Interferon Treatment of Ehrlich Ascites Tumor Cells: Effects on Exogenous mRNA Translation and tRNA Inactivation in the Cell Extract

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We reported earlier that in cell extracts that were prepared from interferon-treated Ehrlich ascites tumor cells and preincubated and passed through Sephadex G-25 (S30$_{INT}$), the translation of exogenous mRNA (viral and host) was impaired and the impairment could be overcome to a large extent by adding a crude tRNA preparation from Ehrlich ascites tumor cells but not from *Escherichia coli*. We find now that the rate of inactivation of some tRNA's (especially those specific for leucine, lysine, and serine) but not those of many others is faster in S30$_{INT}$ than in corresponding extracts from control cells. This increased rate of tRNA inactivation may perhaps account for the need for added RNA to overcome at least partially the impairment of translation in S30$_{INT}$. The relationship of the increased rate of tRNA inactivation to the antiviral effect of interferon is unclear. So far no significant difference has been detected in the amount of tRNA needed to overcome the impairment of encephalomyocarditis virus RNA translation in S30$_{INT}$ between tRNA from interferon-treated cells and tRNA from control cells. Furthermore, no difference was found in the rate of inactivation in S30$_{INT}$ between leucine-specific tRNA's from interferon-treated and from control cells. tRNA's specific for leucine and lysine were not inactivated (unless very slowly) during incubation under our conditions in an extract from interferon-treated (or from control) cells unless the extract had been passed through Sephadex G-25 or dialyzed. The translation of exogenous mRNA was, however, impaired in an extract from interferon-treated cells that had not been passed through Sephadex G-25. This impairment was apparently not overcome by added tRNA.

Interferons are macromolecules (presumably glycoproteins) that are formed in various animal cells upon viral infection or some other stimuli. They are released from the producing cells, interact with other cells, and make these inefficient in supporting the multiplication of a large variety of animal viruses. The nature of the block in virus replication in interferon-treated cells is under investigation (9). In the case of reovirus, the adsorption and penetration into the cell and the uncoating of the virus are apparently not inhibited in interferon-treated cells, whereas the accumulation of virus-specific proteins and RNA is (14, 17, 43; M. E. Wiebe and W. K. Joklik, Abstr. Am. Soc. Microbiol. 1973, V97, p. 210; R. Galster et al., manuscript in preparation). We do not know whether the block in viral protein accumulation is a consequence of an impairment of viral RNA metabolism (e.g., of transcription, processing, or turnover) or of the translation of viral mRNA or both.

We have been studying the effect of treating cells (L-929 mouse fibroblasts and Ehrlich ascites tumor [EAT] cells) with a partially purified mouse interferon preparation on the capacity of their extracts to translate exogenous and endogous mRNA's (17, 18). The extracts from interferon-treated and control cells were usually "preincubated" and filtered through Sephadex G-25 (S30$_{INT}$ and S30$_C$) to decrease protein synthesis resulting from the translation of endogenous mRNA. Under our conditions the translation of viral and cellular mRNA's was less in S30$_{INT}$ than in S30$_C$. In S30$_C$ the translation of encephalomyocarditis (EMC) virus RNA proceeded at a linear rate for over 90 min, whereas in S30$_{INT}$ the translation proceeded usually at a somewhat lower rate than in S30$_C$ for about 30 min and then ceased. The impairment of translation in S30$_{INT}$ was apparently due to the action of one (or more) inhibitor loosely bound to the ribosomes (18). Some time ago it was established that the translation of

exogenous mRNA in S30$_{INT}$ (which ceased after about 30 min of incubation) could be restored by the addition of crude tRNA (7, 19). (We established recently that a preincubated Sephadex-treated extract from EAT cells that have been treated with the interferon inducer poly-inosinic-polycytidylic acid was also impaired in its capacity to translate EMC RNA. This impairment could also be overcome partially by the addition of crude tRNA [B. Lebleu, unpublished data].) Further characteristics of this tRNA effect in S30$_{INT}$ are described in this communication. Data are also presented on the competition for translation between a viral and a cellular mRNA in S30$_C$ and S30$_{INT}$ and on the difference in the extent of inhibition of translation between these mRNA's in S30$_{INT}$. Finally, characteristics of an inhibition of exogenous mRNA translation in untreated (i.e., not preincubated and not Sephadex treated) extracts from interferon-treated cells are described.

MATERIALS AND METHODS

Chemicals. Radioactive amino acids of the highest specific activity available were obtained from New England Nuclear Corp., Sephadex G-25 was from Pharmacia, and BioGel P-6, 50 to 100 mesh, was from Bio-Rad.

Cells. EAT cells (42) were grown in suspension cultures at 37 C in Eagle minimal essential medium (Grand Island Biological) supplemented with 7% fetal calf serum.

Interferon. A partially purified mouse interferon preparation was obtained by infecting EAT cells with Newcastle disease virus and purifying the interferon according to the procedure of H. Weideli et al. (in preparation). The preparation had a specific activity of 2×10^7 NIH mouse reference standard units per mg of protein. This corresponds to 2×10^6 vesicular stomatitis virus plaque reduction units per mg of protein. The interferon units throughout the paper are vesicular stomatitis virus plaque reduction units (43).

Treatment of cells with interferon. Growing suspension cultures of EAT cells were diluted to a density of 4×10^5 cells/ml and treated (unless otherwise specified) with 60 U of interferon per ml for 18 h.

Preparation of cell extracts (S30). S30 from control cells (S30$_C$) and from interferon-treated cells (S30$_{INT}$) was prepared as described before (18). The following types of extracts were used (both from interferon-treated and control cells) as indicated in the text: (i) preincubated, Sephadex-treated extracts, which were incubated at 37 C for 45 min and passed through a column of Sephadex G-25; these procedures were performed to lower endogenous protein synthesis; (ii) not preincubated, but Sephadex-treated extracts; (iii) not preincubated, not Sephadex-treated extracts.

mRNA. EMC virus and mengo virus were grown, and viral RNA was extracted according to Aviv et al.

(1). Mouse globin mRNA was prepared according to Soreq et al. (41).

tRNA. tRNA was purified according to the methods of Aviv et al. (1) from interferon-treated and from control EAT cells.

