Biochem. J. (1979) **179**, 221–225
Printed in Great Britain

Interferon Effect on Ribosomal Ribonucleic Acid Related to Chromosome 21 Ploidy

By LEONARD E. MAROUN
Department of Medical Sciences, Southern Illinois University School of Medicine, P.O. Box 3926, *Springfield, IL* 62708, *U.S.A.*

(*Received* 2 *August* 1978)

Antiviral and cell-growth-inhibitory activities of human interferon were shown to be related to the activity of a gene or genes present on chromosome 21. The 18S rRNA is vital to cell growth; it is capable of a viral-mRNA-recognition function and it is coded for by genes a portion of which are present on chromosome 21. A previously reported ability of human interferon to affect rRNA metabolism is characterized by a decrease in the sucrose-gradient-peak ratio of radiolabelled 28S to 18S rRNA in extracts from the cytoplasm of interferon-treated human fibroblasts. In the present report, interferon dose–response curves are presented demonstrating a direct relationship between a decrease in this ratio and interferon concentrations in the media. By using this virus-independent cytoplasmic rRNA assay, eight human fibroblast lines, differing in chromosome 21 ploidy, were tested for sensitivity to human interferon. Two monosomy-21, two euploid-21 and four trisomy-21 cell lines were tested. The monosomy-21 cell populations were significantly less sensitive to interferon than the other six cell types tested. Of the cell lines tested, the most sensitive, by a wide margin, was a trisomy-21 line. Trisomy-21 cell monolayer sensitivity, however, varied widely within the range from normal to supersensitive. These observations suggest that interferon's ability to affect rRNA metabolism is related to the activity of a gene or genes present on chromosome 21.

Both the antiviral (Tan, 1975) and the cell-growth-inhibitory (Tan, 1976) activity of human interferon have been reported to be dependent on chromosome 21 ploidy. These observations have resulted in the assignment to the long arm of this chromosome (Tan & Greene, 1976; Epstein & Epstein, 1976) of a hypothetical antiviral gene whose activity is envisaged to be responsible for the ability of a cell to develop the antiviral state. Chromosome-21 dependency, however, could occur if this chromosome carried a gene which coded for a human interferon-specific cell-surface receptor (Revel *et al.*, 1976). Evidence has been reported both for (Wiranowska-Stewart & Stewart, 1977; Revel *et al.*, 1976) and against (De Clercq *et al.*, 1976) this receptor hypothesis. Unlike the antiviral and cell-growth-inhibitory activities, other reported biological activities of human interferon appear to be unrelated to chromosome 21 ploidy (De Clercq *et al.*, 1975).

It is well established that approx. 20% of a human cell's complement of rRNA genes are present in the satellite regions of chromosome 21 (Dittes *et al.*, 1975), and 18S rRNA has been implicated in mRNA recognition and binding (Kabat, 1975). Conditions have now been reported whereby human interferon can be observed to affect rRNA metabolism markedly (Maroun, 1978). This effect is characterized by a decrease in the ratio of [^{3}H]uridine-labelled 28S to 18S rRNA in extracts from the cytoplasm of interferon-treated human fibroblasts.

The purpose of the present paper is to give dose–response curves demonstrating a direct relationship between decrease in this ratio and interferon concentrations in the media and the results of a survey of eight human fibroblasts differing in chromosome 21 ploidy which suggests that interferon's ability to affect this rRNA-ratio decrease is related to the activity of a gene or genes present on chromosome 21.

Experimental

Cell culture, RNA extraction and sucrose-gradient-analysis procedures have been previously reported (Maroun *et al.*, 1971; Maroun & Miller, 1977; Maroun, 1978). Briefly, human fibroblasts are grown to confluency and labelled with [^{3}H]uridine for 24h beginning 12h after interferon addition. RNA is then extracted with phenol from the post-nuclear supernatant of cells hypo-osmotically swelled and homogenized with a tight-fitting Dounce homogenizer.

Unless otherwise noted, all experiments reported here utilized human fibroblast interferon prepared

and assayed by Meloy Laboratories, Springfield, VA, U.S.A. This FS-4 human fibroblast interferon was superinduced with poly(I)·poly(C) and had a final titre of 85000 international units/ml (calibrated to N.I.H. standard no. G-023-901-527 assayed with vesicular-stomatitis virus).

