts shows that the effect is localized in the fraction which can be washed off the ribosomes by high salt. These results establish that interferon induces a block in genetic translation in noninfected L cells.

Cells pretreated with interferon are incapable of supporting the multiplication of many RNA and DNA viruses. Entry and decapsidation of the viruses are unimpaired, and the block is clearly in the synthesis of viral macromolecules (21, 29). The treated cells continue to carry out their own macromolecular synthesis, although interferon may produce some reduction in the rate of cell division (11, 28) and cellular protein synthesis (15).

Many studies have been concerned with the mechanism of action of interferon and, although the effect on viral multiplication is very clear, the level at which viral gene expression is affected has not been elucidated. Blocks in viral RNA synthesis (1, 23, 24, 27) and in viral protein synthesis (3, 25, 26) have both been observed in vivo, and it has been suggested that these are responsible for the antiviral action of interferon. The mechanism by which interferon induces these blocks is, however, not known. Furthermore, it is not clear if these alterations result from changes induced in the cell by interferon treatment before infection or if they are expressed only when virus infection is in progress. These questions can be answered only by using cell-free systems in which the influence of interferon treatment on macromolecular synthesis can be directly studied.

We recently reported (6) a complete inhibition of the in vitro translation of Mengo virus RNA in cell-free extracts from interferon-treated L cells. Friedman et al. (9) have also observed a block in the translation of encephalomyocarditis virus (EMC) RNA, but only in L cells which, in addition to interferon treatment, were infected with vaccinia or EMC virus. Since both viruses have direct effects on host protein synthesis, it is hard to discriminate in their results the part played by viral infection from that due to interferon. In our conditions (6) an effect of interferon treatment on the translation of natural exogenous mRNAs was obtained even in noninfected cells. This system allows us, therefore, to investigate the effect of interferon by itself on genetic translation.

The present report demonstrates the quantitative relationship between the antiviral effect and the translation blocking effect of interferon in mouse L cells. It is essential to establish this correlation, since even the best preparations of interferon are only partially purified. Experi-

ments directed at analyzing which part of the translation machinery is affected are also presented. The relevance of these effects on protein synthesis for the mechanism of action of interferon is discussed.

MATERIALS AND METHODS

Cell cultures. Mouse L cells, strain CCL1, were grown in suspension culture in Eagle minimal essential medium (MEM) (Laboratoire Eurobio, Paris) supplemented with 8% decomplemented calf serum and 0.1% methyl cellulose. Penicillin and streptomycin were added at standard concentrations; occasionally gentamicin (40 mg/liter) was added. The volumes of the Spinner cultures varied from 0.5 to 10 liters, with stirring speeds from 130 to 200 rpm and gas (5% CO_2–95% air) circulations from 3 to 10 liters/h, depending on the volume of the cultures. In these conditions, the cells reached a density of 1.3×10^6 to 1.5×10^6 cells/ml and were diluted every second day. For experiments, 2 to 3 liters of culture were adjusted to 10^6 cells/ml, and culture was continued with or without interferon.

Interferon preparation and purification. L cell-NDV interferon: Confluent monolayer cultures of CCL1 grown in Eagle MEM with 8% calf serum were infected with Newcastle disease virus (NDV, Hertz strain) at a multiplicity of 20 PFU/cell for 1 h, repeatedly washed with phosphate-buffered saline (PBS) (0.85% NaCl in 0.01 M phosphate buffer, pH 7.2) and supplemented with medium without serum. After 18 h, the supernatant fluid was centrifuged to eliminate the cells, adjusted to pH 2, and concentrated 10-fold by vacuum dialysis. Treatment at pH 2 was continued for 5 days, and the precipitate was discarded by ultracentrifugation. After dialysis against 0.1 M phosphate buffer (pH 5.8), interferon was partially purified by chromatography on carboxymethyl Sephadex (C 25) with a pH gradient from 5.8 to 8.0. Fractions containing the activity (pH 6.3–7) were pooled and concentrated by vacuum dialysis. Protein concentration measured by the method of Lowry (22) was usually 30 to 40 μg/ml. Interferon titer, determined by inhibition of the cytopathic effects of vesicular stomatitis virus (VSV), indicated specific activities of 10^6 to 2×10^6 reference units/mg of protein. Before storage at −80 C, bovine serum albumin (fraction V) was added as stabilizing agent.

Mouse brain-West Nile virus interferon was prepared and partially purified as described previously (8). Preparation of human lymphocyte (5) interferon induced by NDV in circulating lymphocytes cultures was detailed elsewhere (7).

