The Effect of Hypertonic Salt on Interferon and Interferon mRNA Synthesis in Human MG63 Cells

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SUMMARY

After infection with Sendai virus or Newcastle disease virus (NDV) strain F, human osteosarcoma MG63 cells produced large amounts of interferon-β. Both interferon production and overall protein synthesis were strongly inhibited by hypertonic salt. Interferon mRNA synthesis, however, was little affected by hypertonic salt up to twice normal salt concentrations, although cellular RNA synthesis was inhibited under these conditions. The results are compared to those obtained with polyriboinosinic acid:polyribocytidylic acid copolymer [poly(rI).poly(rC)] inductions of MG63 cells.

It has been suggested that interferon mRNA behaves like some virus mRNAs in that it can be translated more efficiently than other cellular mRNAs under suboptimal conditions such as hypertonic salt (Garry & Waite, 1979). We have tested this hypothesis for interferon production under hypertonic salt conditions in virus-induced human MG63 cells. We have also investigated whether interferon mRNA synthesis was affected by hypertonic salt. A further object of the study was to determine optimal conditions for the large scale production of interferon and interferon mRNA from virus-induced MG63 cells.

Human MG63 cells (obtained from Dr A. Billiau, Rega Institute, Leuven, Belgium) were seeded at $5 \times 10^6$ cells and grown to confluent monolayers at $37^\circ C$ in roller bottles with Glasgow-modified minimal essential medium (GMEM) (110 mM-NaCl) supplemented with 10% (v/v) newborn calf serum (NCS) (Flow Laboratories), penicillin (200 units/ml) and streptomycin (100 μg/ml). When fully confluent at 8 to 12 days ($6 \times 10^7$ to $10 \times 10^7$ cells/bottle) the culture medium was removed from the cells, Sendai virus or NDV-F (grown as described by Meager et al., 1979b) added in 14 ml serum-free GMEM, and the infected cultures rolled at $37^\circ C$ for 1 h. Following this, 40 ml of maintenance medium [GMEM + 2% (v/v) NCS] prewarmed to $37^\circ C$ were added to each infected culture without removing the virus inoculum. Incubation was continued at $37^\circ C$ and samples of culture fluid withdrawn at intervals for interferon assays. Excess salt, NaCl, was added into the medium following the 1 h infection period to create hypertonic conditions.

Samples for interferon assays were dialysed for 5 days at $4^\circ C$ against pH 2 buffer to inactivate residual virus. Interferon assays were carried out in human foreskin fibroblasts (HFF) (Atherton & Burke, 1975). Interferon titres are expressed as international units (IU): the interferon-β international reference preparation (G-023 902 527) containing 10000 IU/ml, titrated at 5000 U/ml in HFF.

Protein and RNA synthesis were estimated by incorporation of $[^3\text{H}]$leucine and $[^3\text{H}]$uridine respectively, into trichloroacetic acid (TCA)-insoluble counts. MG63 cells were seeded into small glass vials, grown to confluency and infected with NDV-F under identical but scaled-down conditions, L-$[^4,5\text{H}]$leucine (160 Ci/mmol, 1 μCi/vial) or $[^5\text{H}]$uridine (25 Ci/mmol, 0.2 μCi/vial; Amersham International) were added at various times post-infection and incubation continued at $37^\circ C$ for a further 0.5 h before processing (Meager et al., 1979b).

RNA was extracted from cells at various times after induction; the cell monolayers were washed in situ twice with cold phosphate-buffered saline (PBS), scraped off and pelleted and
Fig. 1. Interferon and interferon mRNA production by MG63 cells after NDV-F induction in the presence or absence of hypertonic salt (220 mm). The interferon yield in the medium (control, 110 mm salt (O) and 220 mm salt (●)) at the times shown, and the interferon yield from *Xenopus laevis* oocytes after injection of total cellular RNA from untreated (△) or salt-treated (▲) induced cultures extracted at the indicated time post-induction are presented.

then lysed in 4 M-guanidinium isothiocyanate, 1 M-2-mercaptoethanol in 20 mM-sodium acetate, pH 5. The RNA was pelleted through 5.7 M-CsCl, extracted as described by Morser et al. (1979) and dissolved at a concentration of 5 mg/ml. Interferon mRNA was assayed by micro-injecting groups of 10 to 12 oocytes from *Xenopus laevis* with 30 nl of RNA solution per oocyte, incubating the oocytes for 24 h at 20 °C and then assaying the incubation medium for interferon (Colman & Morser, 1979).

