

***In vitro* Translation of Natural mRNAs in a Cell-free System Containing Components from Interferon-treated Chicken Fibroblasts and Factor Preparations from Mouse Ascites Cells or Rabbit Reticulocytes**

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The effect of interferon has been studied in a mixed cell-free protein synthesizing system. Hemoglobin (Hb) and Encephalomyocarditis virus (EMC)-RNA can be efficiently translated *in vitro* in a system containing S-30 lysates or run-off ribosomes from primary chick embryo fibroblasts (CEF) and a postmicrosomal supernatant from mouse ascites cells or a ribosomal-wash preparation from rabbit reticulocytes. Ribosomes prepared from CEF pretreated with high doses of homologous interferon (500 units/ml) were able to translate Hb-RNA in the presence of heterologous factors with the same efficiency as ribosomes prepared from control cells. Translation of EMC-RNA was slightly reduced if ribosomes from interferon-treated cells were used in the mixed cell-free system, confirming previous reports. No inhibitory effect caused by interferon treatment of CEF cells could be detected on *in vitro* translation of natural mRNAs if the cells had, in addition to interferon treatment, been infected with vaccinia virus. Possible reasons for the different observations made with our cell-free protein synthesizing system from CEF and with cell-free systems prepared from mouse cells are discussed.

Introduction

In interferon-treated cells, the synthesis of viral proteins is usually preferentially inhibited while cellular protein synthesis is not drastically affected¹. For the purpose of elucidating the mechanism of interferon action, a cell-free preparation from interferon-sensitive cells would be required which would efficiently translate a variety of viral and cellular mRNAs. The aim would be to develop an *in vitro* protein synthesizing system in which components from interferon-treated cells show an inability to translate viral but not cellular mRNA. If such a system were available, the specific regulation-mechanisms of protein synthesis acting in an interferon-treated cell might be elucidated.

Cell-free systems from mouse L-cells, mouse ascites cells and CEF fortified with supernatant

fractions from mouse ascites cells have previously been used to study the influence of interferon treatment on the capacity of these cells to translate various viral and cellular mRNAs²⁻⁸. Lysates from interferon-treated mouse L-cells were found to be restricted in their ability to translate either viral or hemoglobin RNA^{2,5}. It seems, therefore, that in these cell-free systems the characteristic specificity of interferon on protein synthesis is not expressed, and this important aspect cannot be studied. In addition, the experimental conditions under which alterations of the protein synthesizing machinery can be observed with cell-free preparations from mouse cells are still controversial^{4,5,7,8}.

In this report, we describe a cell-free system for the translation of EMC- and Hb-RNA consisting of lysates from primary CEF or ribosomes from these cells and heterologous factor-preparations from mouse ascites cells or rabbit reticulocytes. This mixed system has been used to study the ability of ribosomes from interferon-treated primary CEF to translate natural mRNA.

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Materials and Methods

Creatine phosphokinase and GTP-tri-lithium salt were obtained from Boehringer-Mannheim; creatine phosphate-di-Tris salt and CTP-Tris salt from Sigma; ATP-di-potassium salt from Calbiochem; Poly(U) from Miles Chemical; [^{14}C]phenylalanine (513 Ci/mol) and [^{14}C]leucine (348 Ci/mol) from the Radiochemical Center, Amersham.

Tissue culture, virus and interferon preparation

The preparation of primary chicken fibroblasts and mode of infection with purified vaccinia virus (strain WR) has been described in previous publications⁹. Ascites cells were grown in the mouse intraperitoneal cavity¹⁰. Vaccinia virus was purified according to the method of Joklik¹¹. The preparation of purified interferon and induction of the virus resistant state by exposure to purified homologous interferon has been described^{9, 12}.

