Degradation of Cellular Ribonucleic Acid in Newcastle Disease Virus Infected Cells

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(Accepted 8 August 1968)

SUMMARY

Following Newcastle disease virus infection, the synthesis of both cellular RNA and DNA in chick embryo fibroblast monolayers was inhibited. In such monolayers, Newcastle disease virus infection did not significantly reduce the content of cellular DNA within 10 hr, but did reduce the amount of cellular RNA by about 50% in that time. A part of the RNA was degraded into acid-soluble material and leaked out of the cells. Some fraction of the ribosomal RNA gave rise to small molecular weight fragments which sedimented near the transfer RNA region as determined by sucrose density gradient centrifugation. Evidence is presented indicating that virus-induced proteins, which were synthesized by 6 hr after infection, were involved in the RNA degradation process.

INTRODUCTION

The stability of the nucleic acids in virus-infected cells varies from one cell + virus system to another. The DNA of rabbit kidney cells infected with pseudorabies virus (Kaplan & Ben-Porat, 1963) and the RNA of L cells infected with the Columbia SK group of small RNA viruses (Hausen & Verwoerd, 1963) have been reported to be stable and not degraded as a result of virus infection. On the other hand, the RNA of HeLa cells was degraded into acid-soluble components during poliovirus infection (Salzman, Lockart & Sebring, 1959). The results of our experiments show that cellular RNA but not DNA is degraded in chick cells infected with Newcastle disease virus.

METHODS

Chemicals. 5-[3H]uridine (31,000 mc/mM), [3H]thymidine (13,000 mc/mM), and [14C]Leucine (200 mc/mM) were obtained from New England Nuclear Corp., Boston, Mass. Cycloheximide was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio. Actinomycin D was a gift from Karl Pflister of the Merck, Sharp and Dohme Laboratories, Rahway, N.J.

Cells and viruses. The preparation of chick embryo fibroblast monolayers and the media used were described by Wilson & LoGerfo (1964). The TEXAS (GB) strain of Newcastle disease virus was grown in the allantoic sac of 11-day-old White Leghorn embryos for 48 hr. The virus was collected by two cycles of high- and low-speed centrifugation (26,340 g for 40 min. and 2500 g for 10 min.), and the virus pellets were resuspended in phosphate buffered saline at pH 7.0 before use. The chick embryo fibroblast monolayers were infected with Newcastle disease virus at an average titre of 300 p.f.u./cell. The period of virus adsorption was 30 min. Incubation was at 39°C.
Chemical and isotopic analysis. The synthesis of RNA and DNA in chick embryo fibroblast monolayers was measured by the incorporation of 5-[3H]uridine and [3H]-thymidine into RNA and DNA of the cells at various times after infection. To study the stability of RNA and DNA of infected and uninfected cells, chick embryo fibroblast monolayers were prelabelled with 5-[3H]uridine (0.15 μC/ml.) and [3H]thymidine (0.2 μC/ml.) for 18 hr before infection. The amount of labelled RNA and DNA in the cells was measured at different times after infection. In some experiments, actinomycin D (10 μg./ml.) was included in the overlay medium after infection. To analyse RNA and DNA, the cultures were scraped with a rubber policeman from the bottom of the culture dishes into the overlying culture fluid. The resuspended cells were then transferred to tubes and centrifuged at 2500 g for 10 min. RNA and DNA of the cells were extracted and separated according to the method of Schmidt & Thannhauser (1945). The pellets of the 2500 g centrifugation were extracted once with 10% trichloracetic acid (w/v), once with 5% trichloracetic acid (w/v) at 0°C, once with 95% ethanol and once with ether at room temperature. The pellets were then hydrolysed with 0.3 N-KOH at 37°C for 18 hr. RNA and DNA were separated by precipitation at 0°C with cold perchloric acid. The separation of RNA from DNA was quite complete as shown by the fact that cross-contamination between RNA and DNA, measured by the appearance of [3H]thymidine label in the RNA fraction or 5-[3H]uridine in the DNA fraction, was less than 2% of the total activity. To measure the radioactivity recovered in the acid-soluble fraction of the medium, the supernatant fluid of the 2500 g centrifugation was precipitated with an equal volume of 10% trichloracetic acid. The radioactivity in both RNA and DNA of the cells and the acid-soluble fraction of the medium was determined with a liquid scintillation counter (Nuclear Chicago). Cell protein synthesis was measured by exposing cultures to [14C]L-leucine (0.1 μC/ml.) for 30 min. The cultures were prepared for counting as described above. In some experiments cycloheximide (20 μg./ml.) was added to the overlay medium after infection and removed by washing the cells with phosphate buffered saline immediately before the radioactive leucine was added.

