Relative Quantitative Assay of the Biological Activity of Interferon Messenger Ribonucleic Acid

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SUMMARY
RNA extracted from cells previously stimulated to synthesize the antiviral protein, interferon, causes species-specific interferon synthesis when added to heterospecific cell cultures. Our results confirm the report of De Maeyer-Guignard, De Maeyer & Montagnier (1972). We have used this observation to obtain a relative quantitative assay for the interferon messenger RNA activity. At appropriate RNA concentrations, the yield of interferon is proportional to the concentration of RNA adsorbed to recipient cell cultures.

INTRODUCTION
The chief difficulty in studying the metabolism of eukaryotic messenger RNA (mRNA) has been the lack of means to demonstrate and measure specific mRNA sequences. The problem is one of agreement on a general operational definition of mRNA as a class of molecules. Studies using average properties of mRNA may lend impressions from which specific instances could deviate widely. Such properties as the presence of a 3'-terminal polyadenylate sequence, sensitivity to various inhibitors, rapid labelling, polysomal association and DNA-like base composition may not be invariant features of mRNA. One approach to the identification of specific mRNA sequences has been synthesis in vitro of DNA hybridization probes of high specific radioactivity using previously purified mRNA as template (Kacian et al. 1972, 1973; Ross et al. 1972; Verma et al. 1972; Aviv et al. 1973; Diggelman, Faust & Mach, 1973; Harris et al. 1973). This approach is limited by the availability of specific mRNA templates. A second approach is based on the ability of eukaryotic mRNA to stimulate the synthesis in cell-free systems of polypeptides identified by physicochemical and immunological means but, to date, not by biological activity (Jacobs-Lorena, Baglioni & Borun, 1972; Gross et al. 1973; Mach, Faust & Vassali, 1973; Rhoads, McKnight & Schimke, 1973; Schechter, 1973; Schutz, Beato & Feigelson, 1973; Taylor & Schimke, 1973). A third approach has used the ability of RNA preparations containing specific mRNA sequences to cause the synthesis of specific proteins upon injection of the RNA into amphibian oocytes, eggs or embryos (Gurdon et al. 1971; Moar et al. 1971; Berns et al. 1972; Laskey, Gurdon & Crawford, 1972; Rollins & Flickinger, 1972; Stevens & Williamson, 1972; Brachet, Huez & Hubert, 1973). Again the products have been identified by physical criteria but not by biological activity.

We report here the confirmation and extension of the observation published by De Maeyer-Guignard et al. (1972) concerning a fourth approach. RNA preparations isolated from mouse or monkey cells previously stimulated to synthesize the antiviral protein,
interferon, cause species-specific interferon production when added to heterospecific cell cultures. We have employed the 'RNA transfer' to obtain a relative quantitative assay for the interferon mRNA activity. The mRNA is measured by its ability to cause the synthesis of a stable and functional gene product, i.e. the interferon activity.

METHODS

Buffers. PBS buffer: 0.144 M-NaCl, 2.8 mM-KCl, 10 mM-NaHPO₄, 1.5 mM-NaH₂PO₄, pH 7.4. TD buffer: 0.137 M-NaCl, 5 mM-KCl, 0.7 mM-Na₂HPO₄, 25 mM-tris-HCl, pH 7.4. Tris buffer: 0.137 M-NaCl, 5 mM-KCl, 0.7 mM-Na₂HPO₄, 0.5 mM-MgCl₂, 0.9 mM-CaCl₂, 25 mM-tris-HCl, pH 7.4. DNase buffer: 50 mM-NaCl, 10 mM-tris-HCl, 2 mM-MgCl₂, pH 7.4.

Cell cultures. All cell cultures have been propagated in our laboratory as monolayers in plastic culture dishes using Dulbecco’s modified Eagle medium (hereafter, DME; Dulbecco & Vogt, 1954) without antibiotics and containing bovine serum or foetal bovine serum. Suspension cultures of mouse L cells were maintained in DME with 5% foetal bovine serum under 10% CO₂ at 37 °C on a gyratory shaker.

Viruses. Newcastle disease virus (NDV, strain L-Kansas) was grown in the allantoic cavity of 10 to 11-day embryonated chicken eggs (Duesberg & Robinson, 1965). A large-plaque clone of vesicular stomatitis virus (LP-VSV, New Jersey strain) thrice plaque-purified in L cells was passed once at low multiplicity to obtain challenge stocks used in interferon assays (Kronenberg, 1974).