Mengo virus RNA. Monolayer cultures of L cells were infected with Mengo virus at a multiplicity of 20 PFU/cell. After 60 min at room temperature, 1 ml of medium with 2% calf serum was added for each 3×10^6 cells. The medium was collected 18 h later, and debris was eliminated by centrifugation at $10,000 \times g$. Titers obtained ranged from 3×10^9 to 5×10^9 PFU/ml. Virus was centrifuged for 150 min at 152,000 $\times g$ and resuspended in PBS with a Dounce homogenizer. Aggregates were eliminated by centrifugation for 10 min at $10,000 \times g$. The virus suspension was treated for 1 h at 37 C with 50 μg of RNase A per ml, for 1 h with 20 μg of DNase per ml at 2.5 mM Mg^{2+}, and for 30 min with 20 μg of trypsin per ml. The virus was pelleted in a Spinco SW41 rotor through a 1.5-ml cushion of 40% sucrose in PBS for 120 min at 108,000 $\times g$, resuspended with a Teflon homogenizer in 0.5 to 1 ml of TBS (35 mM Tris-hydrochloride [pH 7.4], 140 mM NaCl) to a titer of 10^{12} to 10^{13} PFU/ml, and banded in a CsCl gradient at a density of 1.33 g/ml. RNA was extracted from the purified virus by phenol treatment at 45 C by the method of Kerr et al. (18). Rabbit hemoglobin 9S mRNA was prepared as described previously (13, 19).

Preparation of cell-free extracts. One-liter suspension cultures of CCL1 (10^6 cells/ml), diluted with fresh medium one day before the experiment, were treated for 18 h (unless otherwise indicated) with the amount of interferon indicated in each experiment. In some cases, control cultures received a mock preparation. The antivival effect of the interferon pretreatment was checked in each experiment by infecting 20 ml of culture (2×10^7 cells) with 10 PFU/cell of Mengo virus and measuring the yield of virus after 24 h of incubation under Spinner conditions. Alternatively, cells were returned to monolayer cultures and, after 1 h, were infected with 50 PFU of Mengo virus, overlaid with agarose, and incubated for 24 h at 37 C. The reduction in the number of plaques is a direct measure of the antiviral effect. The remainder of the control and treated cultures was rapidly chilled on ice, washed three times in TBS, and pelleted at 5,000 rpm for 5 min. The pellets were suspended in 2 vol of hypotonic buffer A (10 mM Tris-hydrochloride [pH 7.5], 10 mM KCl, 1.5 mM Mg acetate) for 5 min at 0 C and homogenized by hand about 20 times in a tight-fitting Dounce homogenizer. Concentrated buffer was added to give a final concentration of 20 mM Tris-hydrochloride (pH 7.5), 120 mM KCl, 5 mM Mg acetate, and 7 mM β-mercaptoethanol (buffer B), and the lysate was centrifuged at $10,000 \times g$ for 10 min. For preincubation, the supernatant extract (S-10) was adjusted to 1 mM ATP, 0.1 mM GTP, 10 mM creatine phosphate, 160 μg of creatine kinase per ml, and 40 μM each of the 20 amino acids. After incubation for 45 min at 37 C, the lysate was clarified at $10,000 \times g$ for 10 min and filtered through a Sephadex G25 column. The material excluded from the column was pooled and stored frozen in liquid nitrogen at a concentration of 25 to 35 A_{260} units/ml. Extracts were thawed only once before use.

Cell-free protein synthesis. Reaction mixtures contained, in 0.05 ml, 30 mM Tris-hydrochloride (pH 7.5), 3 to 4 mM Mg acetate, 80 to 100 mM KCl, 7 mM β-mercaptoethanol, 1 mM ATP, 0.1 mM GTP, 10 mM creatine phosphate, 8 μg of creatine kinase, 40 μM each 19 amino acids, 0.125 μCi of ^{14}C-leucine (150 mCi/mmol), 1 to 2 A_{260} units of preincubated S-10, and 3 μg of Mengo RNA, or 2 to 5 μg of globin 9S mRNA. When poly U, 10 μg, was used, 0.125 μCi of ^{14}C-phenylalanine (260 mCi/mmol) was added instead of amino acids, and the Mg^{2+} concentration was raised to 5 mM. For endogenous L-cell mRNA translation, nonpreincubated S-10 was used and ^{14}C-leucine incorporation was measured as above without adding any exogenous mRNA.

After 60 min at 37 C, 40 μliters were applied to a Whatman 3 MM filter paper (2.5-cm disk), dipped in cold 10% trichloroacetic acid for 15 min, then in 5% trichloroacetic acid at 90 C for 15 min, rinsed twice in 5% trichloroacetic acid for 5 min, once in alcohol-ether (1:1), and dried before counting by toluene-based scintillation.

