Inhibition of RNA and Protein Synthesis in Interferon-treated HeLa Cells Infected with Vesicular Stomatitis Virus

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SUMMARY

Synthesis of vesicular stomatitis virus RNA and protein is almost completely inhibited in HeLa cells treated with relatively high doses of human fibroblast interferon. With lower concentrations of interferon virus replication is inhibited, but near normal amounts of virus RNA are found in cells infected at a m.o.i. of 10. All the virus RNA species are found in these cells with the exception of genomic size RNA. In contrast, synthesis of all the virus proteins is equally inhibited in proportion to the interferon concentration used to treat the cells. This inhibition is due to a decline in the rate of protein synthesis, which occurs in interferon-treated cells sooner after infection than in untreated cells. The decreased rate of protein synthesis is accompanied by a change in the polysome pattern of infected cells, characterized by polysome run-off and increase in 80S ribosomes. At the same time, a larger proportion of the virus poly(A)-containing RNA is not associated with polysomes in interferon-treated cells than in control cells. The non-polysomal virus RNA has a sedimentation rate identical with that of polysomal virus RNA. Possible causes for the decline in the rate of protein synthesis observed in interferon-treated cells are discussed.

INTRODUCTION

In interferon-treated cells infected by vesicular stomatitis virus (VSV), accumulation of virus transcription products is reduced (Marcus et al. 1971; Manders et al. 1972; Repik et al. 1974; Baxt et al. 1977; Marcus & Sekellick, 1978; Thacore, 1978), synthesis of virus proteins is decreased (Baxt et al. 1977; Thacore, 1978) and virus replication is inhibited. The mechanisms involved have not yet been clarified. By infecting Vero cells with a ts mutant of YSV at a non-permissive temperature, Marcus & Sekellick (1978) showed that the rate of primary transcription is reduced by interferon treatment. Primary transcription is carried out by virion-associated transcriptase even in the absence of virus protein synthesis, as for example in cells infected in the presence of inhibitors of protein synthesis. Marcus et al. (1971) and Manders et al. (1972) had previously shown that VSV primary transcription is inhibited in interferon-treated cells in the presence of cycloheximide. Any reduction in the accumulation of primary transcripts in a negative-strand virus-like VSV, would result in a marked reduction in virus protein synthesis. Baxt et al. (1977) reported, however, that near normal amounts of virus RNA are present in interferon-treated monkey and human cells infected with VSV; only those virus RNA species, the synthesis of which is dependent on virus protein synthesis, were found in much reduced amounts. These results
are consistent with an inhibition of virus protein synthesis in interferon-treated cells (Baxt et al. 1977). In agreement with these observations, Thacore (1978) reported that in a simian cell line interferon did not inhibit VSV primary transcription though virus protein synthesis was completely inhibited. The action of interferon on virus transcription and/or translation may therefore depend to a large extent on the particular cell line studied (Thacore, 1978).

We have examined virus RNA and protein synthesis in HeLa cells treated with human fibroblast interferon and infected with VSV. Our purpose was to define the characteristics of the inhibition of virus RNA accumulation and protein synthesis in this system. We have obtained evidence for both a reduced rate of accumulation of virus transcripts and inhibition of protein synthesis in interferon-treated cells to an extent dependent on the m.o.i. and on the dose of interferon.

**METHODS**

**Cells.** HeLa cells were grown in suspension cultures in Joklik's modified minimal essential medium (MEM) with 5% horse serum. Cells in logarithmic growth were collected at about 5 × 10⁵/ml, concentrated by centrifugation and resuspended in fresh medium for virus infection.