Confluent monolayer cultures of MG63 were infected with varying amounts of Sendai virus or NDV-F to determine optimum conditions for interferon production. Maximum yields were obtained from cultures grown 8 to 10 days from seeding and by infecting with Sendai virus at 10³ haemagglutinating units (HAU) per 10⁶ cells and NDV-F at 4 × 10² HAU per 10⁶ cells. Amounts of interferon produced were variable from one induction to the next, and ranged from 4 to 4.8 log₁₀ IU/ml for Sendai virus and 4.5 to 5.2 log₁₀ IU/ml for NDV-F. Interferon was first detected in the culture medium at about 1.5 h post-induction and was maximal by 7 to 8 h post-induction (Fig. 1).

Garry & Waite (1979) showed that chick interferon production in poly(rI).poly(rC) plus DEAE-dextran-induced chick cells was relatively unaffected by hypertonic salt, although the level of cellular protein synthesis was inhibited by up to 95% of control cultures. We attempted to confirm whether this was also true for interferon production by MG63 cells. Inductions of MG63 cells were carried out using NDV-F or poly(rI).poly(rC) (50 μg/ml) plus DEAE-dextran (50 μg/ml) in serum-free medium for 1 h at 37 °C before adding medium containing 2% NCS and hypertonic salt concentrations. Protein and RNA synthesis, as measured by incorporation of [³H]leucine and [³H]uridine into TCA-precipitable counts at 3 to 3.5 h post-induction, were inhibited by salt concentrations above the normal salt concentration of 110 mm (Table 1). Interferon production with either inducer was inhibited
### Table 1. Effect of hypertonic salt on macromolecular synthesis, interferon production and interferon mRNA activity in induced MG63 cells

<table>
<thead>
<tr>
<th>Salt concentration (mM)</th>
<th>Protein and interferon synthesis</th>
<th>Inducer</th>
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<tbody>
<tr>
<td></td>
<td>[1^H]leucine incorporation†</td>
<td>NDV-F</td>
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<tr>
<td></td>
<td>[3H]uridine incorporation‡</td>
<td>NDV-F</td>
</tr>
<tr>
<td>Interferon production</td>
<td>NDV-F</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Poly(rI).poly(rC) + DEAE-dextran</td>
<td>100</td>
</tr>
<tr>
<td>Interferon mRNA activity§</td>
<td>NDV-F</td>
<td>100</td>
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<tr>
<th></th>
<th>110†</th>
<th>143</th>
<th>165</th>
<th>220</th>
<th>275</th>
<th>330</th>
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<tr>
<td>[1^H]leucine incorporation</td>
<td></td>
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<td>[3H]uridine incorporation</td>
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<tr>
<td>Inducer</td>
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<tr>
<td>NDV-F</td>
<td>100</td>
<td>74</td>
<td>50</td>
<td>10</td>
<td>1.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Poly(rI).poly(rC) + DEAE-dextran</td>
<td>100</td>
<td>97</td>
<td>80</td>
<td>30</td>
<td>15</td>
<td>9</td>
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<tr>
<td>NDV-F</td>
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<td>ND</td>
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* The results are quoted as percentages and represent the average values taken from at least three experiments.
† Normal salt concentration.
‡ [1^H]leucine and [3H]uridine incorporation measured between 3 and 3.5 h post-infection.
§ RNA extracted at 3 h post-induction from induced cultures.
‖ ND, Not done.

by salt concentrations above 143 mM (Table 1). Interferon was produced only very slowly in cells induced by NDV-F in medium containing twice the normal salt (220 mM) (Fig. 1). If high salt was removed at 3 h post-induction and replaced by normal salt, protein, RNA and interferon synthesis apparently recovered to normal levels. However, treatment of induced cells longer than 3 h post-induction with hypertonic salt led to lower interferon yields despite a return to normal levels of protein synthesis after salt removal (data not shown).