Other reagents. Polyinosinic-polycytidylic acid duplex (poly(I:C)) as the lyophilized sodium salt was obtained from P-L Biochemicals, dissolved at 1 to 2 mg/ml in PBS buffer and stored at 4 °C (E₂₅° = 115 cm⁻¹) for up to 1 month. L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone (TPCK) treated trypsin (EC 3.4.4.4), deoxyribonuclease I (EC 3.1.4.5) and 5 times recrystallized pancreatic ribonuclease (EC 2.7.7.16) were obtained from Worthington, dissolved in TD buffer at 1 mg/ml and stored at -20 °C. Diethylaminoethyl-dextran (DEAED) was obtained from Pharmacia (5 × 10⁵ average mol. wt.), dissolved in PBS buffer at 40 mg/ml, titrated to pH 7.4 with NaOH and sterilized by filtration, then stored at 4 °C up to 2 months. Sucrose (ultrapure grade) was obtained from Mann. All other substances were reagent grade.

Interferons and interferon assay. Mouse interferon standards were obtained from monolayers of L cells or Balb-C/3T3 cells induced with poly(I:C) or with NDV (Kronenberg, 1974). Monkey interferon standards were obtained from monolayers of BSC 1 cells using poly(I:C) as inducer. Interferons were routinely dialysed five days at 4 °C against several changes (20 vol. each) of water acidified to pH 2.2 with HCl, then dialysed against two changes (20 vol. each) of PBS buffer and stored at -20 °C.

Interferons were assayed by plaque inhibition (Finter, 1973) on confluent 35 mm monolayers of Vero or L cells. Standard volumes (1.3 to 2.0 ml/dish) of serial interferon dilutions were incubated on replicate cultures after washing once with DME. Controls contained no interferon. After 15 to 20 h at 37 °C the cultures were washed once with tris buffer and infected with 50 to 60 p.f.u./dish of the appropriate LP-VSV stock previously grown in the same cell line. Infected cultures were overlaid with DME containing 0.9% Bacto-Difco agar and 2% serum. After 48 to 60 h at 37 °C plaques were stained with neutral red saline. For each interferon dilution, the mean plaque number and standard deviation were plotted against log (interferon dilution). Points lying between approx. 20 to 80% plaque inhibition were fitted to a least squares line and interferon titres were determined by interpolation as the reciprocal of the dilution producing 50% plaque inhibition. One unit of mouse interferon herein corresponds to 1.0 unit of international mouse reference interferon (NIH-NIAID.
Bioassay of mRNA

mouse reference standard, code G002-920-026). In the mouse L cell assay system, the titre of an interferon increases with increasing vol. of the dilutions incubated with cells. Hence interferon activities are given as units per 1·3 to 2·0 ml in various experiments.

Cellular RNA. Monolayers of L cells were induced by 90 min adsorption with NDV (5 p.f.u./cell) at room temperature. BSC and L cells were also induced by incubation for 3 h at 37 °C in DME containing 2 % serum, 10 μg/ml poly(I:C) and 800 μg/ml DEAED. Suspension cultures of L cells were induced for 3 h at 37 °C with DME containing 4 % serum, 5 μg/ml poly(I:C) and 50 μg/ml DEAED. Zero time is always the beginning of induction. After induction the cells were washed twice with DME and the medium replaced with DME containing 2 % foetal bovine serum (BSC cells) or 2 % heated calf serum (L cells). Calf sera were heat-inactivated for 60 min at 56 °C.

RNA was extracted from induced cells as follows: the cells were scraped from monolayers or pelleted from suspension and washed once by resuspending in TD buffer and pelleting. Cells were then suspended at 10⁷ ml⁻¹ in TD buffer, made 0·1 to 0·2 mg/ml bentonite (prepared as in Watts & Mathias, 1967) and 1 % sodium dodecyl sulphate (SDS), vortexed and immediately extracted for 15 min at room temperature with 2 vol. of buffer-saturated phenol-chloroform (1:1, v/v) mixture pre-warmed to 37 °C. Phases were separated by centrifuging for 10 min at 2500 g at 4 °C and the upper, aqueous phase re-extracted once with an equal vol. of phenol-chloroform. Finally 2·5 vol. of 95 % ethanol (kept at −20 °C) was added to the aqueous phase and gently mixed, and the DNA precipitate spooled and discarded. RNA precipitated overnight at −20 °C was collected by centrifuging for 15 min at 27000 g, redissolved in TD buffer and reprecipitated in 70 % ethanol at −20 °C. Finally, precipitates were dissolved in PBS buffer and stored at −20 °C. For the extinction coefficient, in PBS, of cellular RNA, \( E_{1%} = 225 \text{ cm}^{-1} \) was used throughout.