This unitage assignment should be considered an approximation. The variability problems with the bioassay of interferon are discussed in a review by Friedman (1977). Consistent with the reported species specificity of other interferon activities (Friedman, 1977), in addition to the chick-embryo fibroblast control reported previously (Maroun, 1978), this interferon preparation (tested at 500 units/ml) also had no effect on the rRNA ratio of primary rabbit kidney cultures.

In addition to the N.I.H. standard, reproducible rRNA-ratio effects have now been observed with numerous other interferon preparations, including: interferon 226-fold purified by hydrophobic affinity chromatography (9.3×10^5 units/mg of protein, obtained from H.E.M. Research, Rockville, MD, U.S.A.), and both superinduced and non-superinduced interferon secreted into media containing the identical serum lot and other media components used on both mock and test monolayers.

Newcastle-disease virus (strain Roanin) was obtained from the American Type Culture Collection and passed in the allantoic cavity of 10-day-embryonated Cofal chicken eggs (Spafas, Roanoke, IL, U.S.A.). At 12h after the addition of various concentrations of human fibroblast interferon, 100 haemagglutination units of Newcastle-disease virus was added to each 25 cm^2 flask. Then 16h later the medium was removed, the monolayers were washed twice with medium (without serum), and haem-absorption was measured with a 0.5% suspension of guinea-pig erythrocytes (Flow Laboratories, Rockville, MD, U.S.A.; 1h at 23°C).

Results and Discussion

The previously reported effect of interferon on the sucrose-gradient profile of [^{3}H]uridine-labelled cytoplasmic rRNA (Maroun, 1978) is shown in Fig. 1 (inserts). Changes in the relative peak heights of the

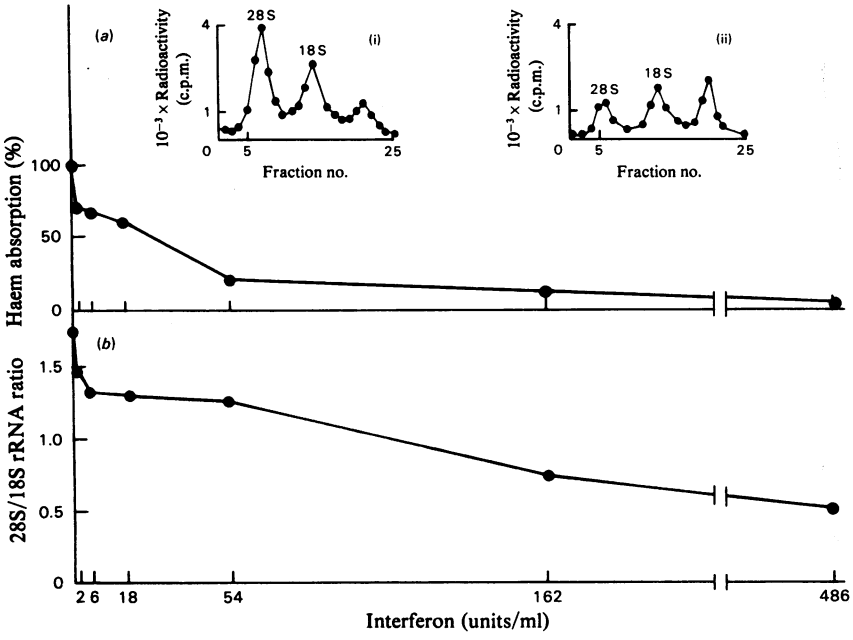

Fig. 1. *Decreasing 28S/18S rRNA ratio, and increasing resistance to Newcastle-disease virus infection, with increasing interferon dosage*

Insets show sucrose-gradient profiles from which the 2 units/ml (i) and 162units/ml (ii) ratios were calculated. (*a*) Increasing resistance to infection by Newcastle-disease virus. (*b*) Decreasing 28S/18S [^{3}H]uridine-labelled cytoplasmic rRNA ratios in parallel CCL84 (passage 29) monolayers. Ratios were calculated by division of the sum of the four highest points for the 28S RNA by the sum of the four highest values for the 18S RNA. In this system (CCL84), N.I.H. standard leucocyte interferon gave the following ratios: untreated, 1.56; interferon (162units/ml) 1.31; interferon (540 units/ml) 1.21; interferon (1000 units/ml) 0.83.