Preparation of subcellular fractions. Crude ribosomes, obtained by centrifugation of 1 ml of S-10 for 3 h at 150,000 × *g*, were resuspended in buffer B. Supernatant fluid was used directly or after concentration by precipitation with 90% ammonium sulfate and dialysis against buffer B containing 20% glycerol. To purify ribosomes, the pellet was resuspended in 0.2 ml of 0.5 M KCl, 20 mM Tris-hydrochloride (pH 7.5), 5 mM $MgCl_2$, and 7 mM β-mercaptoethanol, stirred for 2 h, and centrifuged for 3 h at 150,000 × *g*. The wash fluid was dialyzed before use against buffer B containing 20% glycerol.

RESULTS

Properties of cell-free protein-synthesizing systems from normal and interferon-treated cells. Postmitochondrial supernatant S-10 was prepared from CCL1 cells grown in suspension cultures to a density of 10^6 cells/ml (doubling time about 2 days). The high endogenous ^{14}C-leucine incorporation in these extracts (16 pmol of ^{14}C-leucine per 1 A_{260} unit of L-cell extract) is reduced to about 5% by preincubation at 37 C for 45 min under conditions of protein synthesis. Preincubated L cell S-10 extracts respond to the addition of exogenous mRNAs, natural or synthetic, with at least a 10-fold stimulation in the incorporation of amino acids into protein. Translation of Mengo virus RNA is carried out as shown in Fig. 1, with 3 μg of Mengo RNA, 4 mM (Mg^{2+}), for 60 min at 37 C. Recently, better results were obtained at 30 C. Analysis of the Mengo RNA-directed protein products, by electrophoresis on polyacrylamide gels, shows, as reported also by others (4), the formation of specific products of different sizes

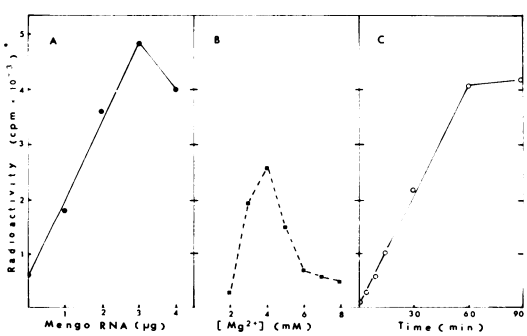

FIG. 1. *Incorporation of ^{14}C-leucine by L-cell extracts in response to Mengo RNA. Conditions are detailed in Methods.*

(the largest 140,000 mol wt, then at 110,000, 90,000, 45,000 and 30,000), which are not found in the absence of mRNA. These products may arise from premature termination of a polypeptide chain initiated at a single site on the RNA as proposed from studies on EMC RNA translation in Krebs ascites cell extracts (17, A. E. Smith, Eur. J. Biochem., in press). As with EMC RNA, synthesis of characteristic sparsomycin amino-terminal methionine peptides (A. E. Smith, Eur. J. Biochem., in press) is observed in L-cell extracts in response to Mengo RNA. Addition of 2 to 5 μg of rabbit reticulocyte 9*S* globin mRNA stimulates the incorporation of 4 to 7 pmol of ^{14}C-leucine per 1 A_{260} unit of S-10. The product synthesized in vitro comigrates with rabbit globin upon electrophoresis on SDS-polyacrylamide gels.

Effects of interferon on in vitro mRNA translation: dose-response to interferon. As reported before (6) the cell-free system described above was used to compare the protein-synthesizing capacity of extracts from normal and interferon-treated L cells. Figure 2 shows that, after pretreatment by interferon for 18 h, the L cells yield an S-10 extract inactive for the translation of Mengo virus RNA. The relationship between this inactivation of translation and the antiviral effect of interferon was studied in the experiment of Fig. 3 in which L-cell interferon, of relatively high specific activity, was used at different concentrations. The dose-dependent inactivation of Mengo RNA translation activity closely parallels the loss of in vivo capacity to support Mengo virus development. It can be seen that 5 to 6 reference units of interferon (measured against VSV) are needed to inhibit by 50% the multiplication of Mengo virus in our system. At this concentration, interferon treatment produces a 60 to 70% inhibition of the activity of extracts to translate Mengo RNA. This quantitative relationship in the dose of interferon required for both in vivo and in vitro effects adds a strong argument to our proposal (6) that the inactivation of translation capacity observed in these noninfected cell extracts is responsible for the antiviral activity of interferon.

Figure 3 confirms our previous observation that translation of a "cellular" mRNA, such as rabbit hemoglobin mRNA, also decreases with doses of interferon which produce the antiviral effect. On the other hand, translation of poly U in this system is only slightly affected and with little relation to the dose of interferon.