Interferon mRNA activity. Interferon mRNA activity has been demonstrated by the technique of De Maeyer-Guignard et al. (1972) with minor modifications. Confluent monolayers of recipient cells in 50 mm culture dishes were washed twice with warm PBS, then incubated 60 min at 37 °C in 1·5 ml/dish of PBS containing DEAED (see below) and induced cell RNA. The inoculum was redistributed at 15 min intervals. After adsorption of RNA, 5·7 ml of DME containing 5 % foetal bovine serum, 150 unit/ml penicillin and 150 μg/ml streptomycin was added and mixed and the plates incubated 12 to 15 h at 37 °C. Media harvested at the end of this incubation were clarified by centrifuging and assayed for interferon using plaque inhibition against LP-VSV (see above). These media will be referred to as 'heterologous' interferons. One unit of interferon activity per standard vol. contained in that dilution, giving 50 % inhibition of plaque formation.

RESULTS

Extraction of cellular RNA after induction of interferon

When mouse L cells were stimulated to produce interferon by either poly(I:C) or NDV, extracellular interferon accumulated with the kinetics shown in Fig. 1. The kinetics of accumulation of the interferon mRNA will be the subject of a separate communication. Since interferon accumulates most rapidly at 15 to 18 h, and since previous reports suggest little lag between intracellular and extracellular appearance of interferon (Tan, Armstrong & Ho, 1971), RNA was extracted from poly(I:C)-induced L cells at 16 h post-induction. RNA was also isolated from poly(I:C)-induced L cells at 3 h, before any extracellular interferon is detectable. A third preparation of RNA was obtained from L cells 16 h after induction with NDV. RNA was also extracted from BSC 1 monolayers 16 h after induction by poly(I:C).
Fig. 1. Kinetics of accumulation of extracellular interferon. Suspension cultures of L cells were induced as described in Methods and at intervals samples of culture medium were removed, clarified by centrifuging, frozen at -20 °C and subsequently assayed for interferon on L cell monolayers.

Assay of interferon mRNA activity

RNA prepared at 16 h after induction of L cells using poly(I:C) was adsorbed to recipient cell cultures using 50 µg/ml DEAE-D as described in Methods. Undiluted heterologous interferons were then incubated overnight on replicate monolayers of L cells; the cells were washed once with tris buffer and infected with 40 to 50 p.f.u./dish of LP-VSV. The results are shown in Fig. 2 using monkey (Vero and BSC 1) and human (HeLa and human foetal fibroblast) cells as recipient cells for mouse cell RNA. Generally 25% plaque inhibition was considered significant. These results show a dependence of the amount of inhibitor produced upon the concentration of mouse cell RNA to which the heterospecific cells are exposed. In no case has interferon mRNA activity been associated with RNA prepared from un-induced or mock-induced L cells, nor is there inhibitory activity associated with culture media from heterospecific cells not treated or mock-treated with mouse cell RNA. Quite similar results were obtained if a number of other cell lines were used as recipient cells for mouse RNA, including primary cultures (mouse embryo fibroblasts, chick embryo fibroblasts), primary lines (adult and foetal human fibroblasts) and lines of established or transformed nature (BHK 21, Balb-C/3T3, Rous sarcoma virus-transformed chick embryo fibroblasts, Moloney sarcoma virus-transformed mouse fibroblasts). These results corroborate those of De Maeyer-Guignard et al. (1972).

