The Effect of Interferon on the Formation of Virus Polyribosomes in L Cells Infected with Vaccinia Virus

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SUMMARY

The effect of interferon treatment of mouse L cells on the fate of virus messenger RNA following infection with vaccinia virus has been studied. The polyribosomes of interferon-treated, infected cells are found to be disaggregated and it is proposed that this results from inhibition of the initiation of virus polypeptide synthesis. Evidence is presented that inhibition of polypeptide chain elongation also occurs. The block in initiation appears to be due to the failure of the small ribosome subunit to attach to the virus messenger ribonucleoprotein complex. The translation of the different vaccinia messenger species is inhibited to a comparable extent.

INTRODUCTION

There is evidence that the interferon mechanism may act in more than one way to inhibit virus replication. Inhibition of both early virus-specific translation and transcription has been reported (Joklik & Merigan, 1966; Levy & Carter, 1968; Oxman & Levin, 1971; Marcus et al., 1971; Bialy & Colby, 1972; Manders, Tilles & Huang, 1972; Metz & Esteban, 1972; Esteban & Metz, 1973b).

In the case of mouse L cells infected with vaccinia virus it seems clear that interferon pre-treatment inhibits the translation of virus messenger RNA (mRNA) but not its transcription (Joklik & Merigan, 1966; Metz & Esteban, 1972). In the untreated cell virus mRNA is rapidly transcribed from the genome of the incoming virus particle by the polymerase within the particle. This mRNA is then translated into virus polypeptides. Extensive virus RNA and protein synthesis is seen by 30 min post-infection. Interferon pre-treatment of the cells effects a substantial inhibition of virus polypeptide synthesis as early as 20 min post-infection. Virus RNA synthesis is not inhibited; indeed it is stimulated. All inhibitors of protein synthesis serve to stimulate vaccinia RNA synthesis, probably by blocking the synthesis of a regulatory protein. Thus, virus mRNA is made, but not utilized, in the interferon-treated cell.

In the preceding paper we have described the formation of virus polyribosomes in vaccinia-infected L cells (Metz, Esteban & Danielescu, 1975). In this paper we report the effect of interferon pre-treatment of the cells on these events and thereby extend the earlier studies of Joklik & Merigan (1966).

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**METHODS**

*Procedures* are as described in the preceding paper (Metz *et al.* 1975) with the following additions.

*Interferon.* Mouse interferon was kindly contributed by a number of workers. Most experiments employed highly purified material prepared by Dr K. Paucker (Paucker *et al.* 1970) having a sp. act. of at least $1.5 \times 10^7$ units/mg protein. We have also used materials with a comparable sp. act. prepared by Dr E. Knight and Dr R. Z. Lockart and some with a somewhat lower sp. act. prepared by Dr C. Bradish. These preparations all give essentially equivalent results as did unpurified mouse serum interferon. Chick interferon was a gift from Dr K. Fantes. Mouse interferon was titrated according to the method of Finter (1969), calibrated using the National Institutes of Health Mouse Serum Interferon Interim Reference Reagent. All units in this paper are expressed in terms of this standard. Approx. 15 such units were required to reduce the yield of vaccinia virus by 50% in a one-step growth cycle on L cell monolayers.

L cells were treated with interferon in suspension, at a concentration of $1.5 \times 10^6$ cells/ml in Eagle’s medium plus 10% calf serum for 4 h at 37°C. The cultures were then diluted with an equal vol. of medium and stirring was continued for a further 16 h. Control cells were treated similarly but with the omission of interferon. Cells were harvested and infected as described.

*Polyacrylamide gel electrophoresis.* The labelling of vaccinia-virus infected cells with $^{35}$S-methionine and the analysis of the virus polypeptides on sodium dodecyl sulphate-polyacrylamide gels has been described (Metz & Esteban, 1972; Esteban & Metz, 1973a). The Joyce-Loebel Densitometer was employed for densitometry of the autoradiographs.

