Interferon Induction by Viruses. XII. Inhibition of Protein Synthesis Renders Aged Chick Embryo Cells Refractory to Interferon Induction

By CHARLES SVITLIK AND PHILIP I. MARCUS*

Microbiology Section, U-44, University of Connecticut, Storrs, Connecticut 06268, U.S.A.

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SUMMARY

The initial stages of interferon induction in mouse L cells do not require protein synthesis; all steps in the induction process up to and including the transcription of interferon mRNA occurred in the presence of inhibitors of protein synthesis. In contrast, interferon induction in primary chick embryo cells aged in vitro does require a reaction that depends upon protein synthesis, unique in that it is not required for virus replication or the action of interferon.

Interferon (IFN) induction by viruses in non-lymphoid cells results from the concerted interaction of the inducer dsRNA and the putative recognition system of the host cell (Marcus, 1983, 1985a). Once an inducer molecule is presented to the host cell some mechanism must exist for its recognition, and for the eventual transmission of a signal to promote transcription of the IFN genes. In addition to post-transcriptional regulation of IFN synthesis (Tan et al., 1970; Vílíček & Havell, 1973), the host cell may regulate IFN induction in response to the number and kinds of virus particles infecting the cell (Marcus, 1982, 1983, 1985a). Clearly, IFN induction is highly regulated.

When mouse cells are induced with Newcastle disease virus (NDV) to make IFN, formation and presentation of inducer molecules to the cellular recognition system and processing to the stage of IFN mRNA transcription does not require protein synthesis, an observation first made by Dianzani et al. (1970) and confirmed in this communication. NDV can complete all of these steps in the induction process during protein synthesis inhibition by cycloheximide (50 μg/ml). After addition of actinomycin D and reversal of the cycloheximide block, a normal yield of IFN is produced by NDV-infected mouse L(Y) cells. Since NDV is a negative-strand RNA virus, this inducing capacity is attributed to formation of an IFN inducer moiety following transcription of the virus genome by the virion-associated transcriptase of the input virus (Marcus et al., 1983).

In mouse L cells neither the viral functions necessary for inducer formation nor the cellular recognition system which mediates the transcription of IFN mRNA, require protein synthesis.

While studying IFN induction by NDV in primary chick embryo cells (CEC) 'aged' in vitro to enhance their IFN producing capacity (Carver & Marcus, 1967), we were surprised to find that NDV did not appear to induce transcription of IFN genes during protein synthesis inhibition. This result was in contrast to that obtained when mouse L cells were used as hosts. We now report that viral IFN inducers of all types (positive-strand, negative-strand or preformed dsRNA) fail to induce efficiently in aged CEC when protein synthesis has been inhibited by the action of cycloheximide for 6 h before or during infection/induction, even though virus replication proceeds normally and the action of IFN is unaffected following removal of the drug.

The data presented in Table 1 compare the ability of different viruses to induce transcription of IFN mRNA in mouse L(Y) and aged CEC in the presence or absence of protein synthesis, as deduced from the yield of IFN produced upon reversal of protein synthesis inhibition. The experimental design is similar to that described by Dianzani et al. (1970). Cell monolayers were infected in the presence of 50 μg/ml cycloheximide (a dose that inhibits protein synthesis by over 99%; Marcus et al., 1971). At 5.5 h post-infection, actinomycin D was added at a concentration that blocked cellular transcription of IFN mRNA, but not its translation. These doses are specific for each cell type, and had no effect on cell integrity or virus replication. Cycloheximide...
Short communication

Table 1. Interferon induction and virus replication in host cells treated with cycloheximide during the first 6 h of infection*

(a) Interferon induction in mouse L(Y) and aged CEC

<table>
<thead>
<tr>
<th>Cell</th>
<th>Virus</th>
<th>No drug</th>
<th>Actinomycin D</th>
<th>Cycloheximide</th>
<th>Actinomycin D + cycloheximide</th>
</tr>
</thead>
<tbody>
<tr>
<td>L(Y)</td>
<td>NDV (NJ LaSota)</td>
<td>59500</td>
<td>58000</td>
<td>55000</td>
<td>57500</td>
</tr>
<tr>
<td></td>
<td>NDV (AV)</td>
<td>12000</td>
<td>12900</td>
<td>12500</td>
<td>13000</td>
</tr>
<tr>
<td>CEC</td>
<td>NDV (AV)</td>
<td>32000</td>
<td>33200</td>
<td>720</td>
<td>790</td>
</tr>
<tr>
<td></td>
<td>VSV (T1026R1)</td>
<td>32000</td>
<td>33000</td>
<td>1200</td>
<td>1100</td>
</tr>
<tr>
<td></td>
<td>VSV (DI-011)</td>
<td>10200</td>
<td>11050</td>
<td>180</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>Sindbis</td>
<td>15000</td>
<td>16000</td>
<td>1800</td>
<td>1500</td>
</tr>
</tbody>
</table>

