Production of Chick Interferon by Reactivating Chick Erythrocytes

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SUMMARY

When chick erythrocytes were fused with mouse L929 or A9 cells and the heterokaryons induced for interferon production on consecutive days, considerable amounts of mouse interferon were produced every day. Small amounts of chick interferon were produced 2 to 3 days after fusion, coincident with the appearance of a chick enzyme, appearance of nucleoli and increase in chick cell nuclear diameter.

When a chick erythrocyte nucleus is introduced into the cytoplasm of a mammalian cell in tissue culture, the almost inactive chick cell nucleus is reactivated through a series of stages which reverse the inactivation occurring during erythropoiesis. In the earliest stages of reactivation, a change in the physicochemical properties of the chick nucleoprotein takes place, followed by an increase in nuclear volume and dispersion of the condensed chromatin. The increase in nuclear volume is due to a migration of host nucleospecific proteins into the chick nucleus and is followed by initiation of chick RNA and DNA synthesis, formation of chick nucleoli and the appearance of a series of chick proteins (for reviews, see Ringertz & Savage, 1976; Appels & Ringertz, 1975).

We wished to determine whether the specific chick protein, chick interferon, could be formed by such reactivated nuclei and, if so, whether the system could be used to study any interaction between mouse and chick interferon production.

To obtain the maximum possible yield of chick interferon it was necessary to achieve a high percentage of heterokaryons. By careful attention to detail, it was possible to obtain cultures in which up to 80% of the mouse cells contained at least one chick nucleus. For fusion, cells were seeded in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% foetal bovine serum (FBS) at a density of 0·8 × 10^6 cells/5 cm Petri dish. After overnight incubation, the cells were washed three times with Earle’s buffered salt solution, pH 8·0, and incubated at 4°C for 15 min with 2 ml of the same buffer. U.v.-irradiated Sendai virus (0·25 ml; haemagglutinating titre 5 × 10^3 units/ml) was added and the cells were held at 4°C for a further 15 min. Then 2 to 4 × 10^7 washed red blood cells from 12-day-old chick embryos in 0·5 ml Earle’s buffered salt solution, pH 8·0, were added. After a further 15 min at 4°C, the cells were transferred to 37°C with a minimum of disturbance and incubated for a further 30 min. The supernatant fluid was removed and 5 ml DMEM containing 10% FBS were added before incubation was continued at 37°C. To increase the yield of heterokaryons further, the fusion procedure was repeated 5 h later. Cultures receiving the same treatment, except that Earle’s salt solution was added in place of Sendai virus, were used as controls. At daily intervals after fusion, coverslips with growing cells were fixed in ethanol: acetone, stained with May Grunwald and Giemsa and mounted for light microscope observation. The number of reactivated chick erythrocyte nuclei per mouse cell nucleus was recorded.

To obtain some information about the degree of reactivation at different time points after fusion, the swelling of the erythrocyte nucleus was measured. The synthesis of a chick protein, the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT), was also...
Short communications

Table 1. Production of mouse and chick interferons and of HGPRT and reactivation of chick erythrocyte nuclei in mouse–chick heterokaryons

<table>
<thead>
<tr>
<th>Cell*</th>
<th>Interferon titre (log(_{10}) units)</th>
<th>HGPRT activity (ct/coverslip/min)</th>
<th>Chick nuclei with nucleoli</th>
<th>Diameter of chick nuclei mean ± s.d. (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mouse</td>
<td>Chick</td>
<td></td>
</tr>
<tr>
<td>Chick erythrocyte</td>
<td>0</td>
<td>3.5</td>
<td>967†</td>
<td>0</td>
</tr>
<tr>
<td>Mouse A(_9)+chick heterokaryons</td>
<td>2</td>
<td>3.1</td>
<td>928±</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>3.0</td>
<td>998±</td>
<td>8</td>
<td>5.5 ± 1.5</td>
</tr>
<tr>
<td>4</td>
<td>3.8</td>
<td>942±</td>
<td>6</td>
<td>5.8 ± 1.5</td>
</tr>
<tr>
<td>5</td>
<td>3.3</td>
<td>1033±</td>
<td>46</td>
<td>5.3 ± 1.4</td>
</tr>
<tr>
<td>6</td>
<td>3.4</td>
<td>1225±</td>
<td>72</td>
<td>6.5 ± 1.4</td>
</tr>
<tr>
<td>7</td>
<td>3.6</td>
<td>1807±</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mouse L(_9)+chick heterokaryons</td>
<td>1</td>
<td>4.3</td>
<td>&lt;1.0</td>
<td>N.D.</td>
</tr>
<tr>
<td>2</td>
<td>4.2</td>
<td>&lt;1.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>4.6</td>
<td>1.7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>4.3</td>
<td>1.3</td>
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<tr>
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<td>4.4</td>
<td>1.3</td>
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</tr>
<tr>
<td>6</td>
<td>4.4</td>
<td>1.3</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* The mouse cells were X-irradiated with 3000 rad.
† Control levels (i.e. fusion without Sendai virus) were all in the range of 35 to 50 ct/min.

measured, in experiments in which chick erythrocytes were fused with HGPRT-deficient A\(_9\) cells. The cells, growing on coverslips, were incubated in the presence of \(^3\)H-hypoxanthine for 3 h; the coverslip was then washed in 5% TCA and ethanol and the amount of incorporated precursor determined (Clements & Subak-Sharpe, 1975).

