MECHANISM OF THE INTERFERON-INDUCED BLOCK OF mRNA TRANSLATION IN MOUSE L CELLS: REVERSAL OF THE BLOCK BY TRANSFER RNA

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1. Introduction

Addition of mouse interferon to L cells makes these cells unable to support the multiplication of viruses by blocking viral gene expression [1]. Interferon is also known to reduce the rate of cell division and the intracellular multiplication of some parasites [2]. To determine the changes induced by interferon in the cell, studies of gene expression in cell-free systems have been undertaken in several laboratories. Evidence for an effect of interferon treatment on mRNA translation is now well established in vitro [3–5, 6, 7]. Our work [3] has shown that cell-free extracts from interferon treated mouse L cells have lost their ability to translate into protein exogenously added natural mRNAs as Mengo RNA or Hemoglobin mRNA. This block in translation appears in uninfected L cell extracts, only under conditions in which interferon induces the antiviral state [4]. Endogenous protein synthesis and poly U translation are not significantly inhibited in interferon treated cell-extracts.

We have previously shown that an inhibitor of translation, which appears to be a protein associated with the ribosomes, accumulates in extracts of interferon treated L cells [4, 5]. We show here that this inhibition of translation can be eliminated by the addition of purified fractions of mammalian transfer RNA. The requirement for tRNA in interferon treated cell extracts is caused by the ribosome associated inhibitor. The tRNA species which restore the translation of Mengo RNA and Hemoglobin mRNA in interferon treated cell extracts have been purified and are not identical, suggesting a possible basis for the selective effects of interferon in virus versus host protein synthesis.

2. Materials and methods

2.1. *Cell cultures and preparation of cell-free extracts*

Mouse L cells (strain CCL 1) were grown in suspension cultures in Eagle's MEM (Gibco) supplemented with 8% decomplemented calf serum, 0.3% bactotryptose phosphate, 0.1% methylcellulose, 3.5 g/l glucose, 40 mg/l gentamycin, 10^5 μ/l penicillin and 0.1 g/l streptomycin. Routinely 2 l of culture to a density of 10^6 cells/ml were treated with 150–200 units/ml of mouse interferon. Interferon was prepared from NDV-infected L cells and purified on CM-Sephadex as described previously [4]. Specific activities ranged from $1–5 \times 10^6$ ref. units/mg protein.

After 35 hr at 37°C cells were harvested, washed and a cell-free extract (S10) was prepared as described before [4] except that 20 mM HEPES buffer pH 7.5, was used instead of Tris–HCl buffer. Preincubated S10 was used to translate Mengo virus RNA and rabbit hemoglobin mRNA as before [4]. Fractionation of S10 into ribosomes, ribosomal wash fraction and high speed supernatant has been detailed before [4]. Cell free extracts from Krebs ascites tumor cells and reticulocyte initiation factors were obtained as before [8]. ^{14}C-Radioactive protein hydrolysate (50 μC/μAt C) or ^{35}S-methionine (over 150 mCi/μmole) were purchased from Radiochemical.

2.2. *Preparation and purification of tRNA*

Krebs ascites cells and L cells tRNA were prepared through phenol extraction, 1 M NaCl and isopropanol precipitation as outlined by Aviv et al. [9]. Chromatography on BD-cellulose was carried out according to Petrissant et al. [10]. Purified rabbit liver $tRNA_{metF}$ after BD cellulose and DEAE-Sephadex was a gift from Dr. G. Petrissant. Total rabbit liver tRNA was a gift from Dr. D. Hatfield and *E. coli* B tRNA was from Schwarz Co. Purified *E. coli* methionyl tRNA synthetase was a generous gift from Dr. J. P. Waller. RNA-free *E. coli* total supernatant enzymes was prepared to charge tRNA as described by Muench and Berg [11] and for formylation [12]. MDMP was a generous personal gift from Dr. Baxter.