**Virus growth and assays.** Wild type VSV (Indiana serotype) was a gift of Dr McSharry, Department of Microbiology, Albany Medical College. The virus was grown in HeLa cells, washed with serum-free MEM and resuspended at 10⁷/ml in MEM containing 14 mM-N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (Hepes)-KOH buffer, pH 7.2, and 2 mM-glutamine. VSV was added for 45 min at 30 °C at a m.o.i. of 0.01 to prepare seed stocks of virus, or at a m.o.i. of 1 to prepare high titre VSV stocks. Ten volumes of the same medium with 10% foetal calf serum were then added, together with 1 μg/ml of actinomycin D (Banerjee & Rhodes, 1973). The cells were incubated for 20 h at 37 °C, frozen and thawed three times and centrifuged at 30000 g for 10 min. The supernatant from cells infected at a m.o.i. of 1 constituted a standard virus stock preparation, which contained 4 to 8 × 10⁶ p.f.u./ml. The virus titre was determined by plaque assay on HeLa cells. Cells (1·5 ml at 1·2 × 10⁵/ml) were infected with 0·1 ml of suitably diluted virus stock and then mixed with 1 ml of 1·3% Noble agar (DIFCO) dissolved in Earle's salts solution and kept at 43°C. The cell suspension was poured over 10 ml of 1% agar in F-13 medium (GIBCO) with 10% foetal calf serum in 10 cm diam. tissue culture dishes. These were incubated for 2 to 3 days in 5% CO₂ before counting plaques.

**Interferon treatment and assay.** Human fibroblast interferon (3 × 10⁵ reference units/mg of protein) was obtained from the Interferon Working Group of the National Cancer Institute, NIH, Bethesda, Md. HeLa cells were treated with the indicated interferon concentrations for 17 h before infection with VSV. The antiviral effect of interferon was assessed by plaque assay and by reduction of infectious virus yield. The plaque assay was carried out as described above with interferon-treated and control cells, and virus dilutions which gave between 200 and 200 plaques per dish with control cells. The yield reduction assay was carried out by treating cells with different interferon concentrations and infecting them at a m.o.i. of 10. Eight h p.i. the cells were broken by freezing and thawing. The clarified (30000 g for 10 min) supernatant was assayed by the plaque method to determine the virus yield.

**RNA synthesis.** One ml of cells infected as described above was incubated for 20 min with 5 μg of actinomycin D and then for 30 min with 10 μCi of ³H-uridine (20 to 40 Ci/mmol). The cells were collected by centrifugation, washed twice with phosphate buffered saline at 0 °C and lysed in 0·5 ml of SDS-buffer (0·1 M-NaCl, 1 mM-EDTA, 0·5% sodium dodecyl
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sulphate and 10 mM-tris-HCl, pH 7.5). A sample was precipitated with an equal vol. of 20% trichloroacetic acid and collected on Whatman GF/A filters for counting. The remainder was applied to 15 to 30% sucrose gradients in SDS-buffer and centrifuged as indicated in the figure legends.

Protein synthesis. One ml of cells infected at a m.o.i. of 10 was collected by centrifugation and resuspended in medium prepared with dialysed horse serum, lacking both methionine and lysine. After 10 min at 37 °C, 10 μCi of either 35S-methionine or 3H-lysine were added. The cells incubated with 35S-methionine were diluted with 10 ml of ice-cold phosphate buffered saline, collected by centrifugation and lysed in 0.2 ml of 10 mM-KCl, 1.5 mM-magnesium acetate, 1 mM-dithiothreitol, 20 mM-Hepes-KOH buffer, pH 7.4, and 1% Triton X-100. The supernatant fluid obtained by centrifuging for 5 min at 30000 g was used for gel electrophoresis analysis. A 0.1 ml sample of cells incubated with 3H-lysine was directly spotted on Whatman 3MM papers filters and counted as previously described (Weber et al. 1975).

Gel electrophoresis. Samples (30 μl) from cells incubated with 35S-methionine were fractionated on 12.5% polyacrylamide gels according to Laemmli (1970). The dried gels were exposed to pre-fogged X-ray films and scanned through a recording microdensitometer (Laskey & Mills, 1975). The areas under peaks of virus proteins were determined and expressed in arbitrary units. Control experiments were carried out with different amounts of labelled proteins and different times of exposure of the autoradiographs to obtain a linear relationship between the amount of radioactive protein and the units of peak areas.