Aminoacyl-tRNA synthetases. A partially purified preparation free of tRNA was prepared as follows. EAT cells grown in suspension culture were harvested, washed in isotonic buffer (35 mM Tris-chloride [pH 7.5], 146 mM NaCl, 12 mM glucose), suspended in 2 volumes of hypotonic buffer (10 mM Tris-chloride [pH 7.5], 15 mM KCl, 1.5 mM magnesium acetate, 6 mM mercaptoethanol, 1 mM dithiothreitol), and disintegrated in a Dounce homogenizer. The resulting homogenate was supplemented with 1/10 volume of a buffer (200 mM Tris-chloride [pH 7.5], 800 mM KCl, 40 mM magnesium acetate, 60 mM 2-mercaptoethanol, and 10 mM dithiothreitol) and centrifuged at $20,000 \times g$ for 20 min. The supernatant fraction was diluted with an equal volume of water and put on a DEAE-cellulose column. (This column had been equilibrated earlier with buffer A [10 mM Tris-chloride, pH 7.5, 1 mM magnesium acetate, and 10 mM 2-mercaptoethanol].) Thereafter the column was first washed with buffer A and then (to elute proteins) with the same buffer containing 0.25 M KCl. The protein-rich fractions were pooled, and the proteins precipitating between 40 and 70% (wt/vol) ammonium sulfate saturation were dissolved in a minimal volume of buffer A. The solution was passed through a column of Sephadex G-25 to remove residual ammonium sulfate. The excluded fraction (containing the aminoacyl-tRNA synthetase) was stored in 50% glycerol at -25 C.

Rabbit reticulocyte ribosomal wash fluid. Rabbit reticulocyte ribosomal wash fluid, serving as a source for initiation factors for globin mRNA translation, was prepared according to Shafritz and Anderson (38).

Antisera. An antiserum against EMC virion proteins was prepared by injecting EMC virion proteins into rabbits, following the procedure of Graziadei et al. (16). An antiserum against mouse globin was a generous gift from Denise Russo (Department of Biology, Yale University).

EMC RNA translation. The reaction mixture contained 25 mM Tris-chloride (pH 7.5), 6 mM 2-mercaptoethanol, 5 mM phosphoenolpyruvate, 1 mM ATP, 0.6 mM CTP, 0.2 mM GTP, 2 mg of creatine phosphate per ml, 0.3 mg of creatine phosphokinase per ml, 19 unlabeled amino acids (except valine), 50 μM each, 10 μM [^{3}H]valine (specific activity, 7 Ci/mmol), and 15 A_{260} units of preincubated, Sephadex-treated S30 per ml. EMC RNA (30 μg/ml) was added where indicated. The concentrations of magnesium acetate and KCl were as specified in the table and figure legends. In not preincubated and not Sephadex-treated S30, the ion concentrations of the cell extracts were taken as being equal to those of extracts that had been passed through Sephadex G-25. The reaction mixtures were incubated at 30 C. The amount of total protein synthesis was determined by precipitation with hot trichloroacetic acid according to the procedure of Bollum (5). The amount of

immunoprecipitable protein was determined essentially according to Gupta et al. (17).

tRNA charging. (i) In S30. Endogenous tRNA's were aminoacylated under the conditions of EMC RNA translation except that: no unlabeled amino acids were added; the concentration of the labeled amino acid (methionine was labeled with [^{35}S], all the other amino acid with [^{3}H]) was 5 to 10 μM; and 200 μM sparsomycin was added to block protein synthesis (39). Aminoacyl-tRNA was determined by the procedure of Bollum (5).

(ii) By partially purified aminoacyl-tRNA synthetase. Total RNA from S30 (extracted by phenol treatment, alcohol precipitation, and dialysis against water) or tRNA purified from EAT cells according to Aviv et al. (1) was aminoacylated by incubation at 30 C for 20 min in a reaction mixture containing 100 mM Tris-chloride (pH 7.5), 25 mM KCl, 5 mM magnesium acetate, 5 mM 2-mercaptoethanol, 10 mM ATP, 0.6 mM CTP, 25 μM ^{3}H-labeled amino acid, 1 to 5 A_{260} units of tRNA per ml, and 0.2 ml of aminoacyl-tRNA synthetase preparation per ml of reaction mixture.

RESULTS

Competition between mouse globin mRNA and mengovirus RNA for translation in cell-free systems from interferon-treated and control cells. In the past we tested the effect of interferon treatment on the translation of a single type of exogenous mRNA at a time (17, 18). In vivo, however, many types of mRNA's are translated at the same time. To get closer to the in vivo situation, we proceeded to examine the influence of the addition of various amounts of mengovirus RNA on the translation of mouse globin mRNA (see also 22; M. B. Mathews and A. Korner, Biochem J. **109**:23P–24P; J. Lucas-Lenard, personal communication). The extracts of interferon-treated and control cells used in these studies had been preincubated and Sephadex treated and were not supplemented with exogenous tRNA. For determining the amount of each of the two sets of proteins (i.e., mengovirus proteins and globins) synthesized in our extracts, we performed immunoprecipitation with an antiserum against EMC virion proteins (which cross-reacted with mengovirus proteins) and with an antiserum against mouse globins.

The translation of globin mRNA was inhibited by mengovirus RNA in both S30$_C$ and S30$_{INT}$ (Fig. 1). In both cases the inhibition increased with the concentration of mengovirus RNA. At a mengovirus RNA concentration of 0.27 pmol/ml (the saturating concentration for mengovirus protein synthesis) the inhibition was 58% in S30$_C$ and 37% in S30$_{INT}$. Thus mengovirus RNA was an effective competitor for translation in S30$_{INT}$ although to a somewhat

lesser extent than in S30$_C$. This was the case though the translation of mengovirus RNA was much more inhibited upon interferon treatment than that of globin mRNA.