(b) Replication of NDV (AV) in aged CEC†

<table>
<thead>
<tr>
<th></th>
<th>Cycloheximide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque titre (PFP/ml)</td>
<td>3·8 × 10⁸</td>
</tr>
<tr>
<td>Virus yield (PFP/10⁷ cells)</td>
<td>2·4 × 10⁵</td>
</tr>
</tbody>
</table>

* Replicate monolayers of 4 × 10⁶ L(Y) cells (aged 4-5 days in vitro) or 1 × 10⁷ primary CEC (aged 6 or 7 days in vitro) were treated with growth medium (MEM + 4% calf serum or AS medium, respectively) with or without 50 μg/ml cycloheximide for 30 min prior to infection and during infection at 37·5 °C for 6 h. At 5·5 h, one set of plates received actinomycin D [L(Y), 0·2 μg/ml; CEC, 0·02 μg/ml]. All monolayers were washed five times at 6 h post-infection with prewarmed medium without serum and then maintained in 3 ml of growth medium lacking serum. The action of cycloheximide was completely reversible, as no residual inhibition of protein synthesis was detected after washout of the drug (data not shown). Actinomycin D treatment sufficed to prevent IFN production in either mouse L(Y) or primary CEC if added at the time of infection. No carry-over of actinomycin D from the induction phase of the experiment to the assay was observed. L(Y) cell cultures were incubated at 37·5 °C and CEC at 40·5 °C for 24 h. Supernatant medium was then harvested and assayed for IFN using a cytopathic effect inhibition assay (Marcus et al., 1983). Our standard procedure for IFN induction and IFN-inducing particle characteristics for each virus–cell system have been described [NDV, Marcus et al. (1983); VSV T1026R1, Marcus & Sekellick (1980); VSV DI-011, Marcus & Sekellick (1977); Sindbis virus, Marcus & Fuller (1979)].

† For plaque formation, replicate monolayers containing 1 × 10⁷ aged CEC were treated, or not, with cycloheximide (50 μg/ml) for 30 min, and were then infected with NDV (AV) for 6 h in the presence of the drug. Monolayers were washed three times with prewarmed AS medium and overlaid. For virus yield, monolayers of aged CEC were infected at mₚₜₚ = 3 NDV (AV) with cycloheximide present (or absent) for 6 h and then anti-NDV serum was added for 30 min and washed out. Virus was harvested 15 h later and assayed on young CEC.

was washed out at 6 h post-infection, and cells were maintained in actinomycin D-containing medium for the duration of the experiment. Thus, induction of IFN mRNA transcription could only occur during a 5·5 h time period, before the addition and irreversible action of actinomycin D. IFN yields were measured 24 h post-infection and represent transcription for 5·5 h followed by 18 h of translation. For each virus–cell system, 6 h was ample time for completion of transcription of IFN mRNA, as was 18 h for translation. Control experiments in which cells were treated with either actinomycin D or cycloheximide are also shown. Cycloheximide treatment alone did not induce IFN (data not shown; Tan & Berthold, 1977).

In mouse L(Y) cells both the AV and NJ LaSota strains of NDV induced normal yields of IFN whether the cells received treatment with actinomycin D, cycloheximide or both. Strain NJ LaSota consistently induced a fivefold higher yield of IFN over that of strain AV (Marcus, 1985b).

As recorded in Table 1, when aged CEC were used as hosts, very different results were obtained. In the absence of cycloheximide, whether actinomycin D was present or not, all viruses tested induced high yields of IFN. However, when cycloheximide was present during the first 6 h of infection, CEC produced 10- to 50-fold less IFN, irrespective of the addition of actinomycin D. These results demonstrate that there is a marked reduction in the efficiency with which viral inducers of IFN register in aged CEC when they are presented during conditions of protein synthesis inhibition. An experiment critical to the interpretation of these results required determining whether this 6 h treatment with cycloheximide also prevented virus
Table 2. Effect on interferon induction, virus replication and interferon action of cycloheximide added 6 h before infection of aged CEC with NDV (AV)*