Preparation of S-30 lysates and supernatant fractions (S-100)

CEF-monolayer cultures were washed three times with cold TBS (35 mM Tris-HCl pH 7.5, 140 mM NaCl); the cells were scraped off and packed by centrifugation at $500 \times g$ for 10 min. The pellet was resuspended in 1–2 volumes of a hypotonic buffer (10 mM Tris-HCl pH 7.5, 10 mM KCl, 1.5 mM Mg^{2+} , 7 mM 2-mercaptoethanol) and allowed to swell for 10 min¹⁰. After homogenization with a Potter-Elvehjem homogenizer (4 min; 1500 rpm) the homogenate was brought to a final concentration of 30 mM Tris-HCl, pH 7.5, 120 mM KCl, 5 mM Mg^{2+} , 7 mM 2-mercaptoethanol by the addition of $10 \times$ concentrated buffer and centrifuged at $30\,000 \times g$ for 10 min. The supernatant was incubated at 37°C for 40 min under conditions of polypeptide synthesis as described by Aviv *et al.*¹³. After recentrifugation at $30\,000 \times g$ for 10 min the preincubated supernatant was passed through a Sephadex G-25 (medium) column equilibrated with buffer containing 30 mM Tris-HCl pH 7.5, 85 mM KCl, 5 mM Mg^{2+} and 7 mM 2-mercaptoethanol (S-30 lysate). Preincubated high-speed supernatant (S-100) was prepared from the appropriate post-mitochondrial cell extracts by centrifugation at $180\,000 \times g$ for 120 min at 2°C . The upper 3/4 of the resulting solution was used. In some experiments a crude factor preparation was isolated from non-preincubated ascites S-100 according to Wigle and Smith by precipitating the proteins with $(\text{NH}_4)_2\text{SO}_4$ between 30–70% saturation¹⁴.

Preparation of ribosomes

For the isolation of run-off ribosomes, the preincubated post-mitochondrial CEF extract was made 0.25 M in sucrose. This preparation was layered onto a cushion of 1 M sucrose in buffer containing 50 mM Tris-HCl, pH 7.6, 25 mM KCl, 5 mM Mg^{2+} , 20 mM 2-mercaptoethanol (buffer A) and centrifuged at $200\,000 \times g$ for 150 min at 2°C . The pelleted ribosomes were resuspended in buffer A by gentle hand homogenization in a loosely fitting homogenizer at a concentration of 60 $\text{OD}_{260}/\text{ml}$ and stored in small samples in liquid nitrogen.

Preparation of rabbit-reticulocyte ribosomal wash

New Zealand white rabbits weighing 2–2.75 kg were injected subcutaneously on 6 successive days with 0.25 ml of a 2.5% solution of phenylhydrazine per kilo of body weight. Two days after the last injection the blood was collected by direct puncture of the heart¹⁵. All subsequent steps were performed at 4°C . The collected blood was poured through 3 layers of gauze into glass centrifuge bottles and centrifuged 10 min at 2000 rpm. The pellet was washed 4 times with 4 volumes of NKM-buffer (130 mM NaCl, 7.5 mM MgCl_2 , 5 mM KCl). Four volumes of lysis solution (4 mM MgCl_2 , 1 mM DTT in H_2O) were then added to the washed pellet and shaken for 45 sec. Lysis was stopped by addition of 1 volume of stop-buffer (150 mM KCl, 1.5 M Sucrose). The suspension was centrifuged for 15 min at $12\,000 \times g$. To sediment ribosomes, the supernatant was centrifuged for 5 hours at $78\,000 \times g$. The polysome pellet was washed twice with a buffer containing 25 mM sucrose, 5 mM Tris-HCl (pH 7.6), 1 mM DTT, 0.1 mM EDTA, 5 mM MgCl_2 and resuspended in the same buffer at a concentration of 250 $\text{OD}_{260}/\text{ml}$. 4 M KCl was added slowly to the solution until a 0.5 M concentration of KCl was obtained. The preparation was stirred for a further 15 min. To separate the polysomes, the solution was centrifuged at $165\,000 \times g$ for two hours. The supernatant was collected and stored at -80°C .

Isolation of EMC-RNA

Purified Encephalomyocarditis virus was obtained from G. D. Searle & Co. The virion RNA was extracted according to the method developed by Kerr *et al.*¹⁶.