Nudel et al. (31) discovered that the translation of globin mRNA (but not of mengovirus RNA) in Krebs ascites cell extracts can be increased severalfold by the addition of ribosomal wash fluid from rabbit reticulocytes and isolated the factor exerting this activity. We tested the effect of reticulocyte ribosomal wash
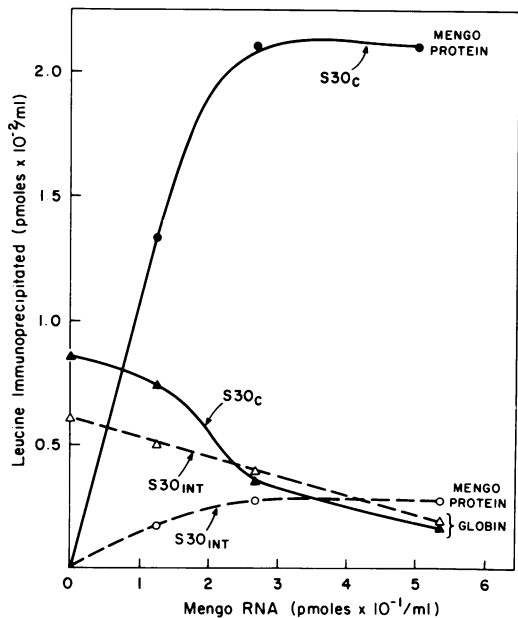

FIG. 1. *Competition between mouse globin mRNA and mengovirus RNA for translation in S30$_C$ and S30$_{INT}$ that had been preincubated and treated with Sephadex. The reaction mixtures contained 3.5 mM magnesium acetate and 95 mM KCl, as well as 200 pmol of mouse globin mRNA per ml and varying amounts of mengovirus RNA as indicated. Other additions were as in the case of EMC RNA translation as described in the text except that [^{3}H]leucine was used as the labeled amino acid. The incubation time was 45 min. The incorporation of [^{3}H]leucine into mengovirus protein and globin were determined by immunoprecipitation with an antiserum against EMC virion proteins (which cross-reacted with mengovirus proteins) and an antiserum against mouse globin. The continuous lines show translation in S30$_C$, and the discontinuous lines show translation in S30$_{INT}$. The amount of labeled material immunoprecipitated from reaction mixtures corresponding in composition to the experimental ones but not containing mRNA was subtracted from the values shown. The subtracted values (in picomoles $\times$ 10^{-2} per milliliter) were: in immunoprecipitates with EMC antiserum, 0.040 in S30$_C$ and 0.067 in S30$_{INT}$; with globin antiserum, 0.034 in S30$_C$ and 0.040 in S30$_{INT}$.*

on the competition for translation between globin mRNA and mengovirus RNA. The data in Table 1 indicate the following.

(i) As shown by Nudel et al. (31), the reticulocyte ribosomal wash boosted globin mRNA translation and did not boost (actually somewhat decreased) mengovirus RNA translation (cf. line 5 with 6 and line 7 with 8). This was the case in both $S30_C$ and $S30_{INT}$, though in the latter the boost of globin mRNA translation was less and the inhibition of mengovirus RNA translation was more pronounced than in the former.

(ii) When both mRNA's were present in the same reaction mixture, the translation of globin mRNA was much more depressed than that of mengovirus RNA in both $S30_C$ and $S30_{INT}$ (cf. line 5 with 9 and line 7 with 11). Thus under these conditions mengovirus RNA is a more efficient competitor for translation than globin mRNA (see also Reference 22). Remarkably, in the presence of added reticulocyte ribosomal wash the difference in the efficiency in competition between the two mRNA's was greatly diminished (cf. lines 5 to 12). These results indicate that, as expected, the outcome of the competition for translation between two mRNA's may be affected by other components (including initiation factors) of the protein-synthesizing system.

(iii) Reticulocyte ribosomal wash did not overcome the inhibition of globin mRNA translation resulting from interferon treatment.

(iv) Mengovirus RNA-directed protein synthesis was much more sensitive to interferon that globin synthesis (see also Fig. 1). These and other data indicate that although the translation of a variety of exogenous mRNA's (also including mRNA's from L cells, reovirus, and EMC virus) is impaired in preincubated Sephadex-treated extracts from interferon-treated cells, the extent of inhibition may vary from mRNA to mRNA.

Several tRNA species are inactivated faster in Sephadex-treated extracts from interferon-treated cells than in corresponding extracts from control cells. We reported earlier that the translation of exogenous mRNA in preincubated, Sephadex-treated $S30_{INT}$ ceased after 30 min and could be restored by the addition of crude tRNA (19; see also 7). We also noted that the capacity of $S30_{INT}$ to attach leucine to endogenous tRNA was lower than that of $S30_C$ and that this capacity of $S30_{INT}$ diminished even more after further incubation (19).

Recently we examined the time course of endogenous tRNA charging with all 20 amino acids in $S30_C$ and $S30_{INT}$ (Fig. 2). In these experiments not preincubated, Sephadex-treated $S30_C$ and $S30_{INT}$ were incubated in reaction mixtures of the same composition as

TABLE 1. *Competition for translation between mouse globin mRNA and mengovirus RNA in preincubated, Sephadex-treated extracts from interferon-treated and from control cells; effect of reticulocyte ribosomal wash on the competition*[a]

No.	Addition to the reaction mixture		Immunoprecipitation with antiserum against:	Leucine incorporated into protein (pmol/ml) in:				Percent inhibition of net mRNA-directed leucine incorporation $\left[\dfrac{(a) - (b)}{(a)} \times 100\right]$
	mRNA	Reticulocyte ribosomal wash		$S30_C$ total	Net (a)	$S30_{INT}$ total	Net (b)	
1	—	—	Globin	3.3	—	9.6	—	
2	—	+	Globin	4.3	—	3.6	—	
3	—	—	EMC	3.0	—	6.3	—	
4	—	+	EMC	2.9	—	3.8	—	
5	Globin	—	Globin		242		135	44
6	Globin	+	Globin		624		251	60
7	Mengovirus	—	EMC		420		52	88
8	Mengovirus	+	EMC		344		18	95
9	Globin and mengovirus	—	Globin		91		68	25
10	Globin and mengovirus	+	Globin		427		220	48
11	Globin and mengovirus	—	EMC		339		69	80
12	Globin and mengovirus	+	EMC		267		37	86