*Measurement of ribosome transit time.* Interferon-treated and untreated cells were infected at a multiplicity of 500 particles/cell and diluted to $2 \times 10^6$ in Eagle’s Spinner medium, buffered with tris-HCl and containing 0.1 of the normal concentration of amino acids and 5% dialysed calf serum. At 45 min post-infection $^{35}$S-methionine was added to $5 \mu$Ci/ml and at 1 min intervals thereafter $1.0$ ml samples were pipetted into tubes of ice-cold Earle’s saline containing $1.5$ mg/ml methionine. Cells were centrifuged at 2000 rev/min for 5 min and resuspended in $1.0$ ml $10 \text{mm}-\text{NaCl}, 10 \text{mm}-\text{tris}, \text{pH } 7.4, 1.5 \text{mm}-\text{MgCl}_2$. Nonidet P-40 was added to 0.5% and, after mixing, the nuclei were removed by centrifuging at 2000 rev/min for 5 min. The cytoplasmic extracts from alternate samples were used to determine the amount of labelled soluble protein released from the polyribosomes by layering over $4.0$ m 15% sucrose in $10 \text{mm}-\text{NaCl}, 10 \text{mm}-\text{tris}, \text{pH } 7.4, 1.5 \text{mm}-\text{MgCl}_2$ and centrifuging in the SW 50.1 rotor at 50000 rev/min for 90 min. The supernatant fluid was decanted. Samples corresponding to equivalent numbers of cells from this high-speed supernatant fluid and from the cytoplasmic extracts which had not been centrifuged (from which the total labelled protein was determined) were adjusted to 0.1 N-NaOH. After a few minutes 100 µg of carrier DNA was added and protein and nucleic acid was precipitated by the addition of perchloric acid to 7%. After 1 h at 0°C, the precipitates were recovered on glass-fibre filters (Whatman GF/C), washed five times with 5 ml 6% trichloracetic acid, once with ethanol and once with ether. After drying in air, the radioactivity on the filters was measured in the scintillation counter.

*Reagents.* L-$^{14}$C-valine (270 mCi/mmol), L-$^{14}$C-isoleucine (310 mCi/mmol), and L-$^{35}$S-methionine (50 to 250 Ci/mmol) were obtained from the Radiochemical Centre, Amersham.
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Fig. 1. Polyribosomes from interferon-treated, vaccinia-infected cells. (a) and (b) Cells were treated with interferon (40 units/ml), or were untreated, as described in Methods. They were infected at a multiplicity of 400 and were diluted with medium containing actinomycin D (1 μg/ml). After 15 min, [3H]-uridine was added (2 μCi/ml) and at 45 min post-infection the cells were harvested. Cytoplasmic extracts from 30 × 10^6 cells were centrifuged through 5 to 30% sucrose gradients in the SW41 rotor at 36000 rev/min for 1.0 h at 4 °C. —— O, ct/min; —— E_260. (a) Untreated cells; (b), interferon-treated cells. In (b) the left-hand extinction scale applies to the left of fraction 9 and the right-hand scale applies to the remainder. (c) Cells were treated with or without interferon (50 units/ml) for 4 h at 1.5 × 10^6 ml. They were then diluted to 0.5 × 10^6 ml and to the interferon-treated cells was added [3H]-uridine (0.04 μCi/ml) and to the control cells [14C]-uridine (1 μCi/ml). After stirring at 37 °C for 16 h, the two lots of cells were mixed together at 37 °C for 20 min and then harvested. A cytoplasmic extract was prepared and centrifuged as in (a, b). △—△, [3H], ct/min; •—•, [14C], ct/min.

RESULTS

Polyribosome profiles from untreated and from interferon-treated, infected cells

The effect of interferon treatment on the polyribosome profile from vaccinia-infected cells is shown in Fig. 1. As originally reported by Joklik & Merigan (1966) interferon treatment (Fig. 1 b) results in an apparent disaggregation of the polyribosomes, compared with the control untreated cells (Fig. 1 a), as judged by the extinction profile. The relative distribution of label in virus RNA between the various size classes of polyribosomes and the 30S RNP (peak at fraction 4) also indicates disaggregation. In the typical experiment shown here the proportion of radioactivity in the larger polyribosomes (trimers and greater) decreased from 22% of the total in the untreated, infected cells to 14% in the interferon-treated, infected cells. There is a corresponding increase in the proportion of label in the RNP. This suggests that the virus RNA is partially inhibited from associating with ribosomes to form polyribosomes.

The extent of polyribosome disaggregation was found to be dose-dependent over the range tested of 5 to 50 units/ml of interferon. If the interferon was inactivated by trypsin treatment or by heating to 80 °C, or if the mouse interferon was replaced by chicken interferon, the disaggregation of polyribosomes was not observed.