Isolation of the rabbit-hemoglobin mRNA

Polysomes isolated from reticulocytes of anemic rabbits were used as starting material for the hemoglobin mRNA preparation. Induction of reticulocytosis by phenylhydrazin, collection of reticulo-

cytes, lysis of reticulocytes and preparation of polyosomes from the lysate was as described by Lingrel¹⁷. RNA was extracted from the polyosomes by EDTA and SDS treatment followed by phenol extraction according to Aviv and Leder¹⁸. Whole polysomal RNA recovered from the extracted lysate by ethanol precipitation was subsequently fractionated by two sucrose gradient centrifugations (Ostertag, personal communication). The first gradient was from 10 to 30% (w/v) in 0.015 M KCl, 0.01 M Tris-HCl, pH 7.4 and was spun from 26 to 30 hours at 27 000 rpm and 4 °C in a Beckman SW 27 rotor. All material sedimenting at a rate from about 7S–16S was collected, precipitated twice with ethanol and layered over 10 to 50% (w/v) sucrose gradients in the above buffer. These were spun for 25 hours at 40 000 rpm and 4 °C in a Beckman SW 41 rotor. The whole material sedimenting from 9S to 16S in the form of several not well resolved peaks was taken as globin mRNA. It was precipitated twice with ethanol, dissolved in water at a concentration of 20 OD₂₆₀ units/ml and stored frozen at –70 °C.

Preparation of tRNAs from embryonic chick leg muscles

Leg muscles of chick embryos (15–16 days) were minced at 0 °C in a Sorvall Omni Mix and the resulting homogenate was extracted for one hour at 4 °C with an equal volume of redistilled phenol saturated with buffer (35 mM Tris-HCl, pH 7.5, 140 mM NaCl, 10 mM MgCl₂). The separation of DNA and fractionation of RNA was performed as described by von Ehrenstein and by Aviv *et al.*^{13, 19}.

Cell-free system

Assay for amino acid incorporation was as described by Aviv *et al.* with the following minor modifications: 85 mM KCl, 3.5 mM Mg²⁺ or as indicated, 50 µM each of 19 non-radioactive amino acids and 5 µM radioactive amino acid ([¹⁴C]leucine or [¹⁴C]phenylalanine as indicated)¹³. 20–30 µl of preincubated CEF extract containing 1–3 mg/ml protein was added per reaction assay. Incorporation of radioactive amino acids into acid insoluble material was proportional to the amount of S-30 lysate used in the reactions. The concentration of natural mRNA was as indicated in the experiments. After incubation for 40 min at 37 °C, the reactions were stopped and the reaction mixture processed as described by Aviv *et al.*¹³.

Characterization of the Hb-RNA directed product of in vitro reaction by sodium dodecylsulfate polyacrylamide gel electrophoresis

The reaction product synthesized *in vitro* was precipitated with 10% TCA (3% leucine), washed

twice with 5% TCA (3% leucine) and once with acetone. The dried precipitate was suspended in 30 µl of 100 mM sodium phosphate pH 7.2 containing 2% SDS, 4 M urea and 1% 2-mercaptoethanol. Authentic rabbit globin was added as carrier. The protein samples were heated for 3 min at 100 °C. Sodium dodecylsulfate gels (14 × 0.6 cm) containing 10% acrylamide and 0.34% methylenebisacrylamide and 0.1% SDS were prepared and run at 4 mA/gel for 15 hours as described by Maizel²⁰. Gels were stained with Coomassie blue for 5 hours. After destaining by diffusion the gels were sliced and prepared for counting as described previously²¹.

Electrophoresis of the tryptic peptides of the in vitro synthesized product

Protein was labeled *in vitro* with [³⁵S]methionine under standard conditions. The incubation was terminated by the addition of EDTA (20 mM final) and digestion with pancreatic ribonuclease (25 µg/ml; 60 min; 37 °C). An equal volume of 10% trichloroacetic acid (3% methionine) was added and the suspension heated for 15 min at 90 °C. After the addition of authentic rabbit globin as a carrier the pellet was washed three times with 5% TCA (3% methionine), twice with ethanol-ether (1:1) and once with ether. The precipitated material was pelleted each time by centrifugation at 5000 rpm for 15 min. Oxidation with performic acid and tryptic digestion was performed as described previously^{22, 23}. The lyophilized samples were dissolved in a small amount of electrophoresis buffer and aliquots spotted on Whatman 3MM paper. The tryptic peptides were separated by one dimensional electrophoresis in pyridin acetic acid buffer pH 6.6. The dried paper was developed with ninhydrin and the [³⁵S]methionine-labeled peptides located by autoradiography.