[a] The reaction mixtures contained, if so indicated, mouse globin mRNA (166 pmol/ml), mengovirus RNA (121 pmol/ml), and reticulocyte ribosomal wash (1.2 mg/ml). The other conditions were as described in the legend to Fig. 1. The net amount of leucine incorporated into protein in reaction mixtures containing added mRNA is due to incorporation caused by the addition of mRNA. The background (incorporation in reaction mixtures with no mRNA added) has been subtracted.
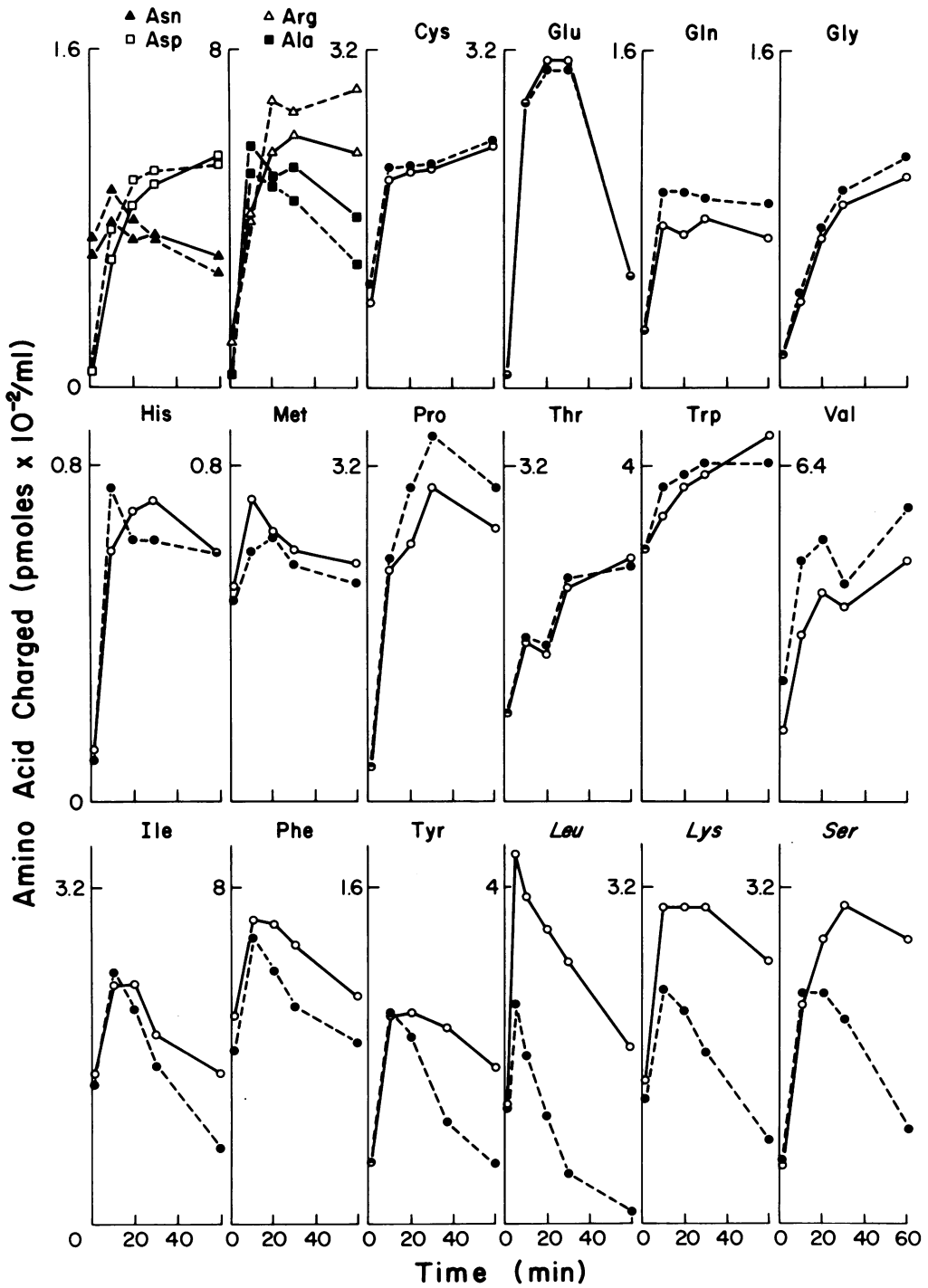
FIG. 2. *Time course of aminoacylation of endogenous tRNA in $S30_C$ and $S30_{INT}$ that had not been preincubated but were treated with Sephadex. The reaction mixtures contained 5 mM magnesium acetate, 80 mM KCl, and 17 A_{260} units of not preincubated but Sephadex-treated $S30_C$ and $S30_{INT}$ per ml of reaction mixture. For further details see the relevant section in the text. Continuous lines show the kinetics of aminoacylation in $S30_C$, and discontinuous lines show those in $S30_{INT}$.*

used for the translation of exogenous mRNA except that only one labeled amino acid was added at a time and protein synthesis was blocked by the inhibitor sparsomycin. (Analogous experiments were performed with preincubated, Sephadex-treated extracts. The results obtained were similar to those obtained with the not preincubated, Sephadex-treated extracts [data not shown].) The amount of cold acid-insoluble product (consisting of aminoacyl-tRNA and perhaps some of its cleavage products, i.e., aminoacyl-oligonucleotides) was determined in aliquots taken at the times indicated in the figure. It can be seen that the time course of the aminoacylation was most strongly affected by interferon treatment in the cases of leucine, lysine, and serine-specific tRNA's.

In $S30_{INT}$ the charging with these three amino acids was less and the amount of aminoacyl-tRNA decreased much faster than in the corresponding $S30_C$. There was a less pronounced effect of interferon treatment in the cases of isoleucine-, phenylalanine-, and tyrosine-specific tRNA's. As far as the charging of all other amino acids is concerned, the effect of interferon treatment was not obvious. (This was the case although the amount of some of these, apparently unaffected, aminoacyl-tRNA's [e.g., glutamyl-tRNA] was decreasing fast in both $S30_C$ and $S30_{INT}$ during incubation.)

Most of the further studies on the effect of interferon treatment on the time course of tRNA charging were restricted to the case of leucine. The curves in Fig. 3A reveal that the agent(s) responsible for the fact that the amount of leucyl-tRNA decreased faster in $S30_{INT}$ than in $S30_C$ was dominant. They clearly indicate also that the amount of leucyl-tRNA decreased even in $S30_C$, only more slowly than in $S30_{INT}$. Moreover, the curves reveal that aminoacyl-tRNA synthetase addition did not restore the charging of tRNA with leucine in a reaction mixture in which the amount of leucyl-tRNA had decreased during earlier incubation. When, however, tRNA (instead of aminoacyl-tRNA synthetase) was added to the reaction mixtures, this became acylated with leucine rapidly both in the case of $S30_C$ and $S30_{INT}$ (Fig. 3B). These results indicate that the decrease in leucyl-tRNA in $S30_{INT}$ and in $S30_C$ was due to the inactivation of leucine-specific tRNA and not of leucyl-tRNA synthetase.