Polysome analysis and isolation of poly(A)-containing RNA. Cell extracts were prepared from 200 ml of cells infected as described above, collected by centrifugation, washed and homogenized as previously described (Weber et al. 1975). Cell extract (0.3 ml) was applied to 10 to 40% sucrose gradients in 10 mM-NaCl, 1.5 mM-MgCl2 and 10 mM-tris-HCl, pH 7.5, and centrifuged for 90 min at 200000 g in the SW41 rotor. The A260 was determined with a recording spectrophotometer. To isolate poly(A)-containing RNA, the cell extracts were fractionated by a modification of the method of Rose & Lodish (1976). Samples of 0.3 ml of cell extract were adjusted to 0.5 M-NaCl and 30 mM-magnesium acetate in a final vol. of 0.4 ml and layered on 4.5 ml 15 to 30% sucrose gradients in the same salts with 20 mM-Hepes-KOH buffer, pH 7.4. The gradients were centrifuged for 3 h at 240000 g in the SW50.1 rotor. Ten fractions were collected through a recording spectrophotometer and the bottom eight fractions containing ribosomal subunits (80S ribosomes are dissociated into ribosomal subunits under these conditions) were combined and used to measure A260 and poly(A)-containing RNA not bound to polysomes. The pellet containing polysomes was dissolved in 1 ml of SDS-buffer to determine A260. An equal vol. of 1% sodium dodecyl sulphate was added to the gradient fractions, and 1/20 or 1/10 vol. of 5 M-LiCl was then added to these fractions and to the re-dissolved polysome pellet respectively. The samples were heated to 60 °C for 1 min and immediately chromatographed on 0.5 x 0.4 cm columns of oligo(dT)-cellulose as previously described (Weber et al. 1979). A sample of the poly(A)-containing RNA was counted, while the remainder was analysed on sucrose gradients.

Experimental conditions. Unless otherwise stated HeLa cells were pre-treated for 17 h with the desired concentration of interferon and infected with VSV at a m.o.i. of 10.

RESULTS

When the antiviral effect of human fibroblast interferon was evaluated in VSV-infected HeLa cells by a plaque reduction test and by a yield reduction assay, the results were in good agreement (Table 1). From these data it can be calculated that about 35 units of the
Table 1. Effect of interferon concentration on vesicular stomatitis virus replication and virus RNA synthesis*

<table>
<thead>
<tr>
<th>Interferon concentration (units/ml)</th>
<th>Plaque reduction† (% of untreated)</th>
<th>Yield reduction‡ (% of untreated)</th>
<th>RNA synthesis§ (% inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>34</td>
<td>36</td>
<td>3</td>
</tr>
<tr>
<td>40</td>
<td>61</td>
<td>46</td>
<td>14</td>
</tr>
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<td>80</td>
<td>90</td>
<td>72</td>
<td>42</td>
</tr>
<tr>
<td>200</td>
<td>95</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

* For details of the experimental procedures see Methods.
† Plaque reduction: mean from several determinations with a VSV input giving 100 to 200 plaques per plate with untreated cells.
‡ The yield of virus was measured after infection at a m.o.i. of 10.
§ Virus RNA synthesis was measured between 1 and 8.5 h p.i. at the same m.o.i. Infected cultures were labelled with ³H-uridine; the RNA synthesis relative to VSV-infected untreated cells was used to calculate % inhibition (100 - ct/min interferon-treated/ct/min untreated × 100).

Human interferon used are equivalent to one PR₅₀ (VSV) unit measured in fibroblasts, indicating that: (i) HeLa cells are less responsive to the interferon used than fibroblasts, and (ii) the effective dose of interferon is about 35-fold less than the units shown in legends and text.