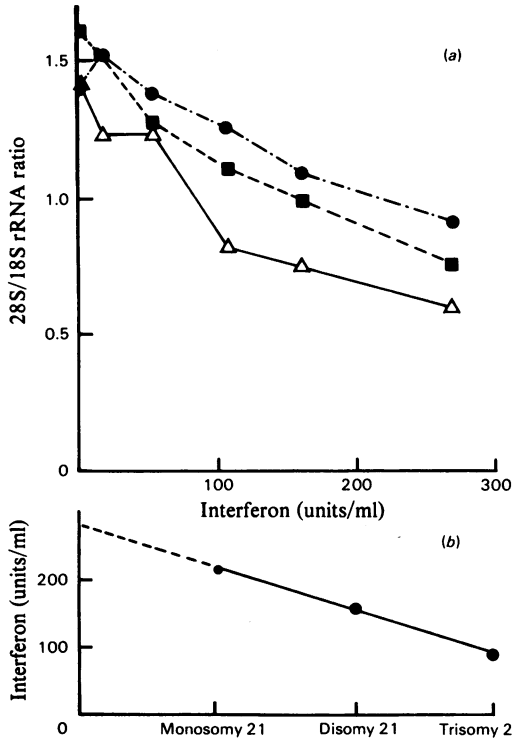

Fig. 2. *Interferon 28S/18S rRNA dose–response curves and chromosome 21 ploidy*

(*a*) ●, GM230 (passage 15) monosomy-21 fibroblasts, and ■, GM408 (passage 13) euploid normal fibroblasts, both obtained from the Institute for Medical Research, Camden, NJ, U.S.A., and △, CCL84 (passage 18) trisomy-21 Down's-syndrome fibroblast obtained from the American Type Culture Collection, Rockville, MD, U.S.A. (*b*) Graph of interferon dose required to give a 28S/18S rRNA ratio of 1.00 (chosen arbitrarily).

28S and 18S rRNA can be expressed as a 28S/18S ratio calculated by addition of the four highest radioactivity values for each peak and division of the 28S by the 18S radioactivity totals. These ratios were quite reproducible (particularly in euploid cells), owing, in part, to their independence from varying RNA extraction yields. The progressively decreasing values of this 28S/18S ratio with increasing interferon concentrations in the media are presented in Fig. 1(*b*). Fig. 1(*a*) presents the increasing resistance to Newcastle-disease-virus infection seen in parallel CCL84 monolayers.

All interferon preparations tested effected a reproducible change in the rRNA ratio in the 20–200 units/ml range. Significant disruption of rRNA metabolism becomes detectable in the 200–2000 units/ml range. These latter interferon concentrations match well with those needed to demonstrate interferon's cell-growth-inhibitory activity (Tan, 1976).

The observation that this rRNA ratio could be used as a quantitative indicator for interferon's effect on rRNA metabolism provided an opportunity to examine the role, if any, that chromosome 21 played in this rRNA phenomenon. Fig. 2(*a*) presents the complete dose–response curves for the initial monosomy-, disomy- and trisomy-21 cell lines tested. It should be noted, for comparative purposes, that the curve for trisomy-21 cells is essentially a repeat of that for the CCL84 cells in Fig. 1.

The sucrose-gradient curves used to calculate the ratios for the GM408 cells are presented in Fig. 3. In contrast with the gradient profiles observed with monosomy and euploid cell lines, gradients of RNA extracted from the CCL84 trisomy-21 cell line often contain a disproportionately high 3–6S radioactivity peak (Maroun, 1978; Fig. 1, insets).

To obtain a 28S/18S rRNA ratio of 1.00 (chosen arbitrarily), the monosomy-, disomy- and trisomy-21 cells required 214, 156 and 84 units of interferon/ml respectively. These values are plotted in Fig. 2(*b*). As nullsomy-21 and tetrasomy-21 human cell lines are not available, this graph is necessarily limited to three points and can be interpreted only with considerable caution. With this limitation in mind, these data suggest that a nullsomy-21 human cell line could respond to human interferon yielding an rRNA ratio of 1.0 at an interferon concentration of 270 units/ml in the medium. No special significance should be ascribed to the value 270 units/ml. This extrapolated value would likely be different if the data were analysed at 28S/18S rRNA ratios other than 1.0.

The extension of these observations to include additional cell types (tested at 270 units/ml) is presented in Table 1. Seven of the eight cell monolayers tested could be arranged in a pattern of increasing interferon-sensitivity, with increasing chromosome 21 ploidy. The monosomy-21 cell populations (GM137, GM230) were significantly less sensitive to interferon than the other six cell types tested ($P < 0.005$). The relative insensitivity of the GM230 monosomy cell line was confirmed by using a dual-label mixing protocol designed to confirm the absence of RNA degradation during the RNA extraction and analysis procedures (Maroun, 1978: ^{3}H-labelled GM230 cells, control ratio = 2.06; ^{32}P-labelled GM230 cells, treated with 270 units/ml, ratio = 0.91).