Results

Endogenous protein synthesis in lysates (S-30) from primary CEF cells

Endogenous protein synthesis in S-30 lysates from primary CEF is insensitive to the initiation-inhibitor pactamycin (Table I). This indicates that nearly all the incorporation of radioactive amino acids represents elongation of peptide chains. As has been previously reported for CEF and for L-cells, the establishment of the antiviral state in these cells by preexposure to homologous interferon has little effect on endogenous protein synthesis (Table I)²⁴.

Table I. Endogenous protein synthesis in S-30 lysates from primary chick fibroblasts with and without interferon treatment.

Exp.No.	Inhibitor	[¹⁴ C]phenylalanine incorporated into acid insoluble material [cpm/100 μ g protein]*	
		10 min	20 min
1	— —	424	496
	Pactamycin (10 ⁻⁶ M)	382	419
2	— —	1720	2200
	CEF-interferon (500 units/ml)	1700	2260

* Pretreatment of cells with interferon and lysis of cells as described in Materials and Methods. Non-preincubated S-30 lysates were passed through a Sephadex G-25 column and used for assay of endogenous protein synthesis under standard conditions. Assays were carried out at 6 mM Mg²⁺ and 85 mM K⁺. Pactamycin was added to the reaction mixture before starting the incubation at 37 °C. Protein content of S-30 lysates: Exp. 1 — 5.5 mg/ml; Exp. 2 — control cells: 0.95 mg/ml, interferon-treated cells: 0.8 mg/ml.

The translation of EMC-RNA and hemoglobin RNA in a mixed cell-free system

Both mRNAs stimulate the incorporation of amino acids into acid insoluble material 10–15 fold in CEF S-30 lysates if the reaction is fortified with heterologous factors (Fig. 1). If supernatant

fractions from primary CEF were prepared and added in varying amounts to the reaction mixture no stimulation could be seen, which might be explained by an instability of these factors in chicken tissue. In the presence of rabbit reticulocyte ribosomal wash factor, S-30 lysates from interferon-treated CEF translated Hb-RNA with the same efficiency as S-30 lysates from control cells. Polypeptide synthesis stimulated by EMC-RNA was to some extent reduced in extracts from interferon-treated cells (Fig. 2).

Kerr⁶ was able to translate EMC-RNA with ribosomes from CEF in the presence of ascites supernatants. A 2.5-fold stimulation of amino acid incorporation was observed in his cell-free system. Using run-off ribosomes from CEF and ascites supernatant we achieved a much more efficient translation of natural mRNA (Fig. 3). Ribosomes from interferon-treated cells translated Poly (U) and hemoglobin mRNA with approximately the same efficiency as control ribosomes (Table II). EMC-RNA translation was again inhibited by about 30%, an effect which was previously detected by Kerr⁶.

Regulation of protein synthesis might be executed in interferon-treated cells by an alteration of ionic conditions required for translation of specific mRNAs. Translation of the two mRNAs by cell-free systems from normal and interferon-treated cells was studied for this reason under a variety of conditions.