Purified RNA from BSC 1 cells which had been stimulated to produce interferon by poly(I:C) was assayed for monkey interferon mRNA with mouse L cells as recipient cells and Vero cells for interferon assay, with quantitatively similar results (data not shown). Thus it seems likely that other species of interferon mRNA activity can similarly be demonstrated.
Bioassay of mRNA

Effect of actinomycin D on expression of exogenous mouse interferon mRNA activity

De Maeyer-Guignard et al. (1972) observed that pre-treatment of recipient cells with actinomycin D enhanced severalfold mouse interferon production in heterologous cells exposed to mouse cell RNA. Therefore it was of interest to compare the results above (Fig. 2) with similar experiments in actinomycin-treated recipient cells. Confluent 50 mm cell cultures were incubated in 2 ml/dish DME, 2% serum containing 1 to 2 μg/ml actinomycin D for 4 to 6 h at 37 °C. The cultures were then washed and treated with several concentrations of induced L cell RNA as above, and the undiluted heterologous interferons were assayed for plaque-inhibitory activity on L cell cultures. The consistent result has been that pre-treatment with actinomycin either has no effect or marginally decreases the activity of a given amount of RNA. Control experiments showed in all cases that the actinomycin pre-treatment inhibited incorporation of [14C]-uridine during a 20 min pulse by more than 95%.

Since endogenous interferon production is inhibited by this dose of actinomycin (Kronen-berg, 1974) these results suggest that mouse RNA is not inducing endogenous interferon synthesis in recipient cell cultures. It seems likely that endogenous transcription in recipient cells is not required for the expression of exogenous interferon mRNA. These results do not
confirm the observation that actinomycin pre-treatment enhances the RNA transfer (De Maeyer-Guignard et al. 1972) and this discrepancy is unresolved. Identical pre-incubation of Vero, HeLa and L 929 cell cultures with actinomycin does enhance severalfold the specific infectivity of purified EMC RNA (data not shown), which may suggest that the mechanisms of uptake and expression of infectious virus RNA and interferon mRNA are not identical.

**Effect of DEAED concentration during adsorption of RNA to recipient cell cultures**

The results in Fig. 2 show low titres and marginal inhibition. In an attempt to increase heterologous interferon yields, we examined the effect of DEAED concentration during adsorption of RNA to recipient cell cultures. Replicate recipient cell cultures were treated with 50 μg/ml induced L cell RNA containing various concentrations of DEAED. L 929 cultures were pre-treated 2 h at 37 °C with 2 μg/ml actinomycin D in order to prevent induction of endogenous interferon. For each concentration of DEAED, control cultures were adsorbed using buffer without RNA in order to estimate the effect of residual DEAED on the titre of challenge virus (see below). The resulting undiluted heterologous interferons, prepared as described above, were tested for plaque-inhibitory activity on confluent L cell monolayers against LP-VSV. For each point, the percent inhibition is calculated relative to controls at the same concentration of DEAED. The results are shown in Fig. 3. For constant RNA input, increasing DEAED concentrations up to about 200 μg/ml increase the yield of inhibitor. Above 200 μg/ml DEAED the efficiency is decreased. For the three cell lines tested, the optimum DEAED concentration is approx. 200 μg/ml. The optimum DEAED concentration during adsorption of cellular RNA is about fivefold lower than for interferon
Fig. 4. Effect of DEAE-dextran pre-treatment on plating efficiency of VSV. Maintenance media containing variable amounts of DEAE-dextran were incubated overnight on L cell monolayers and the cells were washed, infected with 10 p.f.u./dish of LP-VSV (relative to controls without DEAE-dextran) and plaques were scored at 60 h p.i.

induction using poly(I:C) (Kronenberg, 1974). In subsequent experiments, 200 µg/ml DEAED was employed during adsorption of induced cell RNA to heterologous cell cultures.

Heterologous interferons prepared as described in Methods contain a residue of DEAED not adsorbed to the recipient cell monolayers. This was true even when recipient cell cultures were washed exhaustively with DME after adsorption of RNA, implying that DEAED slowly elutes from cell monolayers during the subsequent incubation. Its effect must be controlled since assay of heterologous interferons also sensitizes cell monolayers to plaque formation by LP-VSV because of the residual DEAED (Kronenberg, 1974). The dependence of challenge virus titre on the DEAED concentration after overnight incubation is shown in Fig. 4. In our experiments, heterologous interferon controls are heterologous culture fluids from monolayers mock-adsorbed without RNA, or adsorbed with RNA from mock-induced L cells. Although residual DEAED increases the challenge virus titre, it does not change the titre of standard mouse interferons in this system (Kronenberg, 1974).

