TRANSLATION OF RNA BY L CELL EXTRACTS: EFFECT OF INTERFERON

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1. Introduction

In previous studies we have been unable to detect more than a slight effect of interferon treatment of cells in the absence of infection upon the ability of cell-free systems isolated from them to translate a viral RNA [1]. None the less it would appear that in vaccinia virus-infected L cells pretreated with interferon it is virus protein synthesis which is inhibited: virus messenger RNA (mRNA) is synthesized but is not translated [2, Metz and Esteban, in preparation]. Here, therefore, we report the results of an investigation of the effect of interferon pretreatment on the translation of a viral RNA in cell-free systems from vaccinia virus-infected cells. Extracts from mouse L cells translated encephalomyocarditis (EMC) virus RNA and mouse globin mRNA to yield EMC-specific polypeptides and mouse globin. Under the conditions employed, infection or pretreatment of the cells with interferon alone had little effect on the translation of EMC RNA in these systems. With extracts from cells subjected to both interferon treatment and infection, however, the translation of EMC RNA was strikingly inhibited. The bulk of the inhibitory effect appeared to be associated with the ribosome fraction. Inhibition was not seen if the cell-free systems were prepared in the conventional manner by preincubation of the extracts at 37°. Thus, both interferon treatment and infection appear to be required before a clear-cut effect on translation can be seen in the cell-free system.

2. Materials and methods

The growth of EMC virus, the extraction of EMC RNA [3] and of mouse globin mRNA [4], the labelling of the polypeptide products in the cell-free system and their analysis by electrophoresis on polyacrylamide gels in the presence of sodium dodecyl sulphate (SDS) have already been described [5]. Mouse interferon prepared by the method of Paucker [6] contained $> 10^7$ units/mg of protein expressed in terms of the N.I.H. Mouse Serum Interferon Reference Reagent. Interferon treatment (1.5×10^6 L cells/ml) was routinely with 50 units/ml for 4 hr at 37°. The cells were diluted to 7.5×10^5/ml and incubation continued for 17 hr. They were then infected with purified vaccinia virus (500 particles/cell) and harvested at 1 hr post infection [2]. The preparation of the cytoplasmic extracts will be described elsewhere (Friedman et al., in preparation).

Table 1
Amino acid incorporation in response to EMC RNA or mouse globin mRNA in L cell extracts.

	[^{14}C] amino acid incorporation (counts per min per assay)						
mRNA (μg)	0	0.5	1	2	3	4	5
Globin	1,000	–	–	5,700	6,400	6,200	5,900
EMC RNA	1,300	16,000	18,300	29,800	36,300	21,400	–

The cell-free systems from L cells employed post-mitochondrial supernatant fractions which had been preincubated for 45 min at 37° and passed through Sephadex G-25. Amino acid incorporation in the presence of added RNA was over 120 min at 30°.

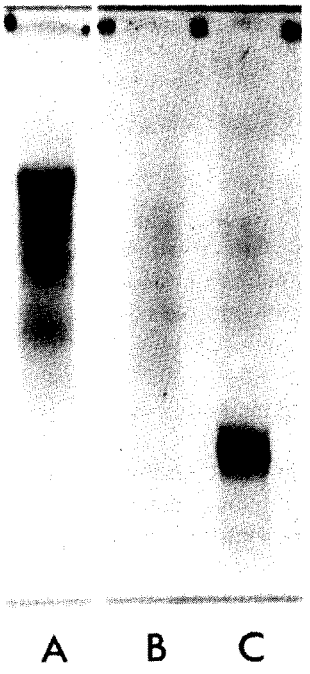


Fig. 1. Analysis of the polypeptide products from the cell-free systems by electrophoresis on SDS-polyacrylamide gels. A) with EMC RNA (5% gel); B) no added RNA (7.5% gel); C) with globin mRNA (7.5% gel). Autoradiographs of the sliced dried gels are shown.

3. Results and discussion

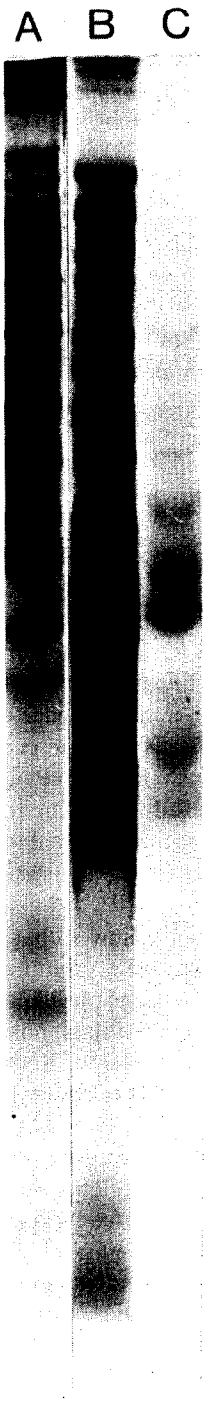
3.1. *Translation of EMC and globin messenger RNA's in the cell-free system – characterisation of the polypeptide products*