Of the cell lines tested, the most sensitive, by a wide margin, was a trisomy-21 line (CCL84). However, with this virus-independent assay, trisomy-21 cell monolayer sensitivity varied widely within the range from normal to supersensitive. Table 1 also presents the extension of the cytoplasmic RNA studies to include simultaneous whole-cell net RNA-synthesis

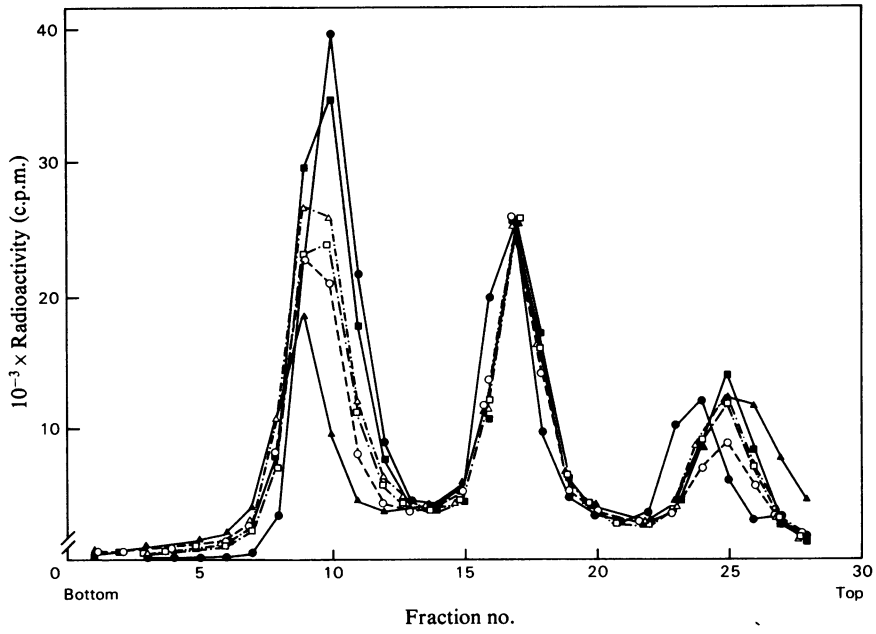

Fig. 3. *Sucrose-gradient radioactivity data for the GM408 monolayer rRNA-ratio dose–response curve shown in Fig. 2(a)* Phenol-extracted RNA was prepared in 0.05 M-sodium acetate (pH 5.2)/0.1 M-NaCl/0.5% sodium dodecyl sulphate, warmed to 37°C for 1 min and layered warm on 5–20% sucrose gradients prepared in 0.01 M-sodium acetate/0.1 M-NaCl, pH 5.2. Centrifugation was at 40000 rev./min for 6 h in a Beckman SW40 rotor. ●, Control (c.p.m. × 1.91); ■, 18 units/ml (c.p.m. × 1.00); △, 54 units/ml (c.p.m. × 1.85); □, 108 units/ml (c.p.m. × 1.61); ○, 162 units/ml (c.p.m. × 3.24); ▲, 270 units/ml (c.p.m. × 3.61).

Table 1. *Decrease in total cellular net RNA synthesis*

The protocol (using 270 units of interferon/ml) was essentially as previously described (Maroun, 1978), except that 0.5 ml of the 3 ml Dounce homogenate was removed before the centrifugation to remove nuclei. The 0.5 ml sample of total cell homogenate was then diluted with 4 vol. of deionized water, sonicated for 3 min at 30 W to break nuclei, and divided into two portions for trichloroacetic acid precipitation. Before this precipitation, one of the portions was digested for 4 h at 37°C with pancreatic ribonuclease (100 μ g/ml; Worthington ribonuclease A pretreated at 95°C for 10 min).