In preliminary experiments HeLa cells were treated before VSV infection with interferon concentrations higher than 100 units/ml. VSV replication was effectively suppressed by this treatment, and virus RNA and protein synthesis were almost undetectable (data not shown). For the following experiments, therefore, the cells were treated with interferon concentrations which, while effective in reducing the yield of infectious virus, still allowed substantial virus RNA and protein synthesis (Table 1 and Fig. 1 to 3).

RNA synthesis

Virus RNA synthesis was measured in VSV-infected cells by incubating the cells for 30 min periods with ³H-uridine after inhibition of host RNA synthesis with actinomycin D (see Methods). In control cells infected at a m.o.i. of 1 the peak of virus RNA synthesis occurred at about 5 h p.i., whereas in cells infected at a m.o.i. of 5 or 10 the peak of virus RNA synthesis occurred at about 3.5 h p.i. (Fig. 1). In interferon-treated cells, synthesis of virus RNA was markedly inhibited. At a m.o.i. of 1 or 5, virus RNA synthesis was considerably delayed and occurred over a longer time period without reaching a well-defined peak (Fig. 2a and b); at a m.o.i. of 10, there was a well-defined peak of synthesis which was delayed about 1 to 2 h relative to untreated cells. The total amount of virus RNA synthesized at this m.o.i. was reduced in proportion to the interferon concentration used to treat the cells (Table 1).

The RNA synthesized by interferon-treated and control cells infected at a m.o.i. of 10 was further characterized by sucrose gradient sedimentation. The patterns of the virus RNA synthesized at 4 to 4.5 and 5 to 5.5 h p.i. are shown in Fig. 2. The virus RNA sedimented in three peaks corresponding to genomic size RNA (38S, peak 1), L protein mRNA (28S, peak 2) and mRNA for the other four VSV proteins (12S to 16S, peak 3). In addition, some small mol. wt. RNA sedimented near the top of the gradients. The amount of virus RNA synthesized between 4 and 4.5 h was similar in interferon-treated and control cells, whereas at 5 to 5.5 h RNA synthesis was greater in interferon-treated cells because their peak of virus RNA synthesis is delayed (Fig. 2c). Virus RNA species of identical sedimentation value were synthesized by interferon-treated and control cells, except that interferon-treated cells did not synthesize appreciable amounts of genomic size RNA even at the peak of
RNA synthesis (Fig. 2e and f), or later (data not shown). Such a failure to synthesize 38S RNA has previously been observed (Baxt et al. 1977). We also observed a slight reduction in the synthesis of 28S virus RNA in cells treated with 80 units/ml of interferon (Fig. 2c and f).

**Protein synthesis**

Virus protein synthesis was next investigated in cells treated with different interferon concentrations and infected at a m.o.i. of 10. Cells were labelled with $^{35}$S-methionine at different times after infection and the labelled polypeptides were fractionated by gel electrophoresis (Fig. 3). The pattern of virus protein synthesis was the same in untreated VSV-infected cells and in cells treated with 20 to 80 units/ml of interferon before infection, but the amounts of the virus proteins synthesized decreased with the interferon concentration used. Virus protein synthesis was also estimated by scanning gel autoradiographs (Fig. 4). The peak areas of three VSV proteins synthesized throughout the first 5.5 h of infection were determined; similar data were obtained for the other two virus proteins (N and L), not shown in this figure.

The virus proteins were found in similar relative ratios in VSV-infected cells whether untreated or treated with different interferon concentrations. The rate of accumulation of virus proteins, however, decreased with the interferon concentration and there was three to
Fig. 2. Sedimentation analysis of the virus RNA synthesized by interferon-treated and control HeLa cells infected with VSV. Control cells and cells treated with 40 to 80 units/ml of interferon were infected at a m.o.i. of 10 and incubated for 30 min with \(^3\)H-uridine at (a) 4 and (b) 5 h p.i. The RNA was fractionated by sucrose gradient centrifugation for 17 h at 70000 \(g\) and precipitated with 5\% trichloroacetic acid for counting. The positions of marker 18S and 28S ribosomal RNAs are indicated by arrows.