Virus RNA in the polyribosomes of interferon-treated, infected cells was 70 to 80% sensitive to EDTA-treatment. The same proportion of polyribosome-associated virus RNA is EDTA-sensitive in untreated, infected cells (Metz et al. 1975).
Fig. 2. The effect of a low dose of cycloheximide on the polyribosomes from interferon-treated, infected cells. Cells were treated with interferon (50 units/ml) and infected at a multiplicity of 500. They were diluted into medium containing actinomycin D (1 μg/ml) and after 15 min were labelled with [3H]-uridine (5 μCi/ml). After a further 10 min cordycepin (40 μg/ml) was added and the culture was divided. To one half there was no addition while to the other cycloheximide (4 μg/ml) was added. After a further 40 min the cells were harvested, cytoplasmic extracts were prepared and centrifuged through 5 to 30% sucrose gradients in the SW 41 rotor at 37000 rev/min for 1 h at 4 °C. ○—○, radioactivity; ——, E₂₅₀. (a) No addition; (b) plus cycloheximide.

A control experiment which demonstrates that interferon treatment does not cause polyribosome disaggregation in uninfected cells is shown in Fig. 1 (c). Interferon-treated cells were labelled overnight with [14C]-uridine and untreated cells with [3H]-uridine. The two lots of cells were mixed together for 20 min at 37 °C, the mixture was then harvested, and a cytoplasmic extract was prepared and was analysed on a sucrose gradient. The polyribosome profiles of the uninfected, interferon-treated and untreated cells are identical.

The effect of interferon treatment on the initiation of polypeptide synthesis

Disaggregation of the virus polyribosome profile which is seen in interferon-treated cells might be caused by one or more of the following: (1) An increase in the rate of polypeptide chain elongation, the rate of chain initiation remaining unaltered. (2) Premature termination of polypeptide chains with attendant loss of ribosomes from messenger. (3) A decrease in the rate of chain initiation with no change in the rate of elongation. Since interferon treatment inhibits polypeptide synthesis (Metz & Esteban, 1972) the first possibility can be eliminated. The polypeptides that are, to a small extent, made in interferon-treated, infected cells are of normal size and do not consist of small fragments (Metz & Esteban, 1972 and Fig. 6).
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Fig. 3. Synthesis of nascent polypeptides on polyribosomes from interferon-treated and untreated, infected cells. Cells were treated with interferon (40 units/ml) or were left untreated, and were then infected at a multiplicity of 500. Infected cells were diluted into Eagle's medium containing 10% of normal amino acids, 5% dialysed calf serum and actinomycin D (1 µg/ml). At 10 min post-infection [3H]-uridine was added (1 µCi/ml). At 28-0 min post-infection [14C]-valine (3 µCi/ml) and [3H]-isoleucine (1-5 µCi/ml) were added and at 30-0 min cells were harvested, cytoplasmic extracts were prepared and these were centrifuged through sucrose gradients as described in Fig. 1. In a parallel experiment (not shown) cells were diluted with normal Eagle's medium and labelled with [3H]-uridine. The polyribosomal profiles were identical to those shown here, where medium with a reduced amino acid content was used. ○—○, [3H]-uridine; ●—●, [14C]-amino acids. (a) Untreated cells; (b) interferon-treated cells.

argues against the second possibility. The third possibility is that chain initiation is inhibited. If this is the case then the superimposition of an inhibition of movement of ribosomes along the mRNA should restore the polyribosome profile to a state approaching that in untreated, infected cells. This was attempted using a low dose of cycloheximide, the effect of which is to inhibit preferentially polypeptide chain elongation (Stanners, 1966; Fan & Penman, 1970; Lodish, 1971; Metz et al. 1975). In the experiment shown in Fig. 2, interferon-treated, infected cells were labelled with [3H]-uridine for 10 min. Then RNA synthesis was blocked by the addition of cordycepin (Metz et al. 1975) and to half the culture there was added cycloheximide (4 µg/ml) (Fig. 2b), while to the other half there was no addition (Fig. 2a). It will be seen that the effect of cycloheximide is to displace label from the RNP and the small polyribosomes into the medium sized polyribosome region of the gradient. Also the size of the single ribosome peak is reduced (as measured by the extinction profile) while the polyribosomal material is increased in amount. This experiment may be performed with the omission of cordycepin, in which case virus RNA synthesis continues during the cycloheximide treatment and thus displacement of mRNA from the RNP region cannot be demonstrated. Nevertheless, a relative increase in the proportion of mRNA in the medium...
Fig. 4. Ribosome transit time determination. The radioactivity in total-acid precipitable material and in soluble protein from the cytoplasm of interferon-treated (a) and untreated (b) infected cells is plotted against the time after the beginning of a pulse of [35S]-methionine. The procedure is described in Methods. Sixty units/ml of interferon were used. O—O, total acid-precipitable radioactivity; ●—●, radioactivity in soluble proteins.