Table 1 gives the results of another experiment in which several concentrations of interferon were compared. There is again a good

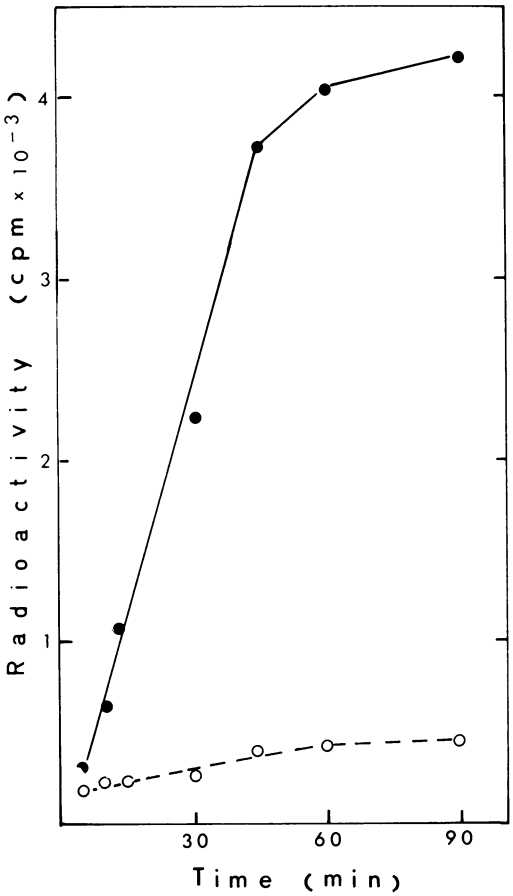

FIG. 2. *Incorporation of ^{14}C-leucine by S-10 extracts from normal and interferon-treated L cells in response to Mengo RNA. One culture of L cells was treated with 100 reference units of interferon (that is 10 times more than the dose which inhibits by 50% the multiplication of Mengo, measured as detailed in Methods). The control culture received a mock preparation. Preparation of S-10 and measurement of Mengo RNA translation as described in Methods. Symbols: O, interferon treated; ●, control.*

correlation between the dose of interferon required to inhibit Mengo virus multiplication in vivo and that needed to block in vitro mRNA translation. At the interferon concentration inhibiting the multiplication of Mengo virus by 50%, there is a 70% inhibition of Mengo RNA translation, a 25% inhibition of hemoglobin mRNA translation, but no effect on poly U translation. At a 10-fold-higher concentration of interferon, the translation of both Mengo RNA and hemoglobin mRNA are inhibited to almost the same extent (83 and 94%, respectively), whereas poly U translation is decreased to a much smaller extent (29%), the same decrease being observed at all Mg^{2+} concentrations.

It is of great interest to know the effect of interferon treatment on translation of endogenous L-cell mRNAs in these extracts. This can be measured in S-10 extracts which have not been preincubated; the major portion of this activity is due to initiation and is sensitive to NaF (unpublished data). Under conditions in which Mengo RNA translation was reduced by over 90%, the decrease in endogenous activity ranged from 15 to 30% only. Although the difference is striking, it does not exclude that interferon treatment affects some cellular protein synthesis. Indeed, at higher concentrations of interferon, some inhibition of amino acid incorporation in L cells in vivo becomes appar-

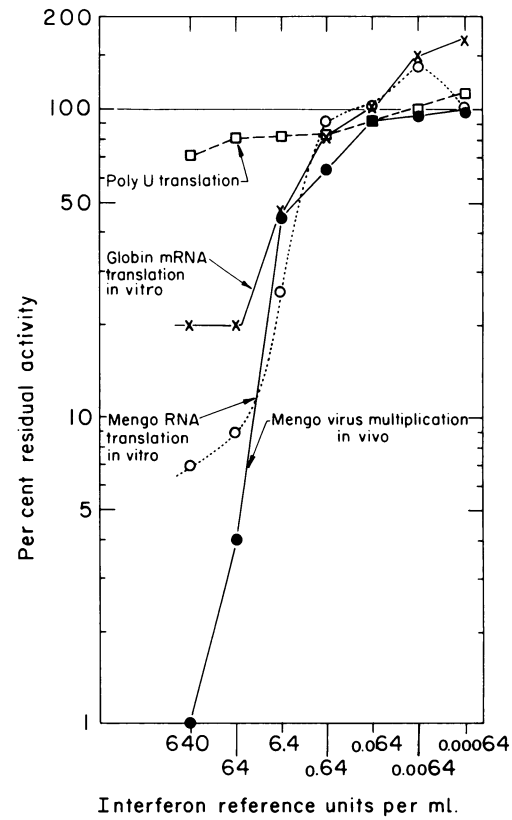

FIG. 3. *Dose-response curve for the interferon-induced inhibition of Mengo virus multiplication and Mengo RNA translation. Highly purified L cell-NDV interferon was added to L-cell Spinner cultures at the final concentration indicated (expressed in reference units against VSV). After 18 h, a sample of each culture was used to measure Mengo virus growth and the remainder to prepare S-10 extracts for measurement of mRNA translation as described in Methods. Results of the different assays are expressed in percent of the activities obtained with an untreated culture. Control values were similar to those in Table 1.*

ent (Martelli, I., Falcoff, R. and Falcoff, E., in preparation).