Fig. 3. Electrophoretic analysis of the proteins synthesized after VSV infection in control HeLa cells and in cells pre-treated with interferon at the indicated concentrations. The cells were pulsed for 30 min with \(^35\)S-methionine at the times indicated. For other details, see Methods. The numbers on the left indicate three unidentified cellular proteins which are well separated from virus proteins. The letters on the right indicate the VSV proteins.
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Fig. 4. Synthesis of VSV proteins in control cells (■—■) and in cells treated for 17 h with 20 (●—●), 40 (▲—▲) and 80 (▼—▼) units/ml of interferon. The peak area was determined from scans of autoradiographs (see Fig. 3) and is expressed in arbitrary units. The cells were pulsed for 30 min with $^{35}$S-methionine at the indicated times after infection. For details of the analysis see legend of Fig. 3.

Fig. 5. Time course of protein synthesis in control cells and in cells treated with interferon and infected with VSV. (a) Control (■—■) and cells treated with 80 units of interferon (▼—▼). (b) Control infected cells (■—■) and cells treated with 40 (▲—▲) and 80 (▼—▼) units/ml of interferon before infection. The cells were pulse labelled with $^3$H-lysine for 1 h at the times shown (see Methods). The data obtained have been plotted as the cumulative amounts of protein synthesized in order to show the change in rate with time p.i. (no change was observed in the first 2 h p.i.).
Table 2. Partitioning of ribosomes and poly(A)-containing mRNA*

<table>
<thead>
<tr>
<th>Cell treatment</th>
<th>Interferon (units/ml)</th>
<th>VSV-infection</th>
<th>% of ribosomes in polysomes</th>
<th>% labelled poly(A)-containing RNA in polysomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>-</td>
<td>62</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>-</td>
<td>52</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>+</td>
<td>66</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>+</td>
<td>38</td>
<td>50</td>
</tr>
</tbody>
</table>

* The cells were infected at a m.o.i. of 10 and labelled between 2 and 5 h p.i. with 10 μCi/ml of 3H-uridine. In the experiments where VSV infection was omitted, the cells were labelled for 2 h with no actinomycin D added. The figures represent averages of duplicate experiments. See Methods for the fractionation of polysomes and the isolation of poly(A)-containing RNA.

four times less virus protein in cells treated with 80 units/ml of interferon than in untreated infected cells. This difference in the accumulation of virus protein most likely results from a different rate of synthesis and, even though we cannot exclude that turnover of virus proteins may also play some role, we will, for convenience, use the term ‘synthesis’ in the following sections.

It is important to note that the reduction in virus protein synthesis in interferon-treated cells is not accompanied by an increased synthesis of cellular proteins. The pattern of proteins synthesized in uninfected cells and in interferon-treated uninfected cells was identical (data not shown). A few clearly-defined bands can be distinguished above the high background due to the complex pattern of cellular protein synthesis. Three of these bands could be followed in infected cells, because they are well resolved from virus proteins (Fig. 3). They were uniformly diminished in both untreated and interferon-treated infected cells, regardless of the relative amount of virus protein synthesis. Therefore, there is a shut-off of host protein synthesis in cells treated with 20 to 80 units/ml of interferon and infected with VSV but, at the same time, synthesis of virus proteins is markedly inhibited.

Protein synthesis was also measured by determining 3H-lysine incorporation. In untreated and interferon-treated uninfected cells, protein synthesis proceeded linearly and at about the same rate (Fig. 5a). In VSV-infected cells, protein synthesis slowed down at about 5 h p.i., and in interferon-treated cells the rate of protein synthesis decreased even further, proportionally to the interferon concentration (Fig. 5b). This is in accordance with the observations that virus protein synthesis is reduced in these cells and that at the same time host protein synthesis is shut off. The overall rate of protein synthesis in interferon-treated cells declined earlier after infection than in untreated cells.