Kinetics of mouse interferon production by Vero cultures exposed to L cell RNA

Replicate confluent Vero cultures were adsorbed as above using 40 µg/ml RNA and 200 µg/ml DEAED, and re-fed 5.7 ml/dish DME containing 2% serum and antibiotics. At intervals the fluids were removed and pooled from duplicate cultures, clarified by centrifuging and stored at −20 °C and subsequently assayed for mouse interferon on L cell monolayers. Fig. 5 shows the kinetics of mouse interferon production by Vero cells in response to induced L cell RNA. The interferon activity accumulates in the extracellular fluid approx. linearly for 4 to 6 h after adsorption of RNA, and the accumulation is complete by about 8 h. Thereafter, interferon in the medium is stable until at least 15 h post-adsorption.

Effect of cycloheximide on heterologous interferon production

Replicate confluent Vero cultures were adsorbed with 40 µg/ml induced L cell RNA and 200 µg/ml DEAED as above. Triplicate cultures were then re-fed 5.7 ml/dish DME, 2% serum with or without 10 µg/ml cycloheximide and incubated 6 h at 37 °C. The fluids were then removed, clarified by centrifuging, dialysed for 5 days at pH 2.2 and back to pH 7.4 and
assayed for mouse interferon on L cell monolayers. While control cultures produced 14 unit/l·3 ml of interferon, cycloheximide-treated cultures produced no detectable interferon (< 0.25 unit).

**Effect of deoxyribonuclease on interferon mRNA activity**

Since the cellular RNA preparations prepared as described in Methods are presumably contaminated with a small amount of DNA not removed by spooling, a control experiment was performed to show that DNase treatment did not alter the biological activity of RNA prepared in this manner. 150 μg of induced L cell RNA in 1 ml DNase buffer was incubated 30 min at 37 °C with 20 μg/ml deoxyribonuclease I. Controls contained no DNase. The samples were then made 0.2 M with sodium acetate, mixed with 2·5 vol. of 95% ethanol and precipitated overnight at -20°C. Precipitated RNA was collected by centrifuging and adsorbed to confluent Vero cell cultures as above, using 30 μg/ml RNA and 200 μg/ml DEAE-D. Undiluted heterologous interferons were then assayed for plaque-inhibitory activity on L cell monolayers against LP-VSV. The results show 50% and 59% inhibition, respectively, for control and DNase-treated RNA preparations, a difference which is assumed to be insignificant. Therefore cellular RNA preparations containing interferon mRNA activity were not routinely treated with DNase.

**Relative quantitative assay of interferon mRNA activity**

The preceding dose–response relationship (Fig. 2) was obtained by adsorbing variable amounts of induced L cell RNA to recipient cell cultures, after which undiluted culture media were tested for plaque-inhibitory activity. Since the degree of plaque inhibition can be chosen at will according to the input RNA concentration, this procedure could provide a means of measuring the message activity, for example, by calculating the concentration of RNA which yields 50% plaque inhibition, i.e. one unit of interferon. This procedure would be valid when the dose–response relationship is linear.
Bioassay of mRNA

Fig. 6. Yield of heterologous interferon versus the concentration of RNA adsorbed to heterologous cell cultures for three preparations of induced L cell RNA: O---O, 16 h RNA (poly(I:C)); △-△, 16 h RNA (NDV); □-□, 3 h RNA (poly(I:C)).

An alternative approach would be to treat recipient cells with a constant concentration of RNA and determine the yield of interferon per constant input RNA concentration. For the data in Fig. 6, replicate Vero cultures were treated with variable amounts of each of the three induced L cell RNA preparations described above, using 200 μg/ml DEAED, and each heterologous interferon was titrated according to the plaque inhibition produced by serial dilutions of the interferon (see Methods). Fig. 6 shows that the yield of heterologous interferon is proportional to the concentration of RNA adsorbed, between approx. 20 and 60 μg/ml for each of the three preparations of RNA. The results indicate that the three RNA preparations do not contain the same amount of interferon mRNA activity per unit mass. Thus the specific mRNA activity is lowest in poly(I:C)-induced L cells at 16 h. It is higher in NDV-induced L cells at 16 h, and higher still in poly(I:C)-induced cells at 3 h, i.e. at the end of incubation with poly(I:C) and before extracellular interferon has begun to accumulate (Fig. 1). Since there is a domain over which the response is linear, this procedure provides a relative quantitative assay for the interferon mRNA activity.

Characterization of the virus inhibitor as mouse interferon

The heterologous interferon activity is not sedimented in 3 h at 100,000 g, is non-dialysable and is stable for at least two weeks at pH 2.2 at 4°C.