The optimal conditions for the stimulation of amino acid incorporation by EMC RNA or by mouse globin mRNA differed slightly. For EMC RNA the K^+ and Mg^{2+} concentrations were, respectively, 110 mM and 4.5 mM; for globin mRNA, 50 mM and 3.5 mM. In both cases, incorporation in response to the RNA was better at 30° than 37°. Table 1 shows the effect of various concentrations of EMC RNA and of mouse globin mRNA on the incorporation of ^{14}C-labelled amino acids in the presence of preincubated extracts of L cells. Incorporation was linear over at least a 2 hr period.

The products formed in such incubation mixtures have been analyzed by electrophoresis on SDS-polyacrylamide gels (fig. 1). In the case of mouse globin mRNA the single polypeptide product (fig. 1) migrated with mouse globin. After 4 hr of incubation in the presence of EMC RNA a polypeptide with a molecular weight of about 140,000 was the major product. A similar large polypeptide has been previously identified as being characteristic of EMC RNA directed protein synthesis in Krebs ascites cell extracts [5]. Moreover, comparison of tryptic peptides from the EMC RNA directed product with those from authentic EMC polypeptides, as described previously [7], established their identity.

3.2. *Interferon mediated inhibition of virus protein synthesis in vaccinia virus-infected cells*

Interferon treatment has no marked effect on RNA or protein synthesis in normal cells [8, 9] whereas in virus-infected cells viral protein synthesis at least is markedly inhibited [2]. Our studies with interferon treated vaccinia virus-infected L cells have confirmed these findings. On normal vaccinia virus infection of cells in the absence of interferon pretreatment, host protein synthesis is inhibited and vaccinia virus speci-



fic peptides can be clearly detected 30 min post infection. By 1 hr after infection protein synthesis is predominantly vaccinia virus directed (fig. 2). In interferon-treated cells, however, all protein synthesis is markedly inhibited (fig. 2). It is not yet clear whether this overall inhibition reflects the sum of the inhibition of host protein synthesis by a component of the vaccinia virion [10] and of viral protein synthesis by interferon, or a blanket inhibition of all protein synthesis in the interferon-treated cell which is triggered by virus infection.

3.3. *Interferon mediated inhibition of virus protein synthesis in cell-free systems from vaccinia virus-infected cells*

Since the antiviral state was clearly established in these interferon-treated vaccinia virus-infected cells, extracts from them were assayed in the cell-free system and a marked inhibition of the translation of EMC RNA was observed (table 2). This was true provided that the extracts were not subjected to a preincubation step to reduce endogenous incorporation. Such preincubation reversed the inhibition and restored the system to full activity (table 2). On fractionation of the extracts the inhibition appeared to be predominantly (70 to 80%) associated with the ribosome fraction although some effect was seen with the cell sap (20 to 30%) (table 3). The polypeptide products synthesized in response to the EMC RNA in the three extracts in table 2 which showed a normal response to the RNA behaved identically on electrophoresis on SDS-polyacrylamide gels to those synthesized in preincubated extracts from *un*infected cells (fig. 1). Those synthesized in the system showing an inhibition of translation, i.e. the non-preincubated system from interferon-treated vaccinia-infected cells, were heterogeneous and of lower molecular weight. It would seem, therefore, that in these cell-free systems from interferon-treated infectec

Fig. 2. Effect of interferon treatment on protein synthesis in vaccinia virus-infected L cells. The polypeptides synthesized in: A) *un*infected cells, B) vaccinia virus-infected, and C) interferon-treated vaccinia virus-infected cells were analyzed by electrophoresis on SDS-polyacrylamide gels (Esteban and Metz, in preparation). Incorporation of [^{35}S]methionine was over a 20 min period 1 hr post infection. Autoradiographs of the sliced dried gels are shown.

Table 2
Translation of EMC RNA by extracts from vaccinia virus-infected cells: effect of interferon pretreatment of cells and of preincubation of the cell-free system.

Cells	Treatment of	Extract	[^{14}C] amino acid incorporation in response to EMC RNA with time (counts per min per 50 μl)*			
Interferon (50 units/ml)	Vaccinia virus infection	Preincubation (37°, 40 min)	10 min	40 min	90 min	150 min
–	+	+	100	680	3050	5070
+	+	+	160	630	3260	4740
–	+	–	500	2100	3420	4310
+	+	–	200	440	670	870

Post mitochondrial supernatant fractions were used without passage through Sephadex. Stimulation of incorporation was with 2 μg of EMC RNA. Incorporation in the absence of EMC RNA did not exceed a maximum of 200 counts per min.
* 50 μl samples were taken from each of four 0.5 ml incubation mixtures.