<table>
<thead>
<tr>
<th>Cycloheximide (50 μg/ml)</th>
<th>Interferon yield (Units/10^7 cells)</th>
<th>Plaque titre (PFP/ml)</th>
<th>Virus yield (PFP/10^7 cells)</th>
<th>Interferon titre (Units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>−</td>
<td>33000</td>
<td>3.2 × 10^8</td>
<td>3.3 × 10^5</td>
<td>3800 ± 170</td>
</tr>
<tr>
<td>+</td>
<td>660</td>
<td>3.5 × 10^8</td>
<td>25.0 × 10^5</td>
<td>3800 ± 200</td>
</tr>
</tbody>
</table>

* Aged CEC were exposed, or not, to 50 μg/ml cycloheximide for 6 h before infection. Following a thorough washout of the drug, the procedures used for infection/induction with NDV were as described in the footnote to Table 1 and in Marcus et al. (1983). IFN action (titre) was measured by a modification of the cytopathic effect inhibition assay (Sekellick & Marcus, 1985), and is expressed as the mean and standard deviation from three different experiments.

replication. The data in Table 1 demonstrate that while cycloheximide inhibited some cellular activity required for IFN induction it did not adversely affect virus replication; in fact, it enhanced it. Thus, cycloheximide treatment which reduced IFN induction by NDV (AV) 44-fold enhanced by 9.2-fold a single cycle yield of virus produced by aged CEC, and had no effect on NDV plaquing efficiency although plaque size was discernibly increased. The enhanced yield of virus most likely reflects a decrease in endogenously induced IFN (Nagai et al., 1981).

Data presented in Table 2 demonstrate that the 6 h period of protein synthesis inhibition that renders aged CEC refractory to IFN induction by viruses need not be restricted to the period of virus infection. When aged CEC were exposed to cycloheximide for 6 h prior to infection (and then washed out) the cell's capacity for IFN induction was reduced severely (50-fold reduction in yield). This same treatment had no detrimental effect on the cell's capacity to replicate NDV; indeed, it was increased 7.6-fold. Plaquing efficiency was unaffected.

Table 2 also shows that IFN action was not affected by the same cycloheximide treatment that blocked IFN induction/production. Thus, expression of the cellular genes associated with the action of IFN appears to be normal after removal of the protein synthesis inhibitor. Exposure of chick cells to cycloheximide during or after IFN treatment stabilizes both 2',5'-oligoadenylate synthetase (West & Ball, 1982) and the resultant antiviral state (Lab & Koehren, 1976).

Since the plaquing efficiency and replication of NDV are not affected adversely by transient protein synthesis inhibition in aged CEC, it seems clear that the early events in virus entry and processing (Svitlik & Marcus, 1984) are not the basis of the observed refractory state to IFN induction. Reactions unique to IFN induction and presumably not concerned with virus replication must be involved: for example (i) formation of the proximal IFN inducer molecule, dsRNA (Marcus, 1983, 1985a), (ii) dsRNA interaction with the cell's putative recognition system of IFN inducer receptors (Marcus, 1983, 1985a), (iii) the generation of any 'signals' therefrom for promoting transcription of the IFN genes, (iv) translation of IFN mRNA, or (v) processing and export of the IFN molecule.

Although not ruled out experimentally, it seems difficult to imagine formation of dsRNA as the rate-limiting step in cells transiently inhibited for protein synthesis, especially since vesicular stomatitis virus (VSV) [±]DI-011 particles (Table 1) with the potential to form dsRNA without any synthetic events (Marcus & Sekellick, 1977) and avian reovirus (data not shown) with its complement of preformed dsRNA (Winship & Marcus, 1980) are rendered ineffective as inducers in cycloheximide-treated cells. We have also observed that the duration of the protein synthesis inhibition which renders aged CEC refractory to the inducers tested varies considerably from one virus to another, and in most cases is less than 6 h. In addition, establishment of the refractory state is not limited to the action of cycloheximide, as puromycin also renders chick cells refractory to IFN inducers (data not shown). Out of ignorance of the nature of the 'signals' used to promote transcription of the IFN genes, and of whether translation of IFN mRNA is affected, we favour some failure in the cell's putative recognition system for responding to dsRNA as the proximal IFN inducer moiety (Marcus, 1983, 1985a). One possibility is that some short-lived factor (protein?) is not readily renewable in aged CEC when its concentration falls below some threshold level. Further study is required to resolve this problem.
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REFERENCES


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