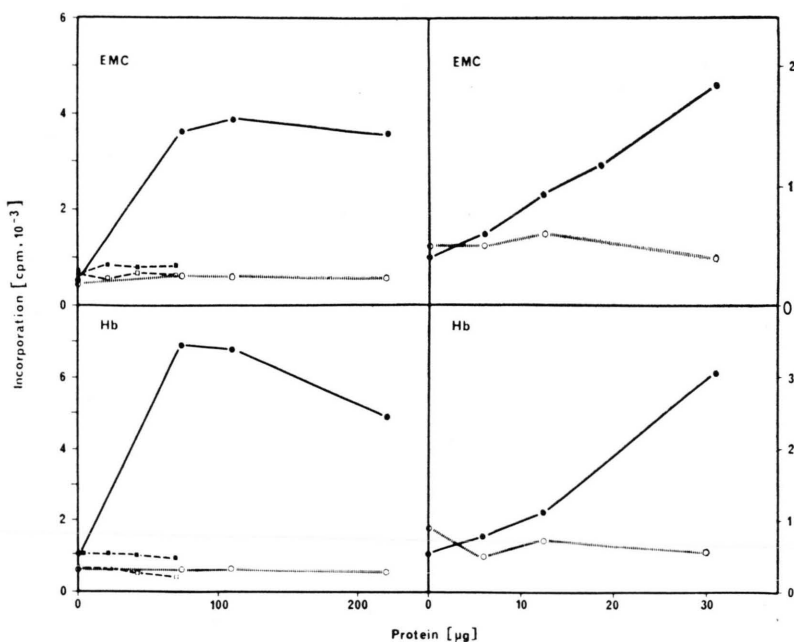


Fig. 1. The stimulation of EMC- and Hb-RNA translation in S-30 lysates from primary CEF by fractionated ascites supernatant or reticulocyte ribosomal wash factors. Each reaction mixture contained about 66 μ g protein of S-30 lysate and varying amounts of S-100 fraction or rabbit reticulocyte factors. EMC-RNA: 2 μ g/50 μ l; Hb-RNA: 3 μ g/50 μ l; conditions of *in vitro* protein synthesis as described in Material and Methods. EMC-peptides were labeled with [¹⁴C]phenylalanine; Hb-peptides with [¹⁴C]leucine. With mRNA —●—; Without mRNA —○—. Right: Rabbit reticulocyte wash factors; Left: Protein fraction of ascites S-100 precipitating between 30–70% ammonium sulfate or S-100 fraction from chick embryos. The reaction in the presence of S-100 from chick embryos was carried out under standard conditions, with mRNA —■—; without mRNA —□—.

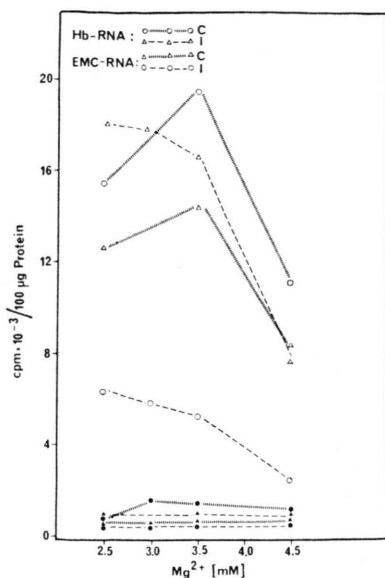


Fig. 2. mRNA directed cell-free polypeptide synthesis in S-30 lysates from interferon-treated (500 units/ml) or untreated CEF in the presence of rabbit reticulocyte ribosomal wash. Assays were carried out under standard conditions. The assays contained: 68 μ g protein of S-30 lysate (control) or 40 μ g protein of S-30 lysate (interferon-treated CEF), 30 μ g protein of rabbit reticulocyte ribosomal wash and mRNA. Hb-RNA: 3 μ g/50 μ l; EMC-RNA: 3 μ g/50 μ l. Assays were run at different Mg^{2+} -concentrations at 85 mM K^+ . Incubation time was 40 min. Open symbols, with mRNA; closed symbols, without mRNA; C, control; I, interferon-treated.

Ionic requirements for translation

In the presence of S-100 from ascites cells or ribosomal wash from reticulocytes, translation of EMC-RNA or Hb-RNA with ribosomes or S-30 lysates from primary CEF was usually optimal in the presence of 2.5 to 3.5 mM Mg^{2+} and 85 to 100 mM K^+ . In the presence of either heterologous factor preparations, the optimal concentration of Mg^{2+} for Hb-RNA may possibly be less than 2 mM but this could not be determined since there was a certain minimum amount of Mg^{2+} ions introduced into the reaction mixture with the S-30 lysate. Addition of tRNA from chick embryo muscles (0.11 OD₂₆₀/assay) stimulated the amino acid incorporation at most 2-fold. Using ribosomes or S-30 lysates from interferon-treated cells did not alter the ionic conditions necessary for optimal translation of natural mRNAs. Incorporation of amino acids into acid insoluble material in the presence of both mRNAs occurred linearly over a period of 45 min with extracts from interferon pretreated and control cells.