It was conceivable that the fact that less leucine could be charged to tRNA in $S30_{INT}$ than in $S30_C$ even initially could have been caused by the faster inactivation of leucine-specific tRNA in $S30_{INT}$ than in $S30_C$ during the initial charging. The results in Table 2 show

that this was indeed the case. For this experiment tRNA was isolated from extracts that had not been preincubated but were passed through Sephadex at 4 C. The data reveal that in these extracts the leucine acceptance and valine acceptance were not much affected by interferon treatment. Other aliquots from the same not preincubated but Sephadex-treated $S30_C$ and $S30_{INT}$ were incubated at 37 C for 60 min and tRNA was isolated from both. The amino acid acceptance of these two tRNA preparations (lines 3 and 4) was very different: (i) leucine acceptance decreased upon incubation by 49% in $S30_C$ and by much more, 96%, in $S30_{INT}$; and (ii) there was much less difference in the decrease in valine acceptance upon incubation between $S30_C$ and $S30_{INT}$ (33% in $S30_C$ and 52% in $S30_{INT}$). Also, valine acceptance decreased much less during incubation in $S30_{INT}$ than leucine acceptance.

Attempts to relate the increased rate of tRNA inactivation in Sephadex-treated $S30_{INT}$ to the antiviral effects of interferon. Finding that tRNA preparations isolated from Sephadex-treated but not incubated extracts from interferon-treated and control cells had similar (if not identical) capacities for accepting leucine prompted us to compare the leucine acceptance of tRNA preparations isolated directly from interferon-treated and control cells. The data in Table 2 reveal that these were the same, within the limits of the accuracy of the assay. Moreover we found no significant difference in leucine acceptance between tRNA preparations isolated from EMC virus-infected cells and tRNA preparations isolated from interferon-treated and EMC virus-infected cells. The same was found to be the case of valine acceptance. These results indicate that the increased rate of tRNA inactivation caused by the treatment of cells with interferon is only manifested in Sephadex-treated and incubated cell extracts.

We also tested whether the RNA species that can restore EMC RNA translation in $S30_{INT}$ (which had ceased after a 30-min incubation) were present in smaller amounts in interferon-treated cells than in control cells. A comparison of the activity of tRNA preparations isolated from interferon-treated and from control cells in boosting the translation of EMC RNA in $S30_{INT}$ revealed no such difference (Fig. 4); each of the two tRNA preparations increased the translation over fivefold, and the saturating concentrations of the two were indistinguishable. However, tRNA isolated from $S30_{INT}$ that had been Sephadex-treated and incubated for 30 min was less active in increasing the translation of EMC
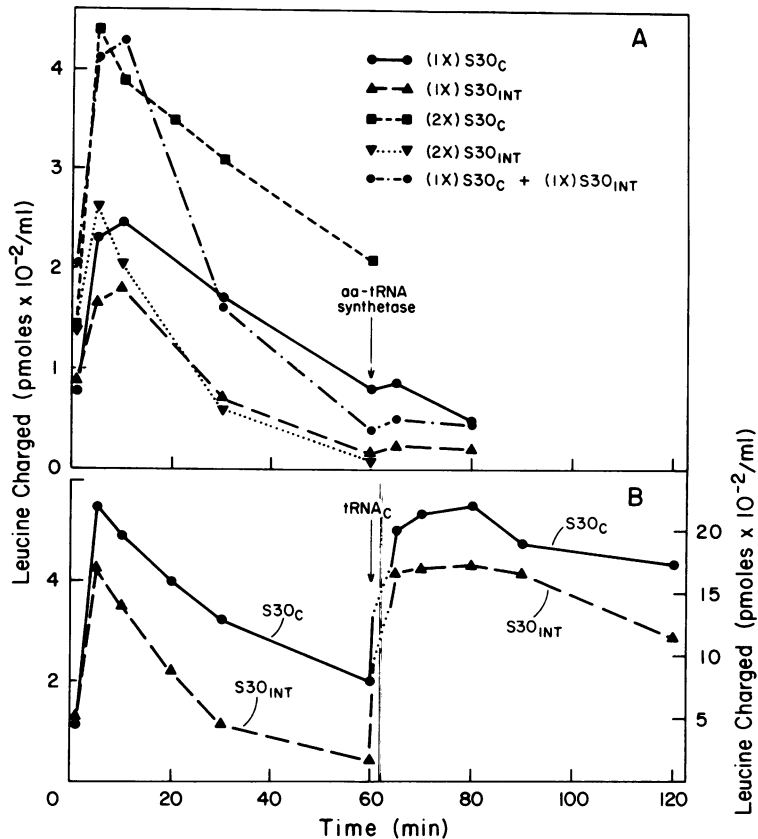
FIG. 3. *Time course of aminoacylation of endogenous tRNA's with leucine in S30$_C$ and S30$_{INT}$ which had not been preincubated but were treated with Sephadex. (A) Effect of adding aminoacyl-tRNA synthetase and effect of mixing S30$_C$ and S30$_{INT}$. (B) Effect of adding tRNA. The reaction mixtures contained 5 mM magnesium acetate and 120 mM KCl. (A) (1×) S30$_C$: 8.5 A$_{260}$ units of S30$_C$ per ml of reaction mixture; (1×) S30$_{INT}$: 8.5 A$_{260}$ units of S30$_{INT}$ per ml; (2×) S30$_C$: 17 A$_{260}$ units of S30$_C$ per ml; (2×) S30$_{INT}$: 17 A$_{260}$ units of S30$_{INT}$ per ml; and (1×) S30$_C$ + (1×) S30$_{INT}$: 8.5 A$_{260}$ units of S30$_C$ + 8.5 A$_{260}$ units of S30$_{INT}$ per ml. A partially purified preparation (0.16 ml) of aminoacyl-tRNA synthetase was added per ml at 60 min where indicated. (This amount of aminoacyl-tRNA synthetase was sufficient to charge in less than 5 min the amount of leucine-specific tRNA present in the reaction mixture at the beginning of the incubation.) (B) Continuous lines indicate aminoacylation with leucine in S30$_C$ (17 A$_{260}$ units/ml) and discontinuous lines indicate the same in S30$_{INT}$ (17 A$_{260}$ units/ml). At 60 min 11.2 A$_{260}$ units of unfractionated EAT tRNA was added per ml. For further details see the relevant sections in the text.*

RNA in S30$_{INT}$ than tRNA isolated from S30$_{INT}$ that had been Sephadex treated but not incubated (data not shown). These results are consistent with the possibility that incubation of Sephadex-treated S30$_{INT}$ results in the inactivation of RNA species involved in translation.