That residual poly(I:C) in the heterologous interferons is not causing the observed inhibition was shown as follows. RNA extracted from L cells induced with NDV also contains the interferon mRNA activity (Fig. 6). Furthermore, the inhibitor synthesized by heterologous
Table 1. Species-specificity of ‘heterologous’ interferons

<table>
<thead>
<tr>
<th>Interferon*</th>
<th>Interferon titre†</th>
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<tbody>
<tr>
<td></td>
<td>L cells</td>
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<td>Mouse interferon standard</td>
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<tr>
<td>Monkey interferon standard</td>
<td>&lt; 16</td>
</tr>
<tr>
<td>3 h L 929 RNA (poly(I:C))</td>
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</tr>
<tr>
<td>16 h L 929 RNA (poly(I:C))</td>
<td>9:6</td>
</tr>
<tr>
<td>16 h L 929 RNA (NDV)</td>
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</tr>
<tr>
<td>16 h BSC RNA (poly(I:C))</td>
<td>&lt; 0:25</td>
</tr>
</tbody>
</table>

* Heterologous interferons are named by the corresponding RNA preparation.
† Titres are expressed as units/1·5 ml.

Table 2. Enzyme susceptibilities of RNAs and ‘heterologous’ interferons

<table>
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<th>RNA</th>
<th>Enzyme</th>
<th>‘Heterologous’ interferon yield†</th>
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</thead>
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<tr>
<td>3 h RNA (poly(I:C))</td>
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<tr>
<td></td>
<td>RNase</td>
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<tr>
<td></td>
<td>Trypsin</td>
<td>29:2</td>
</tr>
<tr>
<td>16 h RNA (poly(I:C))</td>
<td>Mock</td>
<td>10:2</td>
</tr>
<tr>
<td></td>
<td>RNase</td>
<td>&lt; 0:25</td>
</tr>
<tr>
<td></td>
<td>Trypsin</td>
<td>9:6</td>
</tr>
<tr>
<td>16 h RNA (NDV)</td>
<td>Mock</td>
<td>16:1</td>
</tr>
<tr>
<td></td>
<td>RNase</td>
<td>&lt; 0:25</td>
</tr>
<tr>
<td></td>
<td>Trypsin</td>
<td>15:0</td>
</tr>
<tr>
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<td>Mock</td>
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</tr>
<tr>
<td></td>
<td>Trypsin</td>
<td>8·6 × 10⁶</td>
</tr>
<tr>
<td>Mouse interferon standard</td>
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<tr>
<td></td>
<td>RNase</td>
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<tr>
<td></td>
<td>Trypsin</td>
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<tr>
<td></td>
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<td>&lt; 0:25</td>
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</table>

* Heterologous interferons are named by the corresponding RNA preparation.
† Titres are expressed as units/2·0 ml.

Cells in response to mouse cell RNA is resistant, unlike poly(I:C), to digestion by ribonuclease (see below).

The data in Fig. 2 support the conclusion that RNA from induced mouse cells is not inducing endogenous interferon production by the recipient cells: (i) several of the cell lines used as recipients (Vero, HeLa and BHK 21) do not produce interferon in response to double-stranded RNA or NDV (Kronenberg, 1974) and (ii) even if this were the case, standard monkey and human interferons have no antiviral activity in the mouse assay system.
Bioassay of mRNA

employed here. The species-specific nature of the antiviral action was established as follows: three preparations of heterologous interferons were obtained from Vero cultures corresponding to the three mouse RNA preparations described above (see Results, first paragraph) and from L cell cultures treated with induced BSC-cell RNA. Each of these, plus standard mouse and monkey interferons, was titrated on mouse L cells and on Vero cells. The standard interferons provide positive controls for the homologous assays and indicate no detectable cross-reactivity in the heterologous assays (Table 1). Each of the three heterologous mouse interferons was active when assayed on L cells and none was active when assayed on Vero cells. Heterologous monkey interferon was active on Vero cells but not on L cells. Thus the specificity of action of the heterologous interferons reflected the species of origin of the RNA. These results also imply that poly(I:C) in the heterologous interferons is not causing the observed inhibition since Vero cells are not rendered antiviral by poly(I:C) (Kronenberg, 1974).