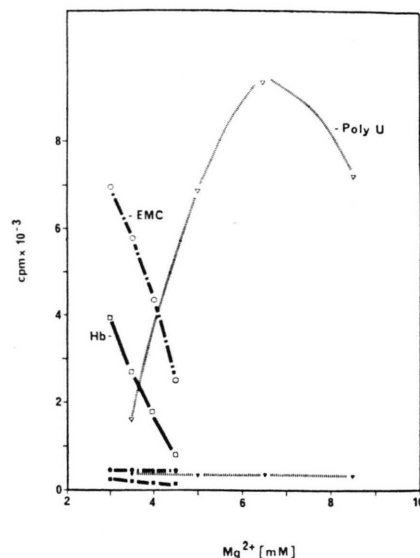


Fig. 3. *In vitro* translation of mRNAs by a mixed reconstituted system containing run-off ribosomes from CEF and a S-100 preparation from ascites cells. Incorporation of [¹⁴C]leucin (Hb-RNA) or [¹⁴C]phenylalanine (Poly U, EMC-RNA) into acid insoluble material was measured at different Mg^{2+} concentrations. Conditions for *in vitro* translation of mRNAs are described in Materials and Methods. Each reaction mixture contained 0.3 OD₂₆₀ of ribosomes and 125 μ g of ascites S-100. Hb-RNA: 3.0 μ g/assay; EMC-RNA: 2.4 μ g/assay; Poly U: 10 μ g/assay. Reactions were run at 85 mM K^+ . Incubation time: Natural mRNA — 40 min, Poly U — 20 min. Open symbols: with mRNA; closed symbols, without mRNA.

Table II. Translation of various mRNAs by a mixed cell-free system containing ribosomes from interferon-treated CEF (500 units/ml) and S-100 from ascites cells*.

Pretreatment of cells	Messenger RNA	Incorporation of radioactive amino acids into polypeptides [cpm/50 μ l] **
untreated	Poly U	9038
	Hb-RNA	3693
	EMC-RNA	6522
CEF-interferon (500 units/ml)	Poly U	9360
	Hb-RNA	3264
	EMC-RNA	4616

* The conditions for treatment of cells with interferon and the preparation of extracts are described in Materials and Methods. Ribosome concentration: 0.3 OD₂₆₀/50 μ l; 125 μ g of ascites S-100 proteins were added to each assay. Hb-RNA: 3 μ g/assay and EMC-RNA: 2.4 μ g/assay; Poly U: 10 μ g/assay; natural mRNA directed polypeptide synthesis was assayed at 3 mM Mg^{2+} ; Poly U translation at 6.5 mM Mg^{2+} . All assays were run at 85 mM K^+ . Incubation time: natural mRNA — 40 min, Poly U — 20 min.

** Incorporation of radioactive amino acids in the absence of mRNA has been subtracted. Incorporation of radioactivity in the absence of mRNA varied between 148 and 489 cpm.