Table 3
Translation of EMC RNA in cell-free systems using ribosome and cell sap fractions from vaccinia virus-infected and interferon-treated vaccinia virus-infected cells.

Ribosomes* from	[^{14}C] amino acid incorporation in response to EMC RNA (counts per min per assay)	
	With cell sap* from	
	Infected cells	Interferon-treated infected cells
Infected cells	11,600	8,800
Interferon-treated infected cells	2,800	2,200

* The pellets (ribosome fraction) from centrifugation of the postmitochondrial supernatant fractions at 140,000 *g* for 1 hr were resuspended in 90 mM KCl, 3.5 mM Mg acetate, 30 mM Tris HCl pH 7.5 and 7 mM β-mercaptoethanol. The supernatants were the cell sap fractions. The incubations were equal amounts of the different ribosome and cell sap fractions for 120 min at 30° in the presence of 2 μg of EMC RNA. The values for amino acid incorporation in the absence of EMC RNA were 2100 and 2500 with the ribosomes from infected cells and 500 and 700 with those from interferon-treated infected cells, respectively, for the cell sap fractions from interferon-treated infected and infected cells.

cells, EMC virus protein synthesis is inhibited, the inhibition apparently affecting both the initiation and the translocation steps in the translation of EMC RNA. It is not yet clear whether the translation of globin mRNA is similarly affected for there is no significant stimulation of incorporation in response to this RNA in any of the nonpreincubated cell-free systems. While preliminary analysis of the product synthesized in the presence of this RNA would suggest that it is translated in all of these systems, a definitive answer will have to await the results of a more detailed study. The translation of polyuridylic acid was not inhibited in the system showing an inhibition in the translation of EMC RNA.

Control experiments showed that the difference in incorporation with and without interferon treatment (tables 2 and 3) cannot be explained on the basis of differences in pool sizes, nor were there gross differences in the levels of RNAase in the systems (L.A. Ball, unpublished results). The reduced response could not be augmented by the addition of excess EMC RNA. That the inhibitory effect on the translation of the EMC RNA was indeed interferon mediated was indicated by the fact that it was obtained with highly-purified interferon preparations from two independent laboratories (the second preparation was generously provided by Drs. E. Knight and R.Z. Lockart). The extent of the inhibition varied with the interferon concentration. A significant inhibition (65%) was observed on exposure of the cells to as little as 5 units/ml of interferon prior to infection. Inactivation of the antiviral

activity of the interferon preparation by heat or trypsin treatment abolished the effect observed in the cell-free system. It was not seen with cell-free systems from Krebs cells which are relatively insensitive to interferon, nor was it seen on treatment of L cells with chick interferon. Finally, the effect was not unique to the vaccinia-infected L cell system. Cell-free systems from interferon-treated L cells infected with EMC rather than vaccinia virus showed a similar reduction in their apparent ability to translate a viral RNA.

The inhibitory effect of interferon pretreatment upon the translation of a viral RNA observed here was dependent upon virus infection of the interferon-treated cell. In the absence of infection interferon-treatment resulted in only a slight inhibition of translocation in these L cell systems (Friedman et al., in preparation). A small effect of interferon pretreatment alone, without infection, has been reported previously from this laboratory for a chick cell-free system [1]. It may well be, therefore, that the antiviral state does not fully develop in the interferon-treated cell until after infection. Certainly the inhibition of translation of EMC RNA observed in these interferon-treated infected cell systems is quantitatively sufficient to account for the antiviral activity of interferon. The basis for this inhibition and its relation to the primary antiviral effect of interferon will, however, have to await the results of further study.

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References

[1] I.M. Kerr, J. Virol. 7 (1971) 448.
[2] W.K. Joklik and T.C. Merigan, Proc. Natl. Acad. Sci. U.S. 56 (1966) 558.
[3] I.M. Kerr and E.M. Martin, J. Virol. 9 (1972) 559.
[4] R. Williamson, M. Morrison, G. Lanyon, R. Eason and J. Paul, Biochemistry 10 (1971) 3014.
[5] I.M. Kerr, R.E. Brown and D.R. Tovell, J. Virol. (July 1972 issue) in press.
[6] K. Paucker, B.J. Berman, R.R. Golgher and D. Stancek, J. Virol. 5 (1970) 145.
[7] P. Dobos, I.M. Kerr and E.M. Martin, J. Virol. 8 (1971) 491.
[8] J.A. Sonnabend and R.M. Friedman, in: Interferons, ed. N.B. Finter (North-Holland, Amsterdam, 1966).
[9] J. Vilcek, Interferon; Virology Monograph No. 6 (Springer Verlag, Wien and New York, 1969).
[10] B. Moss, J. Virol. 2 (1968) 1028.