sized polyribosomes is found following cycloheximide treatment. The experiment lends support to the argument that the polyribosome disaggregation effected by interferon treatment is due to the inhibition of initiation of virus polypeptide synthesis.

The effect of interferon treatment on polypeptide chain elongation

The effect of interferon treatment is to decrease the proportion of virus mRNA in the region of the larger polyribosomes in infected cells (Fig. 1). However, interferon treatment, as noted earlier, stimulates virus RNA synthesis. In the experiment depicted in Fig. 1, for example, there is 15% more radioactivity incorporated into the RNP and polyribosomes from the interferon-treated cells compared with the control. This stimulation compensates, to some extent, for the decreased proportion of label in the region of the larger polyribosomes (22%, reduced to 14% in the trimers and larger in this case). The net result is that the absolute amount of radioactivity in this region in this experiment is reduced by only 25% as a result of interferon treatment. In other experiments stimulation of RNA synthesis was often found to be greater with the result that the amount of label in the larger polyribosomes was barely, if at all, reduced. This occurred when polyribosomal disaggregation was observed as judged both by the extinction profile and by radioactivity in ribosomes following pre-labelling of cells overnight with [14C]-uridine prior to infection. It may be noted that incorporation of exogenous [3H]-uridine into acid-precipitable material has been shown to be a measure of the real rate of virus RNA synthesis in this system (Metz & Esteban, 1972).

This relatively small decrease in the absolute amount of virus RNA from the polyribosome region following interferon treatment was unexpected in view of the substantial inhibition of
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Table 1. Measurement of ribosome transit times in interferon-treated and untreated infected cells

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Interferon (min)</th>
<th>Untreated (min)</th>
<th>Inhibition (%)</th>
</tr>
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<tr>
<td>1</td>
<td>1.4</td>
<td>1.1</td>
<td>36</td>
</tr>
<tr>
<td>2</td>
<td>1.6</td>
<td>1.0</td>
<td>44</td>
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<td>3</td>
<td>1.5</td>
<td>1.0</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>1.7</td>
<td>1.0</td>
<td>66</td>
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*Ribosome transit times were determined as described in Methods at 45 min post-infection. The transit times, in minutes, are given for interferon-treated and untreated cells together with the inhibition of [35S]-methionine incorporation in the former compared to the latter. Expt. 4 in this Table is that depicted in Fig. 3.

The virus protein synthesis observed (Metz & Esteban, 1972 and Fig. 6), and it suggested that virus mRNA in polyribosomes was utilized with reduced efficiency. This implied that the rate of elongation of the nascent polypeptide chains was lower. In the experiment shown in Fig. 3 we examined the relationship between the rate of synthesis of the polyribosome-associated nascent polypeptide chains and the mRNA content of the polyribosomes. Infected cells were labelled with [3H]-uridine for 20 min, and then with [14C]-valine and [14C]-isoleucine for 20 min. In this way the ratios of nascent polypeptide to mRNA were compared. There is incorporation of radioactive amino acids into the region of the larger polyribosomes in the untreated, infected cells (Fig. 3 a) and this is substantially reduced by interferon treatment (Fig. 3 b). We have determined that the rate of uptake of radioactive amino acids into infected cells is not affected by interferon treatment, nor is the size of the endogenous pool of these amino acids altered (measured using the amino acid analyser). Thus, incorporation of radioactive amino acids into the nascent polypeptide chains can be used to compare rates of chain elongation in polyribosomes of comparable size. The effect of interferon treatment in the experiment shown in Fig. 3 is to reduce the ratio of [14C]- to [3H]-acid precipitable radioactivity by 70 % compared to the untreated cells.