**Polysome pattern**

This observation was confirmed by examining the polysome patterns (Fig. 6). Fewer polysomes and more 80S ribosomes were present in interferon-treated infected cells at 5 h p.i. than in the other cells, perhaps because of an imbalance between the rate of polypeptide chain initiation and that of elongation (see Lodish, 1976). Since predominantly VSV proteins are synthesized at this time (Fig. 3), the polysome pattern reflects the relative rate of initiation and elongation of virus polypeptides.

The results presented are representative of repeated experiments. The same pattern of polysome run-off was observed in cells treated with 40 units/ml of interferon and infected with VSV. The distribution of ribosomes between polysomes and the non-polysomal fraction was also determined by a different procedure, which allowed a quantitative measurement of ribosomes as indicated in Table 2. The results were in agreement with those obtained by scanning sucrose gradients. This procedure was also used to prepare poly(A)-containing
RNA from the polysomal and non-polysomal fractions. The relative amount of virus poly(A)-containing RNA associated with polysomes in untreated and interferon-treated cells, and of cellular RNA in uninfected cells was determined by chromatography on oligo-(dT)-cellulose as described in Methods (Table 2). About 95% of the poly(A)-containing RNA was found associated with polysomes in uninfected cells. In cells infected with VSV approx. 65 to 80% of the virus RNA was found associated with polysomes. In cells treated with interferon and then infected with VSV, the proportion of virus RNA associated with polysomes was reduced to 40 to 60% of the total virus poly(A)-containing RNA. The decrease in the relative amount of ribosomes present in polysomes correlated with an increased amount of virus RNA not associated with polysomes.

This was further confirmed by analysing the RNA prepared from the polysomal and non-polysomal fractions of interferon-treated cells. The cells were pulsed with 3H-uridine from 2 to 5 h p.i. and the RNA analysed by sucrose gradient sedimentation to display the 12 to 16S RNA coding for four of the VSV proteins (Fig. 7). Synthesis of virus RNA was reduced in interferon-treated cells during this labelling time (see Fig. 2), but the sedimentation pattern of RNA associated with polysomes was indistinguishable from that of the non-polysomal fraction. The virus RNA from interferon-treated cells sedimented identically to RNA from untreated infected cells, though the ratio of polysomal to non-polysomal RNA was quite different in the two samples analysed. We concluded that treatment with interferon does not appreciably change the size of the virus mRNAs in infected cells, confirming the previous results of Manders et al. (1972), Baxt et al. (1977) and Marcus & Sekellick (1978), but alters their association with ribosomes by mechanism(s) which have not yet been elucidated.

**DISCUSSION**

In interferon-treated HeLa cells infected with VSV, the accumulation of virus transcription and translation products is inhibited in a characteristic way. At a m.o.i. of 1 and at relatively high interferon doses, the accumulation of transcription products is drastically reduced, whereas at a m.o.i. of 5 to 10 and at lower interferon doses virus transcripts are
found in near normal amounts. However, RNA is accumulated at a slower rate relative to control cells.

It seems likely that the inhibition of primary transcription reported by Marcus et al. (1971), Manders et al. (1972) and Marcus & Sekellick (1978) may account for the reduction by interferon in the accumulation of virus transcripts at low m.o.i. Inhibition of primary transcription would result in reduced synthesis of virus proteins and could effectively block the amplification of virus RNA synthesis and production of new virus. This effect on primary transcription, however, is not sufficient to block amplification in cells infected at a higher m.o.i. As previously observed by Baxt et al. (1977) and by Thacore (1978), virus transcription products are accumulated in some cell lines in near normal amounts, but production of infectious virus is effectively inhibited, indicating that antiviral mechanisms other than the inhibition of primary transcription may be involved in reducing virus yield.