In summary, with small doses of interferon a good correlation is found between inhibition of in vitro Mengo virus RNA translation and the antiviral effect. Even with larger doses the translation of Mengo RNA or another exogenous natural mRNA, such as rabbit hemoglobin mRNA, are much more affected than poly U and endogenous L-cell mRNA translation.

The time course and reversibility of the interferon effect. In spite of the partial purification of our preparation of interferon (the results from the dose-response curve of Table 2 were obtained with an interferon preparation containing 30 μg of protein per ml, which at a dose of 39 reference units contributes 0.03 μg of protein/ml of suspension culture), the possibility remained that the inhibition of mRNA translation results from some unspecific effect of an impurity in the interferon preparation. We have, therefore, carried out several experiments to further establish the relation between the antiviral effect and the observed effect on in vitro translation.

As already reported (6), after interferon treatment the rate of development of the antiviral state in vivo coincides with the inactivation of in vitro translation activity.

Up to 4 h after addition of interferon, both Mengo virus multiplication and in vitro Mengo RNA translation are normal as in untreated cells, while from 4 to 18 h a rapid loss of both activities is visible. If actinomycin D (2 μg/ml) is added to the culture, neither the antiviral state nor the effect on mRNA translation appears (6). Interferon, therefore, induces the block in mRNA translation by some active slow process, similar to that responsible for the establishment of the antiviral effect (29). Furthermore, after removing interferon, one can demonstrate that the cells are not permanently

TABLE 1. *Effect of various doses of interferon on mRNA translation activity*

L-cell treatment[a]	Antiviral effect[a] (Mengo virus multiplication in vivo)		Translation in vitro of:[b]		
	PFU/ml	%	Mengo RNA[c]	Hemoglobin mRNA[c]	Poly U[d]
No interferon	1.7×10^8	100	3,725 (100)	2,820 (100)	8,100 (100)
Interferon, 390 reference units/ml	2.1×10^5	0.1	225 (6)	490 (17)	5,780 (71)
Interferon, 39 reference units/ml	7.3×10^7	43	1,135 (30)	2,110 (75)	7,770 (94)
Interferon, 3.9 reference units/ml	2.0×10^8	117	3,980 (106)	2,920 (103)	10,205 (125)

[a] Purified interferon specific activity: 1.3×10^6 reference units/mg of protein; 39,000 reference units/ml determined by inhibition of cytopathic effects of VSV. The antiviral effect is the Mengo virus yield measured as described in Methods.

[b] Cell-free mRNA translation in S-10 extracts of L cells treated for 18 h as indicated. Incorporation measured in counts per minute. Background incorporation (C = 280; I = 120 counts/min) in the absence of mRNA was substracted. Percentage of control activity is given in parentheses.

[c] Incorporation of ^{14}C-leucine.

[d] Incorporation of ^{14}C-phenylalanine.

TABLE 2. *Blocking of the effect of interferon on in vitro translation by anti-interferon serum*

Treatment	Mengo virus multiplication in vivo[a] (PFU/ml)	Translation in vitro of:[b]		
		Mengo RNA	Hemoglobin mRNA	Poly U
None	5.5×10^9 (100)	2,930 (100)	2,050 (100)	8,965 (100)
Mouse brain interferon[c]	4.75×10^6 (0.1)	465 (16)	425 (20)	6,310 (71)
Mouse brain interferon[d] + anti(L-cell-NDV interferon) serum[d]	1.25×10^9 (23)	2,430 (83)	835 (41)	9,000 (100)

[a] After 3 cycles. Number in parentheses is percentage of control activity.

[b] Incorporation of ^{14}C-leucine (for Mengo RNA and hemoglobin mRNA)and ^{14}C-phenylalanine (for poly U) measured in counts per minute.

[c] Produced by injection of West Nile virus; prepared and used similarly to L-cell-NDV interferon (300 units/ml for 18 h).

[d] An antiserum prepared against L-cell-NDV interferon (a gift from B. Fauconnier) was preincubated for 1 h at 37 C with the mouse brain interferon preparation, before addition to the L-cell culture.

damaged and that the inactivation of mRNA translation, like the antiviral state, is reversible (Fig. 4).

After a 24-h treatment of L cells with interferon, the medium was changed and cells were maintained without interferon at a density of 10^6/ml. At the indicated intervals, the medium was renewed and parts of the culture were used to measure the in vivo multiplication of Mengo and in vitro translation of Mengo RNA and hemoglobin mRNA. After 3 days, a partial, and after 7 days, a complete recovery of both in vivo and in vitro activities was observed. Viability of cells, as determined with trypan blue, remained near 100% in interferon-treated and control cells for the whole period of the experiment, and no significant multiplication of the cells was observed at this high density. The activities of cell-free extracts from the control culture (not treated with interferon) at day 1, 4, and 7, remained constant (see legend to Fig. 4). These experiments demonstrate, therefore, that the cells can recover their ability to translate mRNAs at the same rate as they regain their susceptibility to Mengo virus. This eliminates a possible cytotoxic action of the interferon preparation.

