Blockage of Antiviral Induction of Interferon by Homologous Cell Biochemical Activity: Effect of Chicken Embryo Fibroblast Mitotic Cell Cycle Phases on Sindbis Virus Growth

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The antiviral activity of interferon, measured as the reduction of viral yield, was studied as a function of the cell cycle phases. The present study shows that cells which are about to enter DNA replication phase S and cells that are in mitosis phase M are not refractive to viral infection when treated with interferon. The growth of Sindbis virus, used as the challenger, dropped considerably at the G1-S junction, at mitosis phase M, and as cells entered into a deeper quiescent stage.

A considerable variation of the antiviral state induced by interferon (14) was noted when 18- to 24-h-old chicken embryo fibroblasts were treated with identical dosages for the same length of time. This discrepancy led to a search for other factors responsible for this variation. A study of the induction of the antiviral state in synchronized chick cells was conducted to investigate whether a stage of cell growth could be such a factor.

Chicken embryo fibroblasts were obtained by trypsinization of 11-day-old chicken embryos.

Interferon was prepared by infecting confluent, aged (5-day-old) chicken embryo fibroblast monolayers with Sindbis virus at a multiplicity of infection of 0.1 PFU per cell. The monolayers were then incubated for 30 h at 37°C in serum-free medium. The supernatant was collected and centrifuged at 500 × g, treated with normal perchloric acid to a final pH of 2 for 48 h at 4°C, and centrifuged for 30 min at 1,000 × g. The pH was then adjusted to 7 by treating with 5 N NaOH. The preparation was then centrifuged at 100,000 × g for 1 h (16). Interferon preparations were assayed by the method of plaque reduction (23, 27) and by reduction of viral yield (16) in comparison with a laboratory standard and were found to have an activity of 300 to 400 units per ml (1 unit is the 50% plaque-depressing dose [23]), which was thermostatable (56°C for 30 min).

Proliferating tertiary chick cells were arrested in phase G0 by control of the serum concentration and pH of the medium (22, 25, 26). At the starting time of the experiment, the cultures were divided into two groups: one group was stimulated to grow, and the other group was held in phase G0. Cultures from the stimulated and phase G0 groups were treated with homologous prewarmed interferon (7) at regular intervals for 2 h. Control cultures of both groups were incubated simultaneously without interferon treatment. After each treatment, cells treated with interferon and untreated control cells were challenged with Sindbis virus. The virus was harvested after 12 h of incubation, and the viral yield was assessed by plaque formation (16).

The progress of the cells through the mitotic cycle was monitored by tritiated thymidine incorporation into acid-insoluble material and by cell count. Similar monitoring was carried out in cells in the quiescent condition.

Table 1 shows that an appreciable reduction of viral yield, calculated as log reduction = log yield of control cells − log yield of treated cells, was obtained throughout the experiment (20 to 28 h) in cells in the quiescent condition. A comparative value for the antiviral state was also obtained in cells in the prereplicative G1 and S phases.

At phase M, when cells increase in number, the value for the antiviral state, as measured by the reduction of viral yield, dropped practically to zero. When treatment occurred at the G1-S junction, just before the sharp rise in [%H]thymidine incorporation, the value for the antiviral state dropped significantly (60 to 90% of the value in G1 phase cells). Both effects can clearly be seen when plotted against cell cycle phases (Fig. 1).

To ascertain that interferon treatment under these experimental conditions did not interfere with cell growth (11) or alter the length of the cell cycle phases (1), which would cause a discrepancy between treated and untreated cells, monitoring by tritiated thymidine incorporation was carried out simultaneously in growing, interferon-treated cells. Figure 2 indicates that no such discrepancy occurred.