The heterologous mouse interferons as well as the RNA's causing their synthesis were also characterized by their susceptibility to digestion by (TPCK)-trypsin and ribonuclease. Each of the three heterologous mouse interferons and the corresponding mouse RNA preparations was (i) mock-digested 30 min at 37 °C in TD buffer, (ii) digested for 30 min at 37 °C with 40 µg/ml ribonuclease, and (iii) digested for 30 min at 37 °C with 100 µg/ml (TPCK)-trypsin. Parallel digestions were carried out on purified EMC virus RNA and on standard mouse interferon in order to serve as positive controls and to check on enzyme specificity. The digestes were then tested for residual interferon activity or for infectivity (EMC RNA) on mouse cells, or for interferon mRNA activity on Vero cells. The results are given in Table 2. The message activity of each RNA preparation (and the infectivity of EMC RNA) was abolished by ribonuclease and unaffected by trypsin. The inhibitory activity of heterologous interferons as well as that of standard mouse embryo interferon is destroyed by trypsin but is unaffected by ribonuclease. In addition to its sensitivity to ribonuclease, the message activity of mouse RNA preparations is completely abolished by 0.3 M-NaOH (12 h at 37 °C).

DISCUSSION

The results presented here confirm the reports of De Maeyer-Guignard et al. (1972) and Orlova et al. (1974). De Maeyer-Guignard et al. (1972) reported that RNA extracted from mouse cells which have been induced to yield interferon causes mouse interferon synthesis when applied to heterospecific (non-mouse) cell cultures, and they characterized the product as mouse interferon. We have shown that the yield of mouse interferon from heterospecific cell cultures is proportional to the concentration of mouse RNA adsorbed. We do not know why there appears to be a ‘lag’ in this dose–response curve (Fig. 6). However, the response is reproducibly linear above approx. 20 µg/ml RNA. Therefore, this observation provides a simple relative quantitative assay for interferon mRNA activity.

There are few unambiguous instances in which uptake of functional cellular RNA by cell cultures has been shown. Recently it has been reported that RNA from progesterone-stimulated chick oviduct, when instilled into non-stimulated tissue in vivo, causes appearance of proteins characteristic of stimulated tissue (Tuohimaa, Segal & Koide, 1972) and also that RNA from myeloma cell cultures is taken up and expressed by lymphocyte cultures in vitro (Bhoopalam et al. 1972). The interferon system is distinguished by several useful features. Firstly, one requires that the recipient cells produce biologically active product, since the interferon protein cannot yet be detected by direct physical means. Secondly, the interferon assay is extremely sensitive and probably can be made 100 to 1000 times more
sensitive than the plaque inhibition assay used here, because of the synergy between interferon and its inducers in rendering cells antiviral (Kronenberg, 1974). Finally, the species-specific action of many interferons is an effective genetic marker which precludes the objection that one has induced the endogenous gene product in recipient cells.

We do not know the efficiency of the mRNA assay, per molecule of mRNA, because we know neither what fraction of the total RNA is interferon mRNA nor the specific biological activity of pure mouse interferon. This latter number is probably of the order of 10^8 unit/mg or greater in the assay system used here (Yamamoto et al. 1974). On this basis and assuming an average mol. wt. of 30,000 for mouse interferon (Stewart, 1974) we calculate an average yield of 40 to 50 interferon molecules per recipient cell under the conditions we have used. Thus, expressed as yield per cell, this amounts to 1 to 5% of the yield of endogenous interferon induction.

Using the mRNA assay we have partially purified and begun to characterize the interferon mRNA. We have found that 86% of the mRNA activity is associated with polysomes at 13 h post-induction. The sedimentation coefficient of the mRNA activity isolated from L cells at 16 h post-induction is approx. 8 S, and the mRNA activity is not retained by filters containing immobilized polyuridylic acid (Kronenberg, 1974, and in preparation). These results will be reported in detail elsewhere.

We are endeavouring to extend this assay to other mRNA activities. This type of assay may allow characterization and purification of 'minor' mRNA species independently of immunochemical means. When coupled with cell fractionation and kinetic experiments, it may allow a more detailed examination of the metabolism of specific mRNA sequences. Similarly, this assay should allow analysis of controls of the expression of the interferon gene, in particular, and of the paradoxical effects of antimetabolites on the expression of this and other inducible eukaryotic genes (Vilcek & Ng, 1971; Tomkins et al. 1972; Kronenberg, 1974) since the mRNA can be measured independently of the gene product itself. Finally, since cellular mRNA is evidently taken up and expressed in cell cultures, it is possible that the 'RNA transfer' may provide an alternate route to genetic transformation of mammalian cells in vitro.

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REFERENCES


Bioassay of mRNA


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