Confirmation that the rate of chain elongation was decreased in interferon-treated, infected cells was provided by measuring ribosome transit times according to the method of Fan & Penman (1970). The transit time is the length of time required for a ribosome, after having become attached to a messenger RNA, to complete translation and release a finished polypeptide; it is independent of the rate of initiation (Fan & Penman, 1970). Fig. 4 shows the results of one experiment to measure transit times. Infected cells were pulse-labelled with radioactive methionine and at 1 min intervals after addition of the label samples were removed from the culture. Total radioactivity incorporated into protein and radioactivity incorporated into the soluble protein that had been released from the ribosomes were determined on alternate samples. The plots of these two quantities as a function of time yield parallel straight lines. It may be shown that the separation of these two lines, measured at the intercept with the time axis, is equal to half the weight-average transit time (Fan & Penman, 1970). Fig. 4(a) shows the result of a measurement for interferon-treated, infected cells which yields a weight average transit time of 1.7 min while Fig. 4(b) gives a figure for untreated, infected cells of 1.0 min. In this experiment the inhibition of amino acid incorporation due to interferon treatment was 66 %. The results from a series of such experiments are shown in Table 1. There is consistently a longer transit time for interferon-treated, infected cells and this is approximately proportional to the degree of inhibition of protein synthesis. A direct comparison of these transit times can only be made if the size distributions of the poly-
Fig. 5. Buoyant density analysis of fractions from polyribosome gradients prepared from interferon-treated or untreated, infected cells. Cells were treated with interferon (30 units/ml) or were untreated. Both lots were labelled with $[^{14}C]$-uridine (0.03 μCi/ml) during the overnight treatment. The cells were infected at a multiplicity of 500 and were diluted into medium containing actinomycin D (1 μg/ml). After 30 min they were each labelled with $[^{3}H]$-uridine (10 μCi/ml) for a further 20 min. The cells were harvested, cytoplasmic extracts were prepared and were centrifuged through 5 to 30% sucrose gradients in the SW 41 rotor at 39000 rev/min for 2-7 h at 4 °C. Fractions were collected in the cold and samples taken for estimation of acid-precipitable radioactivity. The 74 S ribosome peak had moved two-thirds the length of the gradient. A series of adjacent fractions were taken, from corresponding positions in the two sucrose gradients, across the region between 60 S and 80 S and these were fixed with glutaraldehyde and analysed on caesium chloride gradients. Buoyant density patterns of three adjacent fractions from the sucrose gradients of the interferon-treated cell material ((a), (b) and (c)) and the untreated cell material ((d), (e) and (f)) are shown. These fractions were taken from the leading edge of the 74 S ribosome material, with sedimentation coefficients decreasing from (a) to (c) and from (d) to (f). ○—○, $[^{3}H]$, ct/min; ●—●, $[^{14}C]$, ct/min.
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peptides made in the two situations are the same (Fan & Penman, 1970). Acrylamide gel electrophoresis of the polypeptides synthesized in interferon-treated and in untreated infected cells indicates that the size distributions are quite similar (Fig. 6) so that the transit time determinations can properly be compared.

The site of the inhibition of initiation

In the previous paper the existence of intermediates in the formation of virus polyribosomes was suggested (Metz et al. 1975). If the proposed interpretation is correct then the effect of interferon treatment on initiation could either be to inhibit the formation of the RNP from the virus RNA, or to block the attachment of the 40S ribosomal subunit, or to block the attachment of the 60S subunit to form the RNP-74S ribosome complex. These possibilities were tested by examining the effect of interferon treatment on the relative amounts of the components containing virus RNA which are detected when fractions from sucrose gradients of cytoplasmic extracts are analysed on caesium chloride gradients as described in the preceding paper (Metz et al. 1975). Polyribosomes were prepared from interferon-treated and control infected cells, both of which had been pre-labelled with [14C]-uridine overnight and briefly labelled with [3H]-uridine following infection. Samples from the sucrose gradient analysis (not shown, but similar to Fig. 1) were fixed with glutaraldehyde and centrifuged to equilibrium in caesium chloride. Typical results are shown in Fig. 5, where three adjacent sucrose gradient fractions from the 75 to 80S region from extracts of interferon-treated cells (a, b and c) and from control cells (d, e and f) were subjected to buoyant density analysis. The amount of the single 74S ribosome material, having density 1.52 g/ml and labelled exclusively with [14C]-uridine, increases from the leading edge of the peak in the sucrose gradient (a and d) to the centre (c and f). The RNP is present in fractions from the interferon-treated cells at the expected density (1.46 g/ml) but the ratio of this component to the other two possible precursors (1.46 and 1.48 g/ml) is significantly greater, compared with the untreated cells, while the ratio of the 1.46 and 1.48 g/ml components to each other appears unchanged, within the limits of resolution of the gradient. This suggests that interferon treatment inhibits the proposed association between the 40S ribosomal subunit and the virus RNP to form the complex with density 1.46 g/ml (Metz et al. 1975). This conclusion will only be valid if the availability of 40S subunits is not limiting. This is probably the case as is indicated by the experiment in which virus RNP could be displaced into the polyribosome region following treatment with cycloheximide (Fig. 2).