Sucrose-gradient analysis. For analysis by sucrose gradient centrifugation, RNA was extracted from the chick-embryo-fibroblast monolayers with phenol and sodium dodecyl sulphate (Scherrer & Darnell, 1962). The extracted RNA was shaken several times with ether to remove the phenol and then precipitated twice with two volumes of 95% ethanol. The precipitates were dissolved in a small volume of 10⁻² M acetate buffer at pH 5.1 containing 5 x 10⁻² M-NaCl, 10⁻⁴ M-NaCl, 10⁻⁴ M-Mg²⁺ and 2 μg./ml. polyvinyl sulphate. The dissolved RNA was layered over a 5% to 20% linear sucrose gradient. After centrifugation at 35,000 rev./min. in the SW 39 rotor of a Spinco Model L ultracentrifuge for 5 hr, fractions were collected. Optical extinction and radioactivity were then determined for each fraction of the gradient.

RESULTS

Stability of nucleic acids in infected cells

The stability of cell RNA following virus infection was examined. Cell RNA was labelled by exposing cultures to 5-[3H]uridine for 18 hr. The isotope was removed from the culture medium at the time of infection. At different times after infection the amount of radioactivity in the RNA of prelabelled cells was measured. The amount
of labelled RNA in uninfected cells showed little change with time, but in virus-infected cells a decrease in labelled RNA was observed at 8 and 10 hr after infection (Fig. 1).

Since cell RNA synthesis is inhibited in cells infected with the Texas strain of Newcastle disease virus (Wilson, 1968), one would expect the amount of cell RNA to decrease with time after infection. However, the virus-induced decrease in cell RNA is not entirely a result of the viral inhibition of cell RNA synthesis. This was demonstrated by adding the inhibitor actinomycin D to the overlay medium (Fig. 1). As expected, the total amount of RNA in the actinomycin-treated cells decreased with time after infection (Wiesner, Reich & Shafig, 1965). However, the amount of RNA in virus-infected actinomycin-treated cells decreased considerably more than in the cells treated with actinomycin alone.

The stability of cell DNA following virus infection was also examined. Cells were prelabelled with $[^{3}H]$thymidine before infection. Experiments were made with and without the addition of actinomycin to the overlay medium. In those cultures which received actinomycin, cell DNA synthesis was inhibited 86% by $1\frac{1}{2}$ hr after infection (Table 1). The retention of radioactivity in all cultures was nearly complete. Thus, neither replicating virus nor actinomycin caused a significant decrease in the total amount of cell DNA.

**Table 1. Stability of DNA in cells infected by Newcastle disease virus**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Uninfected</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>111</td>
<td>99</td>
</tr>
<tr>
<td>+ Actinomycin D</td>
<td>95</td>
<td>93</td>
</tr>
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Fate of the cell RNA

The decrease in the amount of acid-soluble cell RNA following virus infection (Fig. 1) could have resulted from the degradation of cell RNA to acid-soluble fragments or from the escape of acid-soluble molecules from the cell into the culture medium. A considerable portion of the radioactivity associated with the acid-insoluble RNA present in the cells at the time of infection was found in an acid-soluble form in the culture medium at 10 hr after infection (Table 2).

![Sucrose-gradient analysis showing degradation of RNA in cells infected with Newcastle disease virus.](image)

**Table 2. The amount of acid-soluble RNA recovered from the medium of cells infected by Newcastle disease virus and uninfected cells**

<table>
<thead>
<tr>
<th>Counts/min.</th>
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<tbody>
<tr>
<td>Initial total counts</td>
</tr>
<tr>
<td>Initial acid-soluble counts</td>
</tr>
<tr>
<td>Acid-soluble counts 10 hr after infection</td>
</tr>
<tr>
<td>Uninfected</td>
</tr>
<tr>
<td>Infected</td>
</tr>
<tr>
<td>Uninfected and actinomycin D</td>
</tr>
<tr>
<td>Infected and actinomycin D</td>
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</tbody>
</table>

The RNA remaining in infected cells at 10 hr after infection was extracted with phenol and layered on a sucrose gradient (Fig. 2). As a result of virus infection ribosomal RNA was degraded and there was an increased amount of low molecular weight RNA which sedimented in the region of transfer RNA. Eighty per cent of the ribosomal...
RNA was degraded 10 hr after infection as estimated from the optical extinction readings. It is unlikely that viral RNA contributed significantly to the optical extinction or radioactivity profile in Fig. 2 since we have calculated (unpublished data) that the total amount of intracellular viral RNA synthesized by 10 hr after infection is equivalent to less than 1% of the total cell RNA.