We next investigated the synthesis of interferon mRNA in NDV-F-induced MG63 cells under both normal and hypertonic salt conditions. The results (Fig. 1) showed that interferon mRNA was readily detected as early as 1 h post-induction and was maximal by 3 h post-induction. Thereafter, interferon mRNA activity decreased rapidly (Fig. 1). Interferon mRNA synthesis was relatively unaffected by hypertonic salt up to twice the normal level in the medium (Table 1 and Fig. 1). The greater levels of interferon mRNA activity observed in the hypertonic salt-treated cultures in Fig. 1 probably arose because the two experiments were done separately. Such differences were not found when experiments were performed on the same day (Table 1).

The cellular RNA was analysed by sucrose gradient centrifugation (Morser et al., 1979) and a peak of interferon mRNA activity was found at 12S, a result similar to other published results for interferon-β mRNA (Sehgal et al., 1978; Pang et al., 1980; Sehgal & Tamm, 1980). RNA purified from cultures induced under hypertonic salt conditions was very similar, both qualitatively and quantitatively, to that from untreated cells (data not shown).

The interferon produced by oocytes in response to injected RNA titrated poorly in bovine (EBTr) cells giving approx. 4% of its antiviral titre in human cells (Hayes et al., 1979). Ten units of this interferon were fully neutralized by a rabbit anti-human interferon-β antiserum (a gift from Dr J. Vilček, New York University School of Medicine, N.Y., U.S.A.) at between 5 x 10^-3 and 1 x 10^-4 dilutions of this antiserum. The antiserum has no effect on interferon-α.

We have shown that whereas virulent strains of NDV are relatively poor inducers of interferon in MG63 (Meager et al., 1979a), the avirulent NDV strain F and Sendai virus induce high yields. Our method is thus an attractive alternative to the various methods employing poly(rI).poly(rC) and superinduction schedules (Billiau et al., 1977; Meager et al., 1978, 1979a) for the production of interferon-β and its mRNA.

Sehgal et al. (1977) found that interferon-β mRNA levels were at their highest 1 to 1.5 h after induction with poly(rI).poly(rC) of human diploid fibroblasts, whereas we found
maximum interferon mRNA activity in NDV-F-induced MG63 cells at 3 h post-induction. This represents a much more rapid accumulation of interferon mRNA than in NDV-induced human GM258 fibroblasts (Pang et al., 1980) where the maximum was at 20 h. In both poly(rI).poly(rC)-induced human diploid fibroblasts (Sehgal et al., 1977) and our NDV-F-induced MG63 cells interferon mRNA activity decreased rapidly soon after the highest levels were reached. The reason for this rapid decay is not understood, although it is known that under superinduction conditions interferon mRNA activity can be stabilized (Cavalieri et al., 1977; Sehgal et al., 1978; Sehgal & Tamm, 1980). Inhibition of protein and RNA synthesis in MG63 cells induced in hypertonic salt did not prevent the decay of interferon mRNA activity and, therefore, the problem remains as to what is responsible for the apparent irreversible inactivation of interferon mRNA. Hypertonic salt did not inhibit interferon mRNA synthesis suggesting that either interferon mRNA itself, or more likely, some other product of cellular protein or RNA synthesis formed in the first hour in induced cells before the addition of high salt was involved in the eventual interferon mRNA inactivation. Alternatively, a product of NDV protein or RNA synthesis may be involved.

Since interferon mRNA activity was relatively unaffected by hypertonic salt up to twice that concentration contained in normal medium, the potential for interferon synthesis remained intact up to the 3 h post-induction maximum accumulation time point under these conditions. However, interferon production was more strongly inhibited in our induced MG63 cells than was observed for chick interferon production in chick cells similarly treated (Garry & Waite, 1979). Therefore, our results differ from those of Garry & Waite (1979) and do not support their suggestion that interferon mRNA is similar to some virus RNAs and can be translated efficiently even when the overall synthesis of cell proteins was inhibited by 95% with hypertonic salt.

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REFERENCES


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