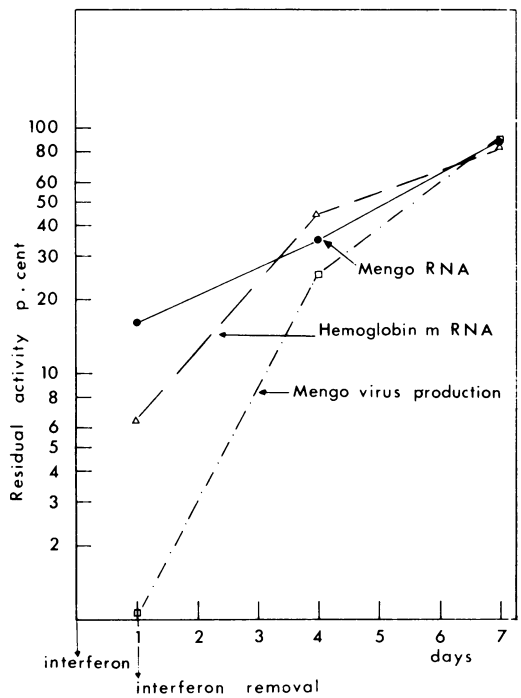

FIG. 4. *Reversion of the antiviral state and reactivation of mRNA translation after removal of interferon. A 1.5-liter Spinner culture was treated with interferon as in Fig. 2, and another identical culture was kept as control. After 24 h, part of each culture was used to measure Mengo virus multiplication (50 ml) and to prepare S-10 extracts (450 ml) for measurement of mRNA translation as described in methods. The remaining cells were centrifuged, washed, and resuspended at 10^6 cells/ml in fresh medium without interferon. The same procedure was repeated at day 4 and day 7, the medium being renewed each time. Results are expressed in percent of the activities obtained with the control culture. The values for the control culture at day 1, 4, and 7 were respectively, for Mengo virus multiplication: 4.5×10^9, 1.6×10^9, and 3×10^9 PFU/ml; for Mengo RNA translation: 1,850, 1,445, and 2,005 counts/min of ^{14}C-leucine; for hemoglobin mRNA translation: 925, 930, and 835 counts/min of ^{14}C-leucine.*

Effects of interferon of different origins. Interferon of several sources were compared. Only murine interferon produced the inactivation of Mengo RNA translation in L cells; human interferon produced by NDV on lymphocytes had no effect (6). A preparation of mouse interferon obtained by intracerebral inoculation of West Nile virus produced inhibitory effects on translation and induced the antiviral state on L cells as does NDV-L-cell interferon. When the brain interferon preparation was first preincubated with an antiserum against NDV-L-cell interferon, both the in vivo and in vitro effects were blocked (Table 2). The yield of Mengo virus and the values obtained in the translation of Mengo RNA and poly U were near that of the control; a significant, although smaller, protection was seen for hemoglobin mRNA translation. Since both preparations of interferon have only in common the biologically active antiviral agent for murine species, this experiment excludes again the role of an unspecific contaminant in the induction of the translation deficiency.

Biochemical characterization of the translational block induced by interferon. The loss of translation activity could be explained by an enhanced ribonuclease activity in extracts from interferon-treated L cells. To check this hypothesis, mixtures of S-10 extracts from control and interferon-treated L cells were incubated as shown in Table 3. Translation of Mengo RNA was compared in each S-10 alone and in various combinations. The results of mixing experiments were found to be similar to the values calculated by simple addition of the activity of each extract. If the interferon-treated S-10 contained an RNase, the activity of the control extract would have been strongly inhibited. This does not, however, exclude an inhibitor which would be strongly bound to ribosomes, for example.

Attempts were made to localize the translation defect in the subcellular components.

Crude ribosomes were obtained from the S-10 by centrifugation at 150,000 × *g* for 3 h. These ribosomes were active for the translation of added Mengo RNA providing that the high-speed cell supernatant fluid and ribosome wash proteins (19) were added. Table 4 shows that the block is in the ribosome fraction: crude ribosomes from interferon-treated cells were much less active than those from untreated L cells. A small reduction in the activity of the supernatant fluid toward Mengo RNA translation (up to 30% in one experiment) may be observed. The crude ribosomes can be separated, by treatment with a 0.5 M KCl-containing buffer, into washed ribosomes and ribosome-associated proteins (ribosomal wash fraction). Washed ribosomes are inactive, but Mengo RNA translation can be partially recovered by adding the ribosomal wash. Table 5 shows that the purified ribosomes from interferon-treated cells are as active as those from control cells. The defect appears to be in the fraction washed-off the ribosomes. This fraction in fact inhibits Mengo RNA translation when added to active S-10 extracts. Table 6 shows the effect of ribosomal wash from normal and interferon-treated L cells on a Krebs ascites cell extract. Ribosomal wash from untreated L cells stimulates, whereas that from interferon-treated cells inhibits, Mengo RNA translation in these extracts. The inhibitory activity of the ribosomal wash increases with the dose of interferon used, with a good correlation to the decrease in the in vivo Mengo virus multiplication. It is important to note that this inhibition is observed even with ribosomes from a cell which itself is quite resistant to interferon treatment. Similar inhibitions are observed if the ribosomal wash is added to extracts of untreated L cells.