To assay for interferon production, cells were infected with 0.4 ml Newcastle disease virus strain F, haemagglutinating titre 10\(^4\) units/ml, for 1 h at 37°C, washed with DMEM plus 2% FBS and incubated overnight at 37°C. The supernatant was dialysed against pH 2 buffer for 5 days and assayed for both mouse and chick interferon by a virus RNA reduction method (Atkins et al. 1974), with mouse L cells and primary chick fibroblasts respectively. The reference research standard for chick interferon (62/4), containing 100 units, showed end-point activity at a dilution of 1/500 (−2.7 log\(_{10}\)) by this method. The reference research standard for mouse interferon (G002-902-026), containing 6000 units, reached the endpoint at 1/6000 in L cells.

Preliminary experiments gave cultures of fused cells which produced high yields of mouse interferon (around 3.5 log\(_{10}\) units) but no detectable chick interferon (less than 0.5 log\(_{10}\) units). However, in these experiments chick nuclei could not be detected for more than 2 to 3 days post-fusion, presumably because such heterokaryons enter mitosis to form mononucleated synkaryons which rapidly eliminate the chick genetic material. To prevent heterokaryons from entering mitosis and thereby losing chick genetic material, the mouse cells used for fusion were X-irradiated prior to fusion. Control experiments showed that doses of up to 6000 rad had no effect on interferon production (data not shown), while doses of 3000 rad or more blocked cell division, without interfering with cell survival.

For X-irradiation, cells were irradiated with 3000 rad, either in suspension in phosphate-buffered saline containing 2% calf serum or in monolayers under the same medium. When such X-irradiated mouse cells were used, the percentage of heterokaryons remained constant for at least 1 week after fusion, and in these cultures chick interferon production could be detected 2 to 3 days after fusion (Table 1). It seems unlikely that the appearance of this antiviral activity in chick cells was due to a hybrid interferon with activity in both chick and mouse cells; species-specific activity is almost certainly controlled by the interferon polypeptide chain rather than by glycosylation and it is difficult to see how a fused polypeptide...
chain could be produced. In addition, in studies with interferon produced by human/mouse cell hybrids, it has been found that the human interferon activity could be neutralized by addition of antiserum to human interferon without any effect on the mouse interferon titre (A. Meager, personal communication), which shows that the activities are associated with distinct molecules. The chick interferon activity appeared at the same time that synthesis of chick HGPRT could first be detected and at the time that the cells first showed an increase in the number of nucleoli and diameter of the chick nucleus. The appearance of HGPRT activity was not due to reactivation of the mouse gene, but to synthesis of a chick cell protein, since the enzyme levels were measured immediately after fusion and the reversion rate of mouse HGPRT is far too low to explain this new activity.

Experiments were carried out to characterize the virus-inhibitory activity. It was not due to a cross-species effect present in the culture fluids as shown by the high titres of mouse interferon (4-3 log_{10} units) and undetectable amounts of chick interferon (< 0-5 log_{10} units) in the fluids induced 1 day after fusion. No activity was detectable if the Semliki Forest virus, used to challenge the interferon assay, was added along with the interferon, or if the chick interferon was added to the cells in the presence of 0-5 μg/ml of actinomycin D. The dose–response curve was exactly parallel with that of authentic chick interferon and the activity also showed the characteristic heat stability of chick interferon: the titre fell by only 0-2 log_{10} units on heating at 65 °C for 2 h, while the mouse interferon activity assayed in the same fluid fell by 3-6 log_{10} units. Thus, in all these respects, the virus-inhibitory activity behaved like chick interferon.

We therefore concluded that the reactivating chick erythrocyte nucleus could produce low titres of chick interferon. This confirms the observation of Guggenheim et al. (1968) who showed that human cells containing a chick erythrocyte nucleus produced a very small amount of chick interferon (log_{10} 0-5) 1 day after fusion. Our results extend their report considerably in showing that the system can produce higher titres of chick interferon, in giving a more detailed characterization of the chick interferon that is formed and in showing that the production of chick interferon is correlated with the appearance of the nucleoli and the increase in nuclear diameter. However, the titres of chick interferon formed under the best conditions we could devise – high fusion rate, X-irradiated cells and a good interferon inducer – were still too low for the system to be exploited further, and several attempts to increase the chick interferon titres were unsuccessful.

There are very few reports about the products synthesized by the reactivated chick erythrocyte and those published describe only the synthesis of constitutive enzymes (HGPRT, thymidine kinase, DNA repair enzymes). Since 12-day-old chick erythrocytes do not produce interferon (data not shown), the production of interferon by the reactivated erythrocyte must be due to a gene that is normally not expressed. This situation resembles that found in differentiating teratocarcinoma cells (Burke et al. 1978).

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