Inactivation of leucine-specific tRNA is very slow during incubation of not Sephadex-treated extracts from interferon-treated or control cells. During our studies on leucine-specific tRNA inactivation in cell extracts, we compared the time course of leucine charging to tRNA in Sephadex-treated and not Sephadex-treated extracts from interferon-treated and control cells (Fig. 5). The tests were run at two concentrations of S30. As shown already in Fig.

2 and 3, leucine tRNA was inactivated faster in Sephadex-treated S30$_{INT}$ than in corresponding S30$_C$; furthermore, the rate of inactivation increased with the S30 concentration, especially in S30$_C$. Unexpectedly, leucine tRNA was inactivated much more slowly (if at all) in not Sephadex-treated S30$_{INT}$ and S30$_C$ under our conditions. Similar results were obtained in studies with lysine tRNA (data not shown). These findings seem to indicate that the fast inactivation of these tRNA species in cell extracts is only triggered by passing the extract through Sephadex G-25. (Passing the extract through BioGel P-6, 50 to 100 mesh, had the same effect; data not shown.)

We attempted to find conditions under which

TABLE 2. *Leucine and valine acceptance by tRNA's isolated from interferon-treated and control cells and their Sephadex-treated, not incubated and Sephadex-treated incubated extracts[a]*

No.	Source of tRNA	Amino acid charged (pmol/ A_{260} unit)	
		Leucine	Valine
1	$S30_C$ not incubated	7.07	3.10
2	$S30_{INT}$ not incubated	6.66	3.10
3	$S30_C$ incubated for 60 min	3.63	2.07
4	$S30_{INT}$ incubated for 60 min	0.25	1.50
5	$Cells_C$	130	45.0
6	$Cells_{INT}$	134	44.3
7	$Cells_C$ infected with EMC virus	120	34.5
8	$Cells_{INT}$ infected with EMC virus	117	33.5

[a] The following RNA samples were charged with leucine and valine by a partially purified aminoacyl-tRNA synthetase: total RNA—extracted from not preincubated but Sephadex-treated $S30_C$ and $S30_{INT}$ that had not been incubated (no. 1 and 2) and that had been incubated (after treatment with Sephadex) at 30 C for 60 min (no. 3 and 4); purified tRNA from: control EAT cells (no. 5); cells treated with 60 U of interferon per ml for 18 h (no. 6); cells that had been infected 4 h earlier with EMC virus at a multiplicity of infection of 10 (no. 7); and cells treated with 60 U of interferon per ml for 18 h and subsequently infected with EMC virus at a multiplicity of infection of 10 for 4 h (no. 8). For further details see the relevant sections in the text.

the inactivation of leucine-specific tRNA in $S30_{INT}$ is slowed down. The agents whose addition to a Sephadex-treated $S30_{INT}$ had such an effect included calcium ions, sodium ions, spermine, spermidine, and ATP; S-adenosylmethionine and S-adenosylhomocysteine had no such effect (G. E. Brown et al., unpublished data).

Translation of exogenous mRNA is impaired even in not Sephadex-treated extracts from interferon-treated cells. This impairment is apparently not overcome by added tRNA. The finding that in not preincubated and not Sephadex-treated extracts from interferon-treated cells tRNA's are quite stable prompted us to test whether translation of exogenous mRNA was inhibited in these conditions. Since in such extracts much endogenous protein synthesis was going on, we had to assay for exogenous mRNA-promoted protein synthesis by immunoprecipitation of the products. The curves in Fig. 6 reveal that EMC RNA was less efficiently translated in such a not preincubated, not Sephadex-treated extract from inter-

feron-treated cells than in a corresponding extract of cells not treated with interferon (see also reference 11). The difference in the rate of translation between the two extracts was manifested from the beginning of the incubation. The curves in Fig. 6A indicate that the impairment of EMC RNA translation in the same $S30_{INT}$ could also be noted by precipitation of the products of the translation by the nonspecific precipitant hot trichloroacetic acid. The addition of tRNA did not seem to overcome the impairment of EMC RNA translation in this $S30_{INT}$ which had not been preincubated and treated with Sephadex (Table 3).

DISCUSSION

tRNA inactivation in an extract from interferon-treated cells that was passed through Sephadex G-25. The data presented reveal that the endogenous amino acid acceptance of some tRNA species decreased faster in $S30_{INT}$ that was passed through a Sephadex G-25 column than in identically treated $S30_C$. This difference in the rate of decrease of endogenous amino acid
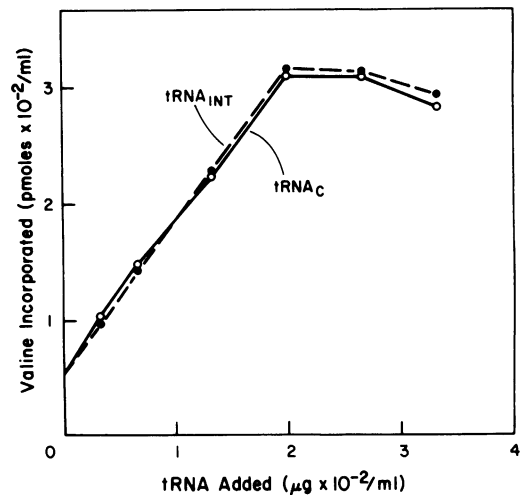