The effect of interferon on different vaccinia virus messenger species

Early vaccinia virus mRNA consists of a heterogeneous mixture of messenger species, each of which is presumed to code for a virus polypeptide. To see if there is any degree of differential sensitivity of the various messenger species to the interferon inhibition we have examined the relative amounts of the virus polypeptides with and without interferon treatment. We have described elsewhere the pattern of virus polypeptides seen when infected cells are labelled with [35S]-methionine shortly after infection and then analysed on sodium dodecyl sulphate-polyacrylamide gels (Metz & Esteban, 1972; Esteban & Metz, 1973a). Fig. 6 shows a densitometer tracing of an autoradiograph of a pair of such gels; one was loaded with extracts from untreated, infected cells labelled between 40 and 60 min post-infection (a), while the other shows material from interferon-treated, infected cells (b). The effect of interferon treatment is to reduce the synthesis of all the polypeptides to approx. the same extent, although the very large virus polypeptides with mol. wt. around 120000 appear to be disproportionately affected. There also appears to be a change in the ratio of a pair of
components having mol. wt. between 35,000 and 40,000 though it is difficult to know if this is a real effect because there may be a contribution from cellular polypeptide synthesis which may be insensitive to interferon treatment. However, in general it appears that the various mRNA species of vaccinia virus all have comparable sensitivities to the interferon mediated inhibition.

**DISCUSSION**

There is at present some uncertainty concerning the mechanism of action of interferon. A central question is whether interferon treatment results in the inhibition of virus trans-
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lation or transcription or both. We have previously presented evidence that, in vaccinia virus-infected cells, transcription is not inhibited by the interferon mechanism while translation of the virus messenger is blocked (Metz & Esteban, 1972; Esteban & Metz, 1973b). In the present paper we have shown that interferon treatment results in an apparent disaggregation of the polyribosomes, as judged by the extinction profile. This confirms the earlier observation of Joklik & Merigan (1966), who proposed that disaggregation of the cellular polyribosomes occurred also in normally infected cells but was masked there by the rapid reformation of polyribosomes on vaccinia virus mRNA. The finding that when virus RNA synthesis is blocked by cordycepin partial disaggregation of cellular polyribosomes occurs is consistent with this view (Metz et al. 1975). The relative distribution of virus mRNA between RNP and polyribosomes also reflects the disaggregation although the total amount of virus RNA made is greater in the interferon-treated cell than in the control cell (Joklik & Merigan, 1966; Metz & Esteban, 1972; Jungwirth et al. 1972).

While interferon treatment results in the disaggregation of polyribosomes in vaccinia-infected cells there is, in general, no such effect on uninfected cells. On occasion, however, a small degree of disaggregation has been observed in uninfected cells and this may be related to the reported ability of interferon preparations, at higher doses than used here, to inhibit cell division (Gresser et al. 1970; Knight, 1973).

The diminished efficiency of virus polyribosome formation in interferon-treated cells may be explained in part in terms of a decrease in the rate of virus polypeptide chain initiation. However, inhibition of initiation with the consequent disaggregation of the polyribosome profile is not sufficient to account for the observed reduction in virus polypeptide synthesis. Although the proportion of virus RNA found as polyribosomes is reduced in interferon-treated cells the stimulation of virus RNA synthesis that results from the inhibition of protein synthesis tends to compensate for this with the result that the absolute amount of virus polyribosomal RNA is only modestly decreased. The apparent discrepancy between the substantial reduction in virus polypeptide synthesis estimated directly from the results of polyacrylamide gel electrophoresis and the small reduction in the amount of polyribosome-associated virus RNA suggests that the rate of virus polypeptide chain elongation must be less in interferon-treated cells. A direct estimation of ribosome transit times in interferon-treated and untreated, infected cells lends support to this view.