The extensive degradation of ribosomal RNA in virus-infected cells may be contrasted with the degradation of RNA which occurred as a result of the inhibition of RNA synthesis. The arrest of cell RNA synthesis by actinomycin and the associated loss of RNA from the cells affected all size classes of RNA and was not selective (Fig. 3). This has also been described for mouse fibroblasts (Wiesner et al. 1965).

The mechanisms of viral degradation of cell RNA

Following infection of cells with Newcastle disease virus, virus-induced proteins are produced which inhibit cell protein and RNA synthesis (Wilson, 1968). The possible role of proteins in the cell RNA degradation process was investigated. An inhibitor of protein synthesis, cycloheximide, was added to culture media at different times after infection. The extent of viral degradation of cell RNA was measured in all cultures 10 hr after infection. When cycloheximide was added to cell cultures 2 or 3 hr after
infection, the viral degradation of cell RNA was prevented (Fig. 4b). Six hr after infection, viral degradation of cell RNA was not prevented by the addition of cycloheximide. Protein synthesis was therefore a necessary step in the process leading to viral degradation of cell RNA. The necessary proteins were synthesized by 6 hr after infection, which is 2 hr before the degradation of RNA becomes noticeable (Fig. 4a). For the sake of comparison, information concerning the viral inhibition of cell protein synthesis is also shown in Fig. 4. The time course of inhibition of cell protein synthesis by Newcastle disease virus is shown in Fig. 4c. Cycloheximide prevented the viral inhibition of cell protein synthesis when the drug was added at the time of infection (Fig. 4d). The proteins involved in viral inhibition of cell protein synthesis were produced about 2 hr earlier than the proteins which are necessary for viral degradation of cell RNA (Fig. 4b, d).

Fig. 4. The role of protein synthesis in cell RNA degradation. In all figures: O, uninfected; ●, virus-infected. (a) Cell RNA degradation measured as in Fig. 1, without actinomycin. (b) Cell RNA degradation at 10 hr after infection when cycloheximide was added at different times. (c) The rate of cell protein synthesis at different times after infection. (d) The rate of cell protein synthesis at 10 hr after infection when cycloheximide was added at different times.

DISCUSSION

The degradation of cellular RNA in Newcastle disease virus infected cells does not appear to be a direct result of the viral inhibition of host cell protein synthesis. Some of the observed degradation of cell RNA may have been a result of the viral inhibition of cell RNA synthesis. Clearly, however, in the presence of actinomycin we observed an additional viral degradation of cell RNA which was not a result of viral inhibition of host cell RNA synthesis. Since the proteins which lead to the degradation of cell RNA are formed by 6 hr after infection and the peak of viral RNA in the cell
NDV-induced degradation of RNA does not occur until 8 hr post infection (Wilson, 1968), we can see that the viral processes which lead to cell death are taking place concurrently with virus production.

Although the mechanism of RNA degradation in cells infected with Newcastle disease virus is unknown, nucleases may play an important role in this process. Allison & Sandelin (1963) have demonstrated that there was a progressive increase in total activity of hydrolytic enzymes, including ribonuclease, in vaccinia and hepatitis-virus-infected monkey kidney cells. Flanagan (1966) has shown that during the course of poliovirus growth in KB cells, ribonuclease was released from lysosomes, but no net synthesis of the enzyme was found.

Thus the proteins induced by Newcastle disease virus which cause degradation of cell RNA may be nucleases, or they may be proteins which stimulate the release of cellular nucleases from lysosomes. Furthermore, it is not known whether these proteins are coded by the cell genome or by the viral genome. If these proteins are coded by the cell genome, then the messenger RNA for them must be present in the cell at the time of infection since actinomycin, added at the time of infection, does not prevent viral degradation of cell RNA. It seems quite likely that these proteins are coded by the viral genome and are 'late' viral proteins.

This research was supported by grants from the National Science Foundation and the Sterling Winthrop Research Institute.

REFERENCES


(Received 13 June 1968)