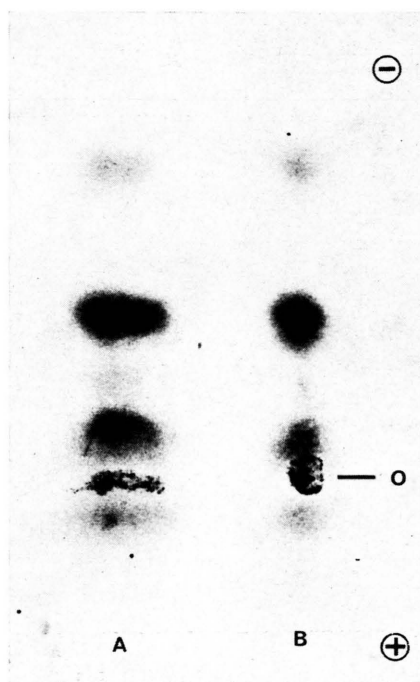


Fig. 5. Autoradiographs of the tryptic digest of the Hb-RNA directed reaction product. [^{35}S]methionine labeled protein was synthesized *in vitro* under standard conditions except that the reaction volume was scaled up 10-fold. The assay mixture contained 520 μg protein of S-30 lysate and 240 μg protein of ribosomal wash. Preparation of [^{35}S]methionine labeled globin from rabbit reticulocytes was carried out following the method of Shapira *et al.*²⁹. Conditions for the isolation of the reaction product and tryptic digestion are described in Materials and Methods. A, globin RNA directed product synthesized *in vitro*. B, [^{35}S]methionine labeled globin from rabbit reticulocytes. Conditions of electrophoresis: 48 V/cm; 50 min.

Characterization of the *in vitro* synthesized product

The product of the EMC-RNA directed reaction by a S-30 lysate from primary CEF and from a mixed cell-free system of CEF ribosomes fortified with an ascites S-100 has been previously characterized by SDS-gel-electrophoresis and two dimensional peptide analysis^{6, 25}. The product of the Hb-RNA stimulated reaction was characterized according to size by SDS-acrylamide gel electrophoresis and by one-dimensional paper electrophoresis of the tryptic peptides. The cell-free system as described above fortified with reticulocyte factors produced a product which co-migrated with authentic rabbit globin (Fig. 4). The product synthesized *in*

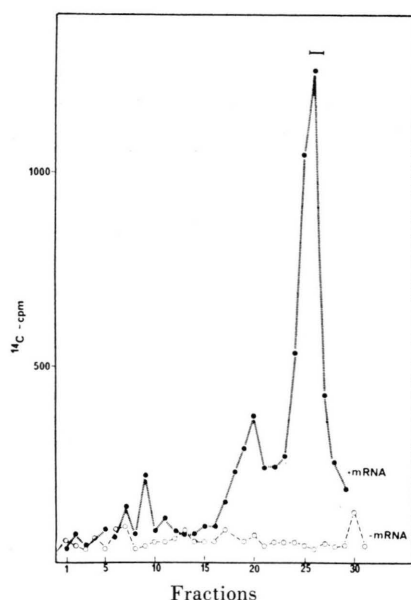


Fig. 4. Characterization of the Hb-RNA stimulated product synthesized by the mixed cell-free system (CEF S-30 + ribosomal wash from rabbit reticulocytes). Protein synthesis in the cell-free system was carried out under standard conditions. The assay contained 34 μ g of S-30 lysate and 24 μ g ribosomal wash preparation. Analysis of the product labeled with [¹⁴C]leucine was done by gel electrophoresis as described in Materials and Methods. Authentic globin was added as internal marker to the reaction product before SDS-acrylamide gel electrophoresis. Gels were run for 16 hours at 4 V/cm. Position of authentic rabbit globin: —|—.

vitro in the presence of an ascites supernatant or by extracts from interferon-treated cells also co-migrated with authentic rabbit globin (not shown). The tryptic peptides of the product synthesized in a preincubated S-30 lysate with Hb-RNA and reticulocyte factors are compared with the peptides from [³⁵S]methionine labeled rabbit globin in Fig. 5*.

Effect of infection on the translational potential of interferon-treated extracts

Several groups have reported that the partial inhibition of cell-free polypeptide synthesis in lysates from interferon-treated mouse L-cells is enhanced by an additional infection with an animal virus^{2, 7}. This observation led to the hypothesis that the antiviral effect of interferon might only fully develop in the cells after infection with the challenge virus⁷. In the mixed cell-free system prepared from interferon-treated CEF, however, no co-operative inhibitory effect of interferon treatment and vaccinia infection on the translation of natural mRNAs could be detected (Fig. 6).