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TABLE 1. Antiviral action of interferon: reduction of viral yield in phase G₀ and growing cells

<table>
<thead>
<tr>
<th>Cell type and expt no.</th>
<th>Yields under indicated conditions&lt;sup&gt;a&lt;/sup&gt;</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>IFN</td>
<td>log red</td>
<td>C</td>
</tr>
<tr>
<td>Phase G₀ quiescent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7.2 × 10⁸</td>
<td>6 × 10⁷</td>
<td>1.1</td>
<td>3.4 × 10⁸</td>
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<tr>
<td>2</td>
<td>3 × 10⁸</td>
<td>3 × 10⁶</td>
<td>2.0</td>
<td>5 × 10⁸</td>
</tr>
<tr>
<td>3</td>
<td>1.8 × 10⁹</td>
<td>1 × 10⁸</td>
<td>1.3</td>
<td>1.6 × 10⁹</td>
</tr>
<tr>
<td>Growing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.3 × 10⁸</td>
<td>8.5 × 10⁶</td>
<td>1.2</td>
<td>2.8 × 10⁷</td>
</tr>
<tr>
<td>2</td>
<td>2.2 × 10⁹</td>
<td>5 × 10⁷</td>
<td>1.6</td>
<td>5.5 × 10⁸</td>
</tr>
<tr>
<td>3</td>
<td>2.6 × 10⁹</td>
<td>7 × 10⁷</td>
<td>1.6</td>
<td>1.2 × 10⁸</td>
</tr>
<tr>
<td>4</td>
<td>3.8 × 10⁹</td>
<td>1.1 × 10⁹</td>
<td>1.5</td>
<td>2.2 × 10⁹</td>
</tr>
</tbody>
</table>

<sup>a</sup> Tertiary chicken fibroblasts, obtained by the trypsinization of 11-day-old embryos, were plated in 30-mm plastic petri dishes (Falcon Plastics) at a density of 4 × 10³ to 6 × 10⁵ cells per dish in Eagle minimum essential medium (MEM; 0011, Eurobio) supplemented with antibiotics–sodium bicarbonate (1.1 g/liter)–25 mM Tricine (Merck Art 8602) containing 8% calf serum. The cultures were then incubated at 37°C in a 10% CO₂ humidified atmosphere. After 48 h the cultures were drained, replenished with MEM plus 0% serum adjusted to pH 6.8 (22), and incubated for 16 h. At the starting time, the cultures were drained once again and either replenished with MEM (plus supplements) containing 0.5% calf serum at pH 7.2 (phase G₀ quiescent cells) or stimulated to grow with MEM (plus supplements) containing 12% calf serum (growing cells) (25, 26). At regular intervals, cells from both phase G₀ and growing groups were treated with prewarmed interferon at a concentration of 30 units per ml for 2 h (IFN in Table 1). The interferon was added directly into the medium without removal of the cells from the incubator. After each treatment, treated cultures, together with untreated control cultures (C in Table 1), were drained, washed with preheated medium, and challenged with Sindbis virus at a multiplicity ratio of 1:1. After an adsorption time of 15 min, the cultures were covered with MEM containing 2% serum and actinomycin D (0.5 μg/ml) to preclude formation of new interferon through infection (28). The cultures were then incubated for 12 h at 37°C, after which the virus growth was halted by freezing the plates at −20°C. Viral yield was assessed by plaque formation. Samples of 10-fold dilutions were used for the assay. Valid counts were those obtained from plates carrying 17 to 170 plaques per plate, confirmed by the count obtained from the next higher dilution (16). Variations between plaque counts of the same dilution did not exceed the values expected from random fluctuation. All values are the mean of duplicate experiment plates.

Abbreviations: I, G₁ phase for growing cells and corresponding cultures of the G₀ group cells (4 to 7 h after starting time); II, G₁-S junction for growing cells and corresponding cultures of the G₀ group cells (8 to 12 h after starting time); III, S phase for growing cells and corresponding cultures of the G₀ group cells (13 to 19 h after starting time); IV, M phase for growing cells and corresponding cultures of the G₀ group cells (20 to 28 h after starting time); log red = log C (C is the yield of control cells) – log IFN (IFN is the yield of interferon-treated cells).
FIG. 1. (a) Antiviral state conferred by interferon and calculated as log reduction of viral yield = log yield of control cells – log yield of interferon-treated cells represented in relation to cell cycle phases in growing cells. Four experiments are represented. Phase M was monitored by cell count in the four experiments shown. (b) Passage of the cells through the cell cycle monitored by tritiated thymidine incorporation into acid-insoluble material. At regular intervals, cultures from the phase G₀ quiescent and growing cell groups were drained, covered with MEM (plus supplements) containing 0% serum and 1 μCi of tritiated thymidine (250 μCi/mmole) per ml, and incubated for 1 h at 37°C. The cultures were then drained, washed three times with phosphate-buffered saline, completely drained, and incubated for 15 min with 0.2 ml of a 0.5% solution of sodium dodecyl sulfate. The cultures were then treated for 10 min with 2 ml of a 10%, cold solution of trichloroacetic acid on ice. The content of every box was filtered (Ap 20; Millipore Corp.), and the filters were washed with absolute ethanol and allowed to dry. Incorporation of tritiated thymidine was determined by scintillation counting in Triton X-