FIG. 4. *Effect of addition of increasing amounts of tRNA isolated from control or interferon-treated EAT cells on the extent of EMC RNA translation in $S30_{INT}$ that had been preincubated and treated with Sephadex. The reaction mixtures contained 5 mM magnesium acetate and 120 mM KCl, as well as 14 A_{260} units of $S30_{INT}$ per ml of reaction mixture, and were incubated for 90 min. Continuous lines show the effect of addition of tRNA isolated from control cells; discontinuous lines show the effect of addition of tRNA isolated from cells treated with 200 U of interferon per ml for 18 h. The addition of tRNA did not boost the incorporation of valine into proteins in reaction mixtures that were not supplemented with EMC RNA. For further details see the relevant section of the text.*
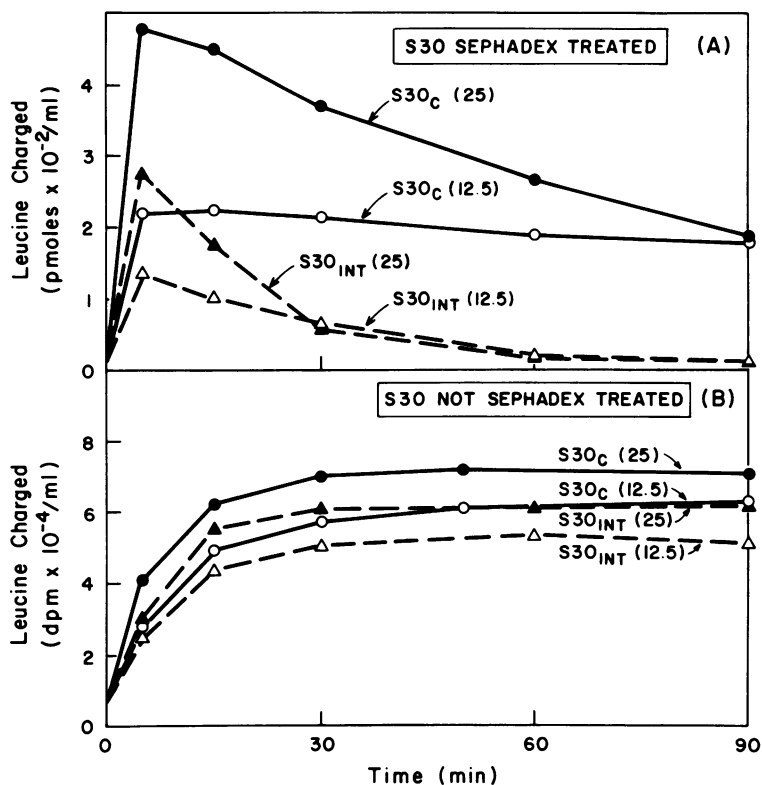
FIG. 5. *Time course of aminoacylation of endogenous tRNA with leucine. (A) In S30$_C$ and S30$_{INT}$ that had been preincubated and treated with Sephadex, and (B) in S30$_C$ and S30$_{INT}$ that had neither been preincubated nor treated with Sephadex. The reaction mixtures contained 5 mM magnesium acetate and 120 mM KCl. For further details see the relevant section in the text (except that in this experiment the sparsomycin concentration was 60 μM, each of the 19 unlabeled amino acids was added at 67 μM concentration, and the S30$_{INT}$ was prepared from cells that had been treated with 300 U of interferon per ml). Continuous lines show leucine charging in S30$_C$, and discontinuous lines show that in S30$_{INT}$. The concentration of S30$_C$ and S30$_{INT}$ (in A$_{260}$ units per milliliter of the reaction mixtures) are indicated in the figure.*

acceptance between S30$_{INT}$ and S30$_C$ was the most pronounced in the cases of leucine, lysine, and serine and less pronounced in the cases of isoleucine, phenylalanine, and tyrosine. No significant difference was detected under our assay conditions in the cases of the other amino acids. In the case of leucine acceptance (the only case examined in detail), the decrease was not due to an impairment of leucyl-tRNA synthetase but to an inactivation of tRNA. Since the acceptance decreased greatly, several (if not all) of the leucine isoacceptors must have been affected. The factor(s) responsible for the faster inactivation in S30$_{INT}$ was dominant.

The products of the inactivation have not yet been identified. tRNA that had been extracted from an incubated, Sephadex-treated S30$_{INT}$ could be charged with less leucine than tRNA extracted from a fresh S30$_{INT}$. Thus the inactivation of tRNA was not due to the binding of an inhibitor that could be removed by phenol treatment. Exogenous leucine tRNA was also inactivated faster in Sephadex-treated S30$_{INT}$ than in corresponding S30$_C$, and moreover there was no difference in the rate of inactivation in S30$_{INT}$ between exogenous tRNA isolated from interferon-treated cells and that from control cells (data not shown). These findings indicate that the difference between Sephadex-treated S30$_{INT}$ and S30$_C$ is not in (or at least not only in) the structure of leucine-specific tRNA's but in some other component(s) of the extracts. It should be emphasized that leucine-specific tRNA was inactivated in Sephadex-treated S30$_C$, too. However, the process was slower in S30$_C$ than in S30$_{INT}$.

Surprisingly, in S30$_{INT}$ (and S30$_C$) that had not been passed through a Sephadex G-25 column, leucine tRNA was stable (or was at least inactivated much more slowly than in corresponding Sephadex-treated extracts). The mode in which Sephadex treatment accelerates
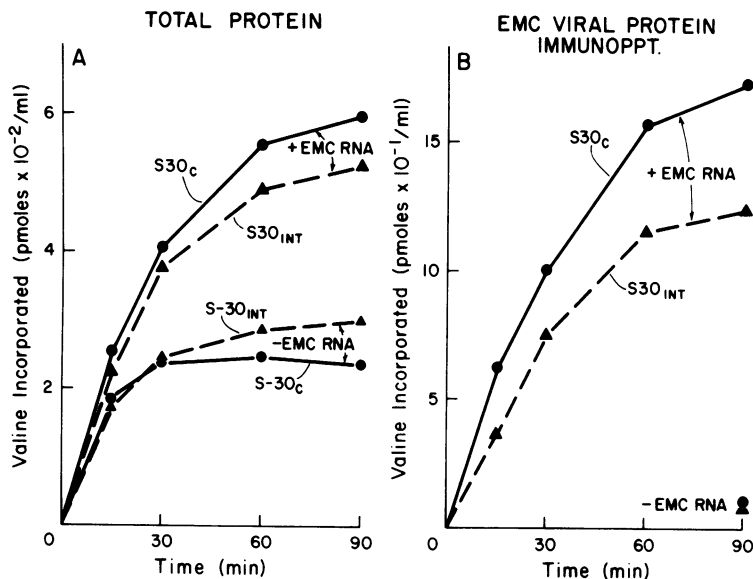
TOTAL PROTEIN

EMC VIRAL PROTEIN IMMUNOPPT.