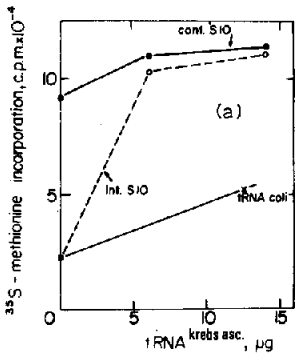
3. Results and discussion

3.1. *Restoration by tRNA of translation activity in interferon-treated extracts*

Cell free cytoplasmic extracts are prepared from L cells, which have been treated in suspension cultures by 200 units of mouse interferon/ml, for 24 hr as described in detail previously [3, 4]. Such extracts, 'interferon S10', translate very poorly Mengo virus RNA in comparison to S10 from normal untreated L cells. This block is reversible: the ability of interferon S10 to translate Mengo RNA can be restored by adding transfer RNA. Fig. 1a shows that addition of Krebs ascites cells tRNA restores over 90% of its activity to the interferon S10. The S10 from control L cells is not significantly stimulated by tRNA.

Periodate treatment of tRNA, which destroys the amino acid acceptance, abolishes its ability to restore the translation of Mengo RNA [5]. *E. coli* tRNA has very little activity (fig. 1, table 1) as compared to mammalian tRNA; when, however, *E. coli* tRNA was first charged with bacterial enzymes, some activity was observed. The restoration by tRNA was obtained in many different extracts from interferon treated L cells, and the activity observed ranged from 75 to 120% of that of the control. Preparations of tRNA from rabbit liver, Krebs ascites cells or L cells showed similar activities.

Restoration of Mengo RNA translation was obtained with tRNA purified from both control



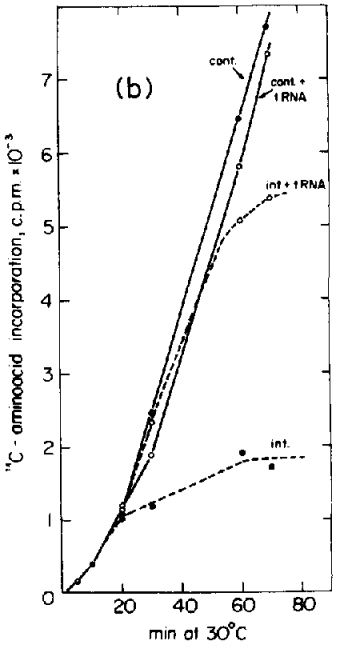


Fig. 1. a. Restoration by tRNA of Mengo RNA translation in interferon-treated L cell S10. Conditions of assay as in Materials and methods with 0.25 A_{260} units of S10 from control or interferon treated L cells and 1 µg Mengo RNA in 0.05 ml. Incubation for 60 min at 30°C. Hot TCA insoluble radioactivity of a 0.04 ml aliquot was determined. b. Kinetic study of the tRNA effect. Conditions as above but with ^{14}C-labelled amino acids. L cell extracts used here were either treated with 150 units mouse interferon per ml culture for 18 hr, or untreated. Rabbit liver tRNA (15 µg) was used and 0.15 A_{280} units ammonium sulfate precipitated L cell S100 were added.

Table 1
Effect of tRNA from various sources on restoration

Additions	^{14}C-labelled aa incorporation		
	Control S10	Interferon S10	
	cpm	cmp	%
None	13 100	1430	(11)
+ tRNA rabbit liver, 6 μg	10 925	8130	(75)
+ tRNA *E. coli*, 4 μg	9575	2210	(23)
+ tRNA L cells, 2.9 μg	8410	5435	(65)
+ tRNA L cells interferon-treated, 2.9 μg	9750	6250	(64)

Conditions as in fig. 1. Incorporation without Mengo RNA was 330 cpm. tRNA was extracted from suspension cultures of control and interferon treated L cells as described in Materials and methods: the same amount of total tRNA was obtained from both cultures.

and interferon treated L cells (table 1). The block in translation induced by interferon does not seem to be due to a drastic reduction in tRNA.