The nature of this ribosome-associated material and the precise reaction which is affected remains to be determined. Preliminary experiments using sparsomycin indicates that some step in peptide chain elongation is inhibited.

TABLE 3. *Effect of mixing control and interferon-treated extracts*

Expt	S-10 extract from control L cells (μliters)	S-10 extract from interferon-treated[a] L cells (μliters)	Mengo RNA translation (^{14}C-leucine incorporation; counts/min)	Calculated results
1	30		2,187	
		10	124	
	30	10	2,367	2,311
2	20		1,412	
		20	319	
	20	20	1,524	1,731

[a] Interferon treatment with 300 units/ml for 18 h.

DISCUSSION

The present experiments and those previously published (6) provide the first demonstration that interferon induces a block in noninfected cells in the translation of exogenous natural mRNAs. This effect is closely related to the development of the antiviral state. Several

TABLE 4. *Effect of interferon treatment on crude ribosomes and cell supernatant fluid*

Expt	Crude ribosomes[a]	Supernatant fluid[a]	Translation of:[b]		
			Mengo RNA	Hemoglobin mRNA	Poly U
1	Untreated	Untreated	855 (100)	575 (100)	4,835 (100)
	Untreated	Interferon	585 (68)	550 (96)	4,675 (97)
	Interferon	Untreated	250 (29)	280 (49)	4,010 (82)
	Interferon	Interferon	270 (31)	265 (46)	3,835 (78)
2	Untreated	Untreated	4,200 (100)	2,875 (100)	3,505 (100)
	Untreated	Interferon	3,575 (85)	3,050 (106)	3,630 (103)
	Interferon	Untreated	490 (11)	590 (20)	1,675 (48)
	Interferon	Interferon	265 (6)	425 (14)	1,795 (52)

[a] Preparation of crude ribosomes and high-speed supernatant fluid from L cells, normal and treated with interferon (300 units/ml, 18 h), is described in Materials and Methods.

[b] Cell-free system as in Materials and Methods with 1 A_{260} unit of crude ribosomes. Ribosomal wash from rabbit reticulocytes was added; in its absence, the incorporation with this reconstituted system was only 10% of the control. (An important loss of activity is observed during fractionation of the S-10 extract and part of the activity is restituted by adding reticulocyte or L-cell ribosomal wash.) Incorporation of ^{14}C-leucine (for Mengo RNA and hemoglobin mRNA) and ^{14}C-phenylalanine (for poly U) was measured in counts per minute.

authors (3, 25) have in the past suggested that interferon acts by blocking translation of viral genes, but until now the lack of active cell-free systems capable of translating mammalian mRNAs made it impossible to demonstrate this effect directly. Since several cell-free systems from mouse L cells, a cell very sensitive to interferon treatment, have been described in the past year (4, 10, 20), we chose this biological system which should be very useful in elucidating the mechanism of action of interferon. The hybrid chick-mouse system described by Kerr (16) is much less active for mRNAs translation. More recently Friedman et al. (9) have also used L cells to study the effect of interferon on EMC translation. However, under their conditions, a block in mRNA translation was obtained only in virus-infected cells (vaccinia or EMC) and the block disappeared upon preincubation of the extracts. This situation contrasts with our results which are obtained in uninfected L cells and in which the translational block is conserved after preincubation of the extracts. These differences may be due to the fact that Friedman et al. (9) used very small amounts of interferon. Indeed, we have also observed that infection with Mengo virus may increase the translational block produced by interferon (unpublished observations). Since, however, viruses as vaccinia or EMC have direct effects on protein synthesis, it is very difficult to determine the part of the effect due to the virus and that due to interferon treatment. In addition, it has been reported (2) that vaccinia virus produces, in interferon-treated cells, cytopathic effects which do not appear in normally infected cells. The same problem is met in the in vivo experiments of Metz and Esteban (26). In both works, the fate of cellular protein synthesis could not be determined since the viral infec-

TABLE 5. *Effect of interferon treatment on ribosomes and ribosome wash fraction*

L-cell ribosomes[a]	L-cell ribosome wash fraction[a]	Mengo RNA translation[b] (^{14}C-leucine incorporation)	
		Counts/min	Δ
Purified ribosomes, untreated	Untreated	940	750 (100)
	Interferon	615	450 (60)
	None	190	
Purified ribosomes, interferon[c]	Untreated	840	760 (102)
	Interferon	450	370 (48)
	None	80	

[a] Preparation of L-cell subfractions as described in Materials and Methods.