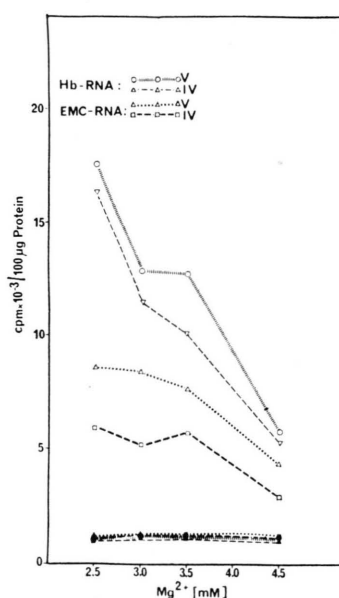


Fig. 6. *In vitro* translation of natural mRNA at different Mg^{2+} -concentrations by extracts from interferon-treated and vaccinia infected cells. S-30 lysates were prepared from CEF treated with interferon (500 units/ml) and infected with Vaccinia virus WR for 2 hours. Multiplicity of infection: 1000 e.b./cell. Conditions of amino acid incorporation as in previous experiments. Each reaction contained 56 μ g of S-30 lysate and was fortified with 3 μ g of reticulocyte ribosomal wash. Hb-RNA: 3 μ g/assay; EMC-RNA: 3 μ g/assay. Incubation time of the reaction: 40 min. V, infected; IV, interferon-treated and infected; open symbols, without mRNA; closed symbols, with mRNA.

Discussion

Interferon-induced alterations of the protein synthesizing machinery are clearly manifest in cell-free extracts from murine cells^{2, 5, 7, 8}. Evidence was

* Fig. 5 see on Table on page 402 a.

obtained that an additional protein appears on ribosomes from interferon-treated cells which prevents the translation of exogenous mRNAs^{4, 5, 8}. The inhibitory effect was expressed even in the presence of added heterologous factors². It is at present not clear why in cell extracts from some L-cells an inhibitory effect of interferon is expressed only after viral infection³.

The observations made with ribosomes from primary CEF in the presence of heterologous factors are in striking contrast to those made with cell-free preparations from mouse cells. Doses of interferon which completely block the replication of various viruses and inhibit virus specific protein synthesis in infected CEF to a high degree, do not cause any drastic alteration of the activity of ribosomes detectable in the *in vitro* protein synthesizing system described above^{9, 12, 26}. Confirming previous results, some inhibition of the translation of EMC-RNA can be detected with extracts from interferon-treated cells⁶. Since EMC cannot be grown in CEF it is impossible to correlate this observation to an antiviral effect of interferon in the infected cell. It will therefore be necessary to see if the mRNA of an interferon-sensitive virus growing in CEF can be translated in the cell-free system described and if the translation is inhibited if extracts from interferon-treated cells are used.

Several possibilities could be thought of to explain why interferon treatment of CEF in contrast to mouse L-cells does not have any strong effects on the *in vitro* translation of hemoglobin and EMC-RNA by CEF extracts. For example, the inhibitory protein of the ribosomes from interferon-treated CEF cells might be unstable and is inactivated

during the preparation of the cell-free extract or the translation-inhibitory effect is, in contrast to mouse cells, only expressed in the presence of homologous factors. To exclude this possibility, attempts are being made to prepare factors from chicken reticulocytes and a variety of other chick embryo tissues.

The translation block in S-30 lysates from interferon-treated mouse cells can be overcome by adding exogenous tRNA^{27, 28}. Even though the interferon-effect was clearly expressed in CEF-cultures the level of tRNA in the cell-free extract might be still sufficient for the *in vitro* translation of exogenous mRNA. In this case only after further fractionation of the cell-free system an impairment of the translation of mRNA might become detectable.

Many differences between interferon-treated chick cells and mouse cells have so far been discovered^{9, 12}. The results reported in this publication further extend these observations. It does appear that phenomena seen in interferon-treated mouse cells cannot be generalized without reservations. In order to discriminate the antiviral principle from other — perhaps unrelated — properties of the interferon preparations in use today, it seems highly desirable to carry out similar studies also in other virus-cell systems.

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