3.2. *Purification of the restoring tRNA for Mengo RNA translation*

The nature of the restoring tRNA was investigated after chromatography on benzoylated-DEAE cellulose according to Petrissant et al. [10]. Fig. 2 shows the chromatography of Krebs cells tRNA and it is clear that the activity is separated from the bulk of the tRNA, eluting late from the BD-cellulose column. The same fractions were used to locate the initiator $tRNA_{metF}$ using *E. coli* purified methionyl tRNA synthetase [13]. This initiator tRNA which elutes early from the BD-cellulose is not active in the restoration of Mengo RNA translation. Further studies with purified $tRNA_{metF}$ from rabbit liver

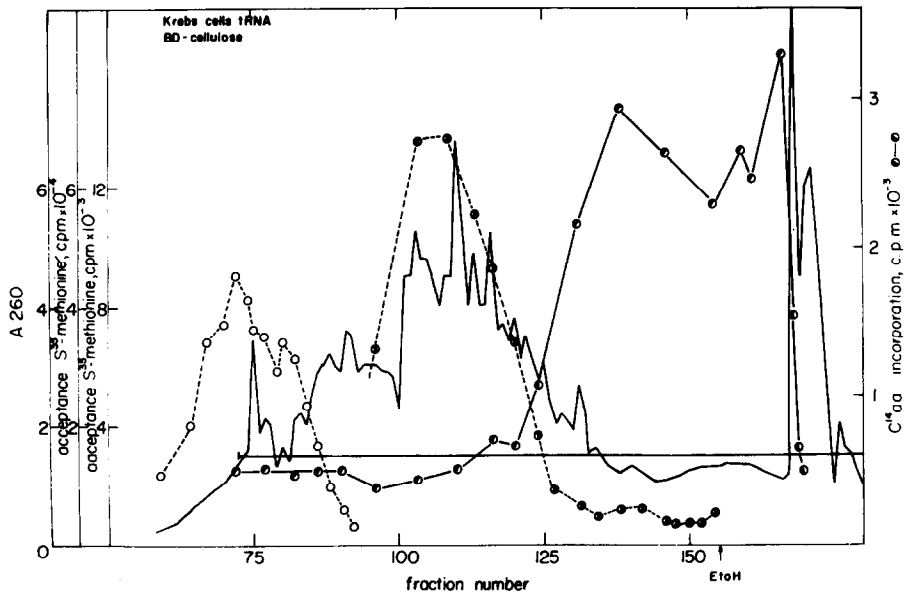


Fig. 2. Chromatography of restoring tRNA on BD-cellulose. A 0.9 × 12.5 cm column of BD-cellulose (Schwartz) was loaded with 12.5 mg tRNA extracted [9] from Krebs ascites cells. The column was washed with 0.35 M NaCl, 0.01 M $MgCl_2$ 0.01 M Na acetate buffer pH 4.5 and eluted with a 100 ml gradient of 0.35–1.0 M NaCl in the same buffer. Fractions of 0.5 ml were collected, dialyzed against 0.02 M HEPES buffer, pH 7.5, 0.12 M KCl, 0.005 M $MgCl_2$, 0.007 M β-mercaptoethanol and glycerol 10% and assayed for their ability to restore Mengo RNA translation in S10 extracts from interferon treated L cells as in fig. 1 (●——●). Fractions were also assayed for acceptance of ^{35}S-methionine with purified *E. coli* methionyl tRNA synthetase (○——○) or L cell S100 (⊕------⊕).