While the precise magnitude of the inhibition of virus polypeptide chain elongation is difficult to establish, partly because of a small but uncertain contribution from residual cellular polypeptide synthesis, it is nevertheless clear that inhibition of elongation contributes in a qualitatively important way to the overall inhibitory effect. The conclusion of Joklik & Merigan (1966) that the primary effect of interferon treatment is the abolition of the ability of virus mRNA and ribosomes to combine to form polyribosomes, while correct, is thus only a partial explanation.

The inhibition of initiation caused by interferon seems to be greater than the inhibition of elongation because the polyribosome profile, which reflects the relative contributions of these factors, is disaggregated compared with untreated, infected cells. If inhibition of elongation were of relatively greater magnitude the profile would be shifted towards the larger components, as is the case following treatment with low doses of cycloheximide. In contrast, the action of interferon seems to resemble that of pactamycin which, at low doses, inhibits initiation to a greater extent than chain elongation (Lodish, Housman & Jacobsen, 1971; Stewart-Blair, Yanowitz & Goldberg, 1971). Indeed the effect of pactamycin on the relative distribution of vaccinia RNA in polyribosome gradients is indistinguishable from that of interferon (D. H. Metz, unpublished observations).
It was suggested in the previous paper that vaccinia polyribosome formation involves virus RNA associating first with cellular proteins to form the RNP complex to which the small and the large ribosomal subunits are added sequentially (Metz et al. 1975). If this is correct then the effect of interferon on initiation might involve a block at any or all of these stages. Analysis of fractions from the sucrose gradients of cytoplasmic extracts of infected cells on caesium chloride gradients indicates that the RNP is present in interferon-treated cells but its association with the small subunit appears to be inhibited.

The net result of interferon treatment in any system is an inhibition of virus yield. In principle it would only be necessary to block the formation of one or a few essential proteins to achieve this result. In the case of vaccinia virus, at least, this is not what occurs. The degree of inhibition of synthesis of all the virus polypeptides appear to be comparable. This implies that all the virus mRNA species have similar sensitivity to the interferon-mediated inhibitor. This inhibitor is thus able to recognize all the vaccinia messages as being viral and distinct from cellular messages. The molecular basis for this discrimination is not yet understood.

The evidence suggesting that chain elongation is inhibited in the interferon-treated cell argues against a mechanism involving only the modification of initiation factors. The results would be consistent with modification of the ribosomes, or some factor that remains associated with them during translocation. It may be that the effect of interferon treatment on elongation is only secondary to the primary action at the level of initiation. A modification of the ribosome that enables it to discriminate against virus messenger may, possibly through some contingent conformational change, affect translocation as well. Pactamycin, which binds to the 40S subunit, may act in this manner (MacDonald & Goldberg, 1970). Alternatively, if there were a general mechanism for tight coupling between translocation and initiation in eukaryotic cells then a primary inhibition by interferon on the former might give rise to the results observed. Other possibilities that have not been excluded are that the virus mRNA is modified after transcription, or that the proteins of the RNP complex are functional and are altered by interferon treatment.

The results of the present investigation do not allow a conclusion to be drawn concerning the precise locus of the action of interferon on the process of virus protein synthesis. Further progress in elucidating the mechanism of action of interferon will require the utilization of in vitro systems which synthesize specific polypeptides in response to added exogenous messenger. Subcellular fractions from interferon-treated cells can then be assayed for their ability to inhibit the translation of virus messenger but not of cellular messenger. The finding that an in vitro system prepared from vaccinia-infected L cells displays an interferon-mediated inhibition of the translation of EMC RNA represents progress in this direction (Friedman et al. 1972). Cell-free systems have also been prepared from interferon-treated but uninfected cells (Kerr, 1971; Falcoff et al. 1973; Gupta, Sopori & Lengyel, 1973). Since the behaviour of such in vitro systems may depend critically on the mode of preparation and assay it will be important to demonstrate that the action of interferon in vitro displays the same characteristics as the infected cell system described here. It is encouraging that the cell-free system prepared from interferon-treated, vaccinia virus-infected L cells displays inhibition of both the initiation and elongation of virus polypeptide chains (Kerr et al. 1974).

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