In agreement with Baxt et al. (1977) and Thacore (1978), we find that synthesis of VSV proteins is reduced even when near normal amounts of virus mRNAs are produced. Therefore, synthesis of genomic size RNA, which is dependent on the synthesis of virus protein, is drastically reduced in interferon-treated cells. Presumably, sufficient amounts of virus protein have to accumulate before replication of genomic size RNA and virus assembly can proceed. For example, the M protein has a regulatory function on transcription: group III ts mutants of VSV synthesize an altered M protein at non-permissive temperatures, which is degraded and cannot act as a regulator; they produce two- to fivefold more virus mRNA than wild type virus without an increase in genomic RNA synthesis (Clinton et al. 1978).

In interferon-treated cells infected with VSV under the experimental conditions such as illustrated in Fig. 5, both cellular and virus protein synthesis are drastically reduced.
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approx. 5 h p.i. This general inhibition of protein synthesis is reflected by the reduced polysome content of interferon-treated infected cells (Fig. 6) and by the failure of a relatively large proportion of virus mRNA to associate with polysomes (Table 2).

This inhibition of protein synthesis may be due to the activation of translational inhibitors in interferon-treated cells. At least two enzymic activities are induced by interferon in a variety of cells, a 2'5'oligo(A) polymerase and a protein kinase (reviewed by Baglioni, 1979). The 2'5'oligo(A) polymerase synthesizes a series of oligonucleotides (Kerr & Brown, 1978) which activate an endonuclease; the protein kinase phosphorylates and inactivates a factor necessary for the initiation of protein synthesis (for references, see Baglioni, 1979). Both these enzymes require the presence of double-stranded (ds) RNA and ATP for activity. A partially dsRNA structure has been isolated from cells infected by VSV under conditions whereby virus RNA replication is the predominant synthetic event (Wertz, 1978) and possibly this may activate the enzymic activities induced by interferon.

The VSV mRNA accumulated in interferon-treated cells is of normal size and we have been unable to detect products of virus mRNA breakdown (unpublished data). This suggests, though by no means proves, that the endonuclease activated by 2'5'oligo(A) is not the primary cause of the inhibition of protein synthesis observed late in the infectious cycle. Perhaps the protein kinase may be involved in this inhibition, since a decreased rate of initiation would promote polysome run-off and decrease the rate of mRNA entry into polysomes, the main features of the inhibited protein synthesis observed in interferon-treated cells at about 5 h p.i. In a recent investigation, however, Gupta (1979) failed to detect increased levels of activated protein kinase in interferon-treated cells infected by VSV. Possibly, under the conditions of these experiments, protein synthesis was not significantly inhibited (no study of the rate of protein synthesis was reported) and the protein kinase was not activated. Perhaps this activation occurs only when enough dsRNA structures involved in virus replication have been formed.

Inhibition of protein synthesis was previously observed by Metz & Esteban (1972) in L cells treated with interferon and infected with vaccinia virus. The polyribosomes were found disaggregated in these cells, and Metz et al. (1975) suggested that this resulted from inhibition of the initiation of polypeptide synthesis. The vaccinia RNA synthesized in infected cells contains a small amount of dsRNA (Colby & Duesberg, 1969; Duesberg & Colby, 1969) and this RNA may be responsible for the reduced protein synthesis observed by Metz & Esteban (1972).

Other explanations for the inhibition of VSV mRNA translation in interferon-treated cells cannot be excluded at this time. An impairment of mRNA cap methylation has been reported by Desrosiers & Lengyel (1977) in interferon-treated L cells infected by reovirus. The virus mRNA synthesized in these cells contains 50% less 'cap II' relative to control cells. Muthukrishnan et al. (1978) have shown, however, that the 2'-o-methylation characteristic of cap II has, at most, a minor effect upon mRNA binding to ribosomes under in vitro conditions, since some preferential binding of vaccinia mRNAs containing cap II was observed only at high input mRNA concentrations. Further analysis of the virus mRNA synthesized in interferon-treated cells infected by VSV is presently in progress in our laboratory. Such an analysis and further investigations on the activity of initiation factors isolated from these cells is necessary to pinpoint the cause(s) of the inhibition of protein synthesis reported here.

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