[b] Conditions as in Materials and Methods with 0.7 A_{260} unit of high-salt purified ribosomes, 0.15 A_{280} unit of ribosomal wash fraction, and 0.15 A_{280} unit of high-speed supernatant fluid (after 90% ammonium sulfate precipitation). Percent residual activity is given in parentheses.

[c] L cells treated with 300 units of interferon per ml for 18 h. The activity of the crude ribosomes, before the high-salt wash, was 45% of that of control ribosomes.

TABLE 6. *Effect of ribosomal wash from normal and interferon treated L cells on Mengo RNA translation in Krebs ascites cell extracts*

Expt	S-10 extract	Ribosomal wash added	Mengo RNA translation[a] (^{14}C-leucine incorporation)		Mengo virus multiplication[b] (% of control)
			Counts/min	%	
1	Krebs ascites	None	4,705		
		Untreated L cells	5,470	100	
		L cells treated by interferon (320 units/ml)	1,460	27	
2	Krebs ascites	None	2,335		
		Untreated cells	4,300	100	100
		L cells treated by interferon:			
		6.4 units/ml	2,400	56	44
		64.0 units/ml	1,230	29	4
		640.0 units/ml	720	17	0

[a] Conditions as in Materials and Methods with 0.3 A_{260} units of Krebs ascites cells S-10 and where indicated, 0.07 A_{280} units of ribosomal wash from normal L cells or 18 h after treatment with the indicated dose of interferon. A background minus mRNA of 175 counts/min was substracted.

[b] Mengo virus multiplication was measured by the plaque reduction assay as described in Materials and Methods in which the 100% value corresponds to 50 PFU.

tion produces a shut-off of host protein synthesis.

Our results suggest that interferon produces a block in the translation of both a viral mRNA as Mengo RNA, and of a cellular mRNA as 9S hemoglobin mRNA. This may be unexpected since interferon is considered to exert a differential effect on viral genes (30). Two alternative possibilities are suggested: (i) Both hemoglobin mRNA and Mengo RNA are foreign to the L cells and are exogenously added to the cell extracts, whereas true cellular mRNAs are processed from the nucleus by a yet undefined mechanism (14). The interferon-induced block may discriminate between such endogenously processed mRNAs and exogenous mRNAs. Indeed, endogenous protein synthesis activity of the extracts is almost unaffected by interferon treatment, in conditions under which Mengo RNA translation is completely abolished. Poly U is an exogenous mRNA, but its translation does not use the initiation mechanism characteristic of natural mRNAs. It is only slightly affected and only at high doses of interferon. Further studies of the mechanism of the interferon-induced block may reveal the basis of the discriminatory effect of interferon between endogenous and exogenous mRNAs. (ii) Alternatively, it is possible that interferon has an effect also on some cellular mRNA translation. Many authors have reported inhibitions of cellular functions by interferon treatment (11, 15, 28) even with well purified preparations of interferon. Johnson et al. (15) observed a partial inhibition of protein synthesis in vivo in monolayer cultures of L cells pretreated with purified interferon; in an independent work, some of us have confirmed these observations (I. Martelli, R. Falcoff, and E. Falcoff, in preparation). These effects become clear, however, only at concentrations of interferon much higher than those needed for the antiviral effect. These doses may nevertheless not be excessive, since Haase et al. (12) have reported that inhibition of virus-induced cytopathic effects requires 1,000-fold more interferon than the antiviral effect.

In this alternative, interferon treatment may affect the translation of certain mRNAs more than that of others, and the sensitivity of different mRNAs should be compared. (We have observed in several experiments that at low doses of interferon, Mengo RNA translation is quantitatively more inhibited than that of hemoglobin mRNA (see for example Table 1). This shows at least that the blocking mechanism does not affect all mRNAs identically.) Would interferon have only such a "relative" discriminatory activity, the more dramatic effect of interferon on viral multiplication than on cellular metabolism may be explained by taking into account that reduction in the production of an essential component of virus multiplication (an enzyme for example) may decrease by several orders of magnitude the final virus yield.

Recent reports by several authors have indicated that interferon acts on viral transcription (1, 23, 24, 27). Interferon may indeed induce in cells pleiotropic effects on both RNA and protein synthesis. But, whatever the mechanism of interferon action may be, the effects on mRNA translation reported here in cell-free systems from cells pretreated with interferon will be very useful to study the control of protein synthesis in mammalian cells.

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