FIG. 2. Effect of a 2-h interferon treatment on entry into the S phase. Cultures of growing cells were exposed to tritiated thymidine at 2-h intervals after stimulation to grow. To study the effect of interferon treatment on the entry of cells into phase S starting at 6 h, parallel cultures were treated with interferon for 2 h, washed three times with medium, and exposed to tritiated thymidine at the same time as the untreated cultures. Incorporation of tritiated thymidine was determined as indicated in the legend to Fig. 1. The time scale indicates the beginning of [3H]thymidine incorporation. Symbols: ○, untreated growing cells; ▼, interferon treatment from 6 to 8 h; ■ interferon treatment from 8 to 10 h; ●, interferon treatment from 10 to 12 h; ▲, interferon treatment from 12 to 14 h; ▽, untreated phase G₀ quiescent cells. The number of hours after stimulation to grow is equal to the number of hours after the starting time.

It should be noted that the antiviral state induced by interferon requires the synthesis of both proteins and RNA (19, 24). During phase M, such syntheses cease (2, 20), making it impossible for the interferon to act.

One aspect of the complex action of interferon

100. Three experiments are shown. The closed symbols indicate growing cells. The open symbols indicate phase G₀ quiescent cells.
The viral yield also appears to vary with the cell cycle phases. The viral yield dropped gradually in quiescent cells as the experiment proceeded and as cells entered into a deeper quiescent state (25, 26). It also dropped noticeably at the G₁-S junction and at the M phase in growing cells (Fig. 3).

Cells in phase G₀ are characterized by a drop in ribosome content (3) and a decrease in the efficiency of protein synthesis (18). The infective virus depends almost totally on cellular protein synthetic procedures for its replication, and therefore, the virus will be in unfavorable conditions for growth during this phase.

At the G₁-S junction, the cells are very active synthetically, and for a short period in the cell cycle, cellular messengers could rule out viral messengers by competition. This would affect only a fraction of an unsynchronized cellular population and would not be perceptible in nor-

generally associated with its antiviral activity is inhibition of protein synthesis (9, 12). Interferon induces the synthesis of a low-molecular-weight inhibitor (15) which degrades mRNA and rRNA (13) by activation of a nuclease (4, 5). Interferon phosphorylates and thus inactivates the peptide chain initiation factor, eIF2 (6), and other proteins (17, 30) by the action of a protein kinase(s) (21). Interferon also inactivates some species of tRNA (8, 29).

A possible explanation for the observed results is that at the G₁-S junction the high translational activity of the cell renders the newly formed tRNA, ribosomes, and initiation factors inaccessible to the action of the interferon-induced enzymes. A second explanation is that at the G₁-S junction the cell develops a block to the inhibition of protein synthesis by interferon, preventing the formation of the interferon-linked enzymes and allowing the cell to proceed unin-

FIG. 3. (a) Viral yield in untreated growing cells represented in relation to cell cycle phases. (b) Viral yield obtained from untreated phase G₀ quiescent cells.

FIG. 4. (a) Growing cells. Symbols: ■, viral yield (PFU per milliliter); ▲, log₁₀ reduction of viral yield. (b) Quiescent cells. Symbols: □, viral yield (PFU per milliliter); Δ, log₁₀ reduction of viral yield.
mally growing cells, in which viral yields are high in most cases.

After division, the cells rearrange their membrane components (10). This could interfere with the adsorption and penetration of the virus, causing a drop in viral yield at phase M.

The relatively low apparent degree of inhibition observed at the G1-S junction and phase M could be considered a reflection of the low control values obtained at this point, but such a hypothesis is overruled by the fact that in G0 phase cells, when the yield of control cultures drops 20 to 28 h after the starting time, the degree of inhibition of the viral yield is unaffected (Fig. 4).

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LITERATURE CITED