FIG. 6. *Kinetics of EMC RNA translation in $S30_C$ and $S30_{INT}$ that had neither been preincubated nor treated with Sephadex. The reaction mixtures contained 4 mM magnesium acetate and 100 mM KCl. The incorporation of [^3H]valine into protein was determined by precipitation with hot trichloroacetic acid. Incorporation of [^3H]valine into EMC viral proteins was determined by immunoprecipitation with an antiserum against EMC virion proteins. For calculating the amount of valine incorporated, we assumed that no endogenous valine was present in the S30 extracts. Continuous lines show valine incorporation in $S30_C$, and discontinuous lines show that in $S30_{INT}$. For further details see the relevant section in the text.*

TABLE 3. *Effect of addition of tRNA on protein synthesis in not preincubated, not Sephadex-treated extracts from interferon-treated and control cells[a]*

| Cell extract | RNA added | | Valine incorporated (pmol × 10^{-2}/ml) into: | |
	EMC RNA	tRNA	Total protein	Viral protein
$S30_C$	−	−	2.24	0.06
$S30_C$	−	+	2.33	0.06
$S30_C$	+	−	5.17	2.01
$S30_C$	+	+	4.92	1.64
$S30_{INT}$	−	−	2.67	0.06
$S30_{INT}$	−	+	2.47	0.06
$S30_{INT}$	+	−	3.21	0.96
$S30_{INT}$	+	+	3.26	0.97

[a] The reaction mixtures contained 4 mM magnesium acetate, 100 mM KCl, and, if so indicated, 170 µg of tRNA per ml purified from EAT cells. They were incubated for 45 min. For calculating the amount of valine incorporated, we assumed that no endogenous valine was present in the S30 extracts. For further details see the relevant section in the text.

the rate of inactivation of some tRNA's in $S30_{INT}$ remains to be established. The treatment results in the removal of small molecules from the rest of the S30; the absence of these might perhaps make the tRNA's more prone to

inactivation or might activate agents inactivating tRNA's.

More information will be needed before we can establish whether and how the tRNA inactivation in cell extracts treated with Sephadex is related to the antiviral effect of interferon. The available data, which may be relevant to this question, include the apparent lack of a difference between tRNA extracted from interferon-treated cells and control cells in leucine acceptance as well as in specific activity in restoring the translation of EMC RNA in Sephadex-treated $S30_{INT}$. These findings do not reveal any obvious correlation between the antiviral effect and tRNA inactivation; however, it is too early to rule out the possibility of the existence of such a correlation.

It is conceivable that tRNA inactivation in extracts (from interferon-treated cells) that is triggered by Sephadex treatment might have to be triggered in interferon-treated cells (i.e., in vivo) by infection with a cytotoxic virus such as vaccinia. Indeed, Metz et al. (29) reported that in interferon-treated, vaccinia virus-infected cells, peptide chain elongation was impaired, and Kerr et al. (21) described similar findings in extracts of such cells. It is possible that this impairment might have been the consequence of a scarcity in certain tRNA species. The infection of interferon-treated cells with EMC

virus did not seem to result in tRNA inactivation, at least not during the first 4 h of infection. It is also conceivable that tRNA inactivation may be induced in interferon-treated, virus-infected cells only later in infection when actual cell death is beginning. If this were the case, the tRNA effect would not be the basis for the selectivity of interferon action. However, according to Content et al. (7), the restoration of globin mRNA translation in $S30_{INT}$ requires a RNA species different from that needed for the restoration of mengovirus RNA translation. Thus, according to these data, the tRNA effect might, at least in principle, account for the selectivity of interferon action. It should be emphasized that the RNA species restoring translation have not been identified; though their size is identical to those of tRNA's, it still remains to be established that they are tRNA species (7).

Some selectivity in the impairment of translation between different mRNA's was revealed in our experiments on the competition for translation between mouse globin mRNA and mengovirus RNA in $S30_C$ and in $S30_{INT}$ that had been passed through a Sephadex G-25 column and were not supplemented with exogenous tRNA. The translation of mengovirus RNA in $S30_{INT}$ was more impaired in all cases (percentage of inhibition 80, 86, 88, and 95, average value 87.25) than that of mouse globin mRNA (percentage of inhibition 25, 44, 48, and 60, average value 44.25). The physiological relevance of the apparent selectivity in these conditions remains to be established.

Impairment of the translation of exogenous mRNA in an extract from interferon-treated cells that was not treated with Sephadex. We were prompted to test the effect of interferon treatment on the translation of exogenous mRNA in cell extracts that had not been treated with Sephadex by finding that in such extracts the tRNA's specific for leucine and lysine were quite stable. The results of the tests indicate that EMC RNA translation was impaired in such an extract of interferon-treated cells and that the impairment could not be overcome by the addition of tRNA. The mechanism of the inhibition of translation in these extracts is under investigation. Preliminary results seem to indicate that the inhibition may be due to one or more dominant inhibitors. It should be noted that not preincubated and not Sephadex-treated extracts from interferon-treated L cells were tested earlier by Friedman et al. (11) for their capacity to translate EMC RNA. These authors described a small (about 10%) inhibition of translation in such extracts

from cells that had been treated with 5 U of interferon per ml. (They reported, however, finding a much more extensive inhibition of translation in such extracts from cells that had been treated with interferon and were subsequently infected with vaccinia virus.)

Problems of elucidating the mechanisms of the antiviral actions of interferons. The understanding of the molecular basis of the (possibly more than one) way in which the replication of different viruses is blocked in various types of interferon-treated cells will have to be based on compatible results from experiments with intact cells and their extracts.

Results of experiments with intact cells reveal the selective nature of interferon action. Thus, uninfected cells can be grown for many generations (30, 32) in the presence of interferon at a concentration that blocks the replication of various viruses, and some enzymes and other proteins can be induced in them (24, 44).

Most of the studies with intact cells point to one of two processes as the primary targets of interferon action: (i) the transcription of early viral mRNA (2, 3, 14, 15, 25, 26, 33; M. N. Oxman, personal communication); and (ii) the translation of early viral mRNA (4, 10, 20, 23, 28, 29, 34, 40). The inhibition of viral RNA synthesis in vitro has not yet been reported, only an accelerated degradation (27). There are several reports on the inhibition of viral mRNA translation in vitro (8, 11, 36), some of which have been cited here.

Many eukaryotic cellular and viral mRNA's (including reovirus mRNA) carry a methyl group attached to their 5' terminal guanylate residue (12, 13, 25, 45). This methyl group is apparently required for translation (6). We found recently that the conversion of unmethylated reovirus mRNA into the methylated form is impaired in extracts of interferon-treated cells (37).

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