(a generous gift of Dr G. Petrissant) or from Krebs cells confirmed this result. The *N*-formyl met $tRNA_{metF}$ prepared using a tRNA free *E. coli* enzyme [11] and formyl donor, had virtually no activity to restore Mengo RNA translation in interferon S10 as compared to the active species from the BD-cellulose column of fig. 2. The other met $tRNA_{metM}$ was also devoid of activity (fig. 2). The species which restored activity to the interferon S10 did not stimulate the control S10. In most experiments we used a mixture of 16 ^{14}C-amino acids as source of label but the same fractions (125–165 of fig. 2) devoid of methionine acceptance, also reactivated Mengo RNA-dependent ^{35}S-methionine incorporation. (With ^{35}S-methionine, a stimulation by $tRNA_{met}$ species may be observed but since control S10 was also stimulated, it probably reflects a low level of this tRNA in the extracts).

The active fractions from fig. 2 were further purified by rechromatography on BD-cellulose which was found to be the most efficient method of purification. The activity for restoring Mengo RNA translation in interferon S10 was again eluted after the main peak of tRNA. The most purified fractions restored at a dose of 0.03 μg tRNA per an amount of S10 estimated to already have 0.8 μg tRNA. We measured acceptance of 16 amino acids by this tRNA and only leucine showed significant charging onto this material. Position on the chromatographic pattern from BD-cellulose corresponded to that of a minor third peak of leu tRNA, but it will be necessary to ascertain the purity of this material before concluding that the Mengo RNA-restoring activity is leu tRNA.

By filtration on Sephadex G-75 and by electrophoresis on polyacrylamide gels in SDS, only 4 S RNA was seen in the active purified material.

3.3. *The Interferon-induced translation inhibitor and the basis for the tRNA effect*

How does interferon treatment produce this tRNA-reversible block in translation? A drastic reduction in tRNA is unlikely since we have already mentioned that the amount and restorating activity of tRNA extracted from interferon treated L cells was not significantly reduced (table 1). We have reported previously [4] that the translation block in interferon S10 is due to the presence in the fraction washed-off ribosomes by 0.5 M KCl of an inhibitor of protein synthesis whose concentration increases with the dose of interferon [4]. This factor (which is not interferon itself, but is induced in the cell by interferon treatment [3], inhibits Mengo RNA and hemoglobin mRNA translation in S10 from untreated L cells but does not affect endogenous protein synthesis in these extracts (table 2). No effect on poly U translation is seen [5]. The factor is precipitated between 70–95% ammonium sulfate, elutes from DEAE cellulose at 0.3 M KCl and is inactivated by heating at 50°C for 7 min [5]. These characteristics show that the inhibitor is at least in part a protein.

Table 2
Effect of ribosomal wash fraction on L cell translation

Additions	L cell endogenous protein synthesis	Mengo RNA translation
	%	%
None	100	100
+ $tRNA^{retic.}$, 5 μg	–	85
+ Control ribosom. wash, 20 μg	72	74
+ Interferon ribosom. wash, 25 μg	63	27
Idem + $tRNA^{retic.}$, 5 μg	–	83

Conditions as in fig. 1. ^{14}C-labelled amino acid incorporation was measured in control L cell S10: non-preincubated for endogenous protein synthesis (100% = 8950 cpm in 60 min) or after preincubation and addition of 1 μg Mengo RNA (100% = 2330 cpm in 60 min).

The inhibition produced by the interferon-induced factor can be overcome by the addition of tRNA (table 2). Actually, the inhibitory factor makes the control S10 become tRNA-dependent, which is normally a characteristic of the interferon S10 (fig. 1). This suggests that this factor causes both the block in mRNA translation and the dependence on tRNA.

A kinetic study of Mengo RNA translation (fig. 1b) shows that in interferon S10, translation of Mengo RNA is initiated as in the control but stops after 20–30 min at 30°C. Addition of tRNA to the interferon S10 allows protein synthesis to proceed for a much longer time at the same rate as the control

Table 3
Effect of the delayed addition of transfer RNA

Extract	Addition at 30 min	^{35}S-methionine incorporation from 30 to 60 min at 30°C	
		cpm	%
Control S10	None	60000	(100)
Interferon S10	None	3750	(6)
Interferon	+ tRNA, 6 μg	62810	(105)
Interferon	+ Mengo RNA, 1 μg	3650	(6)

Conditions as in fig. 1, except that reaction mixture contained from 0–30 min cold methionine, 1 μM. At 30 min, 2.3 μCi of ^{35}S-methionine (195 mCi/μmole) were added. Mengo RNA, 1 μg, was present from time zero.

S10. If amino acid incorporation is measured between 30 and 60 min after addition of Mengo RNA, the complete arrest of translation is clearly apparent (table 3). Preincubation without mRNA did not modify this kinetic and translation still stopped 20–30 min after adding mRNA. Delayed addition of transfer RNA, 30 min after mRNA, still fully restores activity (table 3), which shows that no mRNA degradation takes place; furthermore addition of more Mengo RNA does not overcome the block. If inhibitors of initiation, such as aurintricarboxylic acid or MDMP [14], are added at 30 min together with tRNA, no restoration is observed. This implies that new initiations are required to see the full restoration by tRNA. However, studies to be published elsewhere show that the mechanism of the block is complex: when the block is established, new mRNA is still bound, small peptides may be produced but the polypeptide chains are no more elongated [15].

One possibility is that the inhibitory factor progressively degrades tRNA. We indeed observed some decrease in the tRNA acceptance for methionine and other amino acids in interferon S10. However, when Mengo RNA translation stops, endogenous protein synthesis still proceeds [3, 15]. Furthermore, preincubation of restorating tRNA (from BD-cellulose chromatography in fig. 2) in the S10 extracts shows that it is degraded and loses activity too slowly to account for the rapid block in translation of Mengo RNA [15].

Recent experiments also show that degradation

Table 4
Relative translation of Hemoglobin mRNA and Mengo RNA by interferon-treated L cell S10

Additions	^{14}C-labelled aa incorporation with		Translation ratio Hemoglobin / Mengo
	Hemoglobin mRNA cmp	Mengo RNA cmp	
None	440	720	0.6
+ tRNA$^{\text{rabbit liver total}}$	5106	4505	1.1
+ tRNA$^{\text{Krebs}}$ fractions from BD-cellulose:			
Fraction 110	4265	779	5.5
Fraction 142	3525	2604	1.3

Conditions as in fig. 1. Only interferon S10 was used with 0.5 μg rabbit reticulocyte Hemoglobin mRNA andribosomal wash fraction from reticulocytes [8] or with 1 μg Mengo RNA. BD-cellulose tRNA fractions refer to the chromatography in fig. 2.

of one particular species of tRNA is unlikely and the restoring effect may be more complex. Using rabbit and mouse hemoglobin mRNA, we have observed that translation of these cellular mRNAs in the interferon S10 can also be restored by adding tRNA (table 4) provided that reticulocyte initiation factors are added [8]. The tRNA species from BD-cellulose chromatography which reactivate Hemoglobin mRNA translation are, however, not identical to that which restores Mengo RNA. It is therefore possible to restore the activity of an interferon S10 for hemoglobin mRNA but not for virus Mengo RNA, according to the tRNA species which is present, as shown in table 4. The tRNA requirement of the interferon S10 therefore depends on the nature of the mRNA template and more work is needed to determine how many species of tRNA are involved.

Our results suggest that the requirement for tRNA depends on the codons present in the mRNA. Translation would stop at codons for which too small amounts of the cognate tRNA are present. The ribosome-bound inhibitor may, in this hypothesis, decrease either the availability or the binding efficiency of aminoacyl tRNA to ribosomes.

It is tempting to speculate that in the cell, the discriminatory effect of interferon on cellular and viral mRNA translation [7] depends on the composition of the tRNA population of the cell. Externally imposed variations in tRNA may be useful to potentiate the antiviral effects of interferon. One example may be amino acid starvation [16].

This work has been presented in preliminary form at the Gulbenkian Meeting on Interferon, Lisbon, 1973. Similar results are found in the laboratories of Lengyel (personal communication) and Jocklik (personal communication).

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