Cell designation	Total cellular ribonuclease-sensitive acid ppt. (10^{-3} × c.p.m./10^5 cells) Control	Interferon-treated	Decrease	Cytoplasmic rRNA 28S/18S ratio Control	Interferon-treated	Mean (±S.D.) [Chromosome 21 ploidy]
GM137	252	104	58%	1.53	0.81, 0.87	0.87 (±0.05) [Monosomy]
GM230	—	—	—	1.50	0.87, 0.94	
GM409	133	66	50%	1.44	0.70, 0.72	0.72 (±0.02) [Disomy]
GM408	—	—	—	1.62	0.75, —	
GM201	355	223	37%	1.59	0.71, 0.77	0.66 (±0.08) [Trisomy]
CCL54	132	85	35%	1.48	0.68, 0.70	
CCL66	159	29	82%	1.60	0.67, 0.69	
CCL84	—	—	—	1.56	0.52, 0.54	

measurements. These data suggest that underlying the interferon effect on cytoplasmic rRNA is a consistent decrease in net total cellular RNA synthesis. This observation would not favour, but cannot eliminate, the possibility that the interferon-mediated effects on cytoplasmic rRNA are due to processing or nucleus-to-cytoplasm transport phenomena.

The observation that interferon's ability to affect

rRNA metabolism is related to the activity of a gene or genes present on chromosome 21 immediately suggests the possibility that interferon is affecting the activity of the rRNA genes known to be present in chromosome 21's satellite regions.

An altered chromosome-21 rRNA gene activity represents the simplest explanation available, as it would not require postulating the presence on chromosome 21 of any as yet undiscovered genes. In addition, it would be consistent with the observation that chromosome-21 rRNA genes are 'only rarely open' (Miller *et al.*, 1976) and could serve to link the work on rRNA-gene activity (Miller *et al.*, 1977) with observations on chromosome 21 trisomy (Stoller & Collmann, 1965).

It should be noted that chromosome 21 long-arm localization studies (Tan & Greene, 1976; Epstein & Epstein, 1976) are concerned solely with interferon-sensitivity and are not necessarily in conflict with the possibility that the short arm of chromosone 21 is involved in interferon's multiple biochemical effects. In addition, translocation karyotyping data are not of sufficiently high resolution to exclude the presence of chromosome-21 rRNA genes. For example, a 45,XX, t(15q21q) cell, which by karyotyping analysis would be expected to be lacking one set of chromosome-21 rRNA genes, when analysed by rRNA–DNA hydridization was in fact found to contain the normal complement of rRNA genes (Bross *et al.*, 1973; Dittes *et al.*, 1975). Perhaps notably, these studies also revealed that trisomy-21-cell rRNA gene content is quite variable and often significantly higher than would have been predicted from the simple addition of one extra chromosome 21.

The possible involvement of rRNA in interferon action and interferon receptor and antiviral protein hypotheses should not be considered mutually exclusive possibilities. Models of interferon action can be envisaged which could effectively incorporate any or all of these hypothetical components.

I thank Dr. Dighton Rowan for the Newcastle-disease virus, Ms. Joan Blackwell and Mr. Adeyinka Cole for expert technical assistance, and the Biomedical Communications Department.

References

Bross, K., Dittes, H., Krone, W., Schmid, M. & Vogel, W. (1973) *Humangenetik* **20**, 223–229

De Clercq, E., Edy, V. G. & Cassiman, J.-J. (1975) *Nature (London)* **256**, 132–134

De Clercq, E., Edy, V. G. & Cassiman, J.-J. (1976) *Nature (London)* **264**, 249–251

Dittes, H., Krone, W., Bross, K., Schmid, M. & Vogel, W. (1975) *Humangenetik* **26**, 47–59

Epstein, L. B. & Epstein, C. J. (1976) *J. Infect. Dis.* **133**, 56–62

Friedman, M. (1977) *Bacteriol. Rev.* **41**, 543–567

Kabat, D. (1975) *J. Biol. Chem.* **250**, 6085–6092

Maroun, L. E. (1978) *Biochim. Biophys. Acta* **517**, 109–114

Maroun, L. E. & Miller, E. T. (1977) *J. Cell. Physiol.* **92**, 375–380

Maroun, L. E., Driscoll, B. F. & Nardone, R. M. (1971) *Nature (London) New Biol.* **231**, 270–271

Miller, D. A., Tantravahi, R., Dev, V. G. & Miller, O. J. (1977) *Am. J. Hum. Genet.* **29**, 490–502

Miller, O. J., Miller D. A., Dev, V. G., Tantravahi, R. & Croce, C. M. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4531–4545

Revel, M., Bash, D. & Ruddle, F. H. (1976) *Nature (London)* **260**, 139–141

Stoller, A. & Collmann, R. D. (1965) *Lancet* **289**, 1221–1223

Tan, Y. H. (1975) *Nature (London)* **253**, 280–282

Tan, Y. H. (1976) *Nature (London)* **260**, 141–143

Tan, Y. H. & Greene, A. E. (1976) *J. Gen. Virol.* **32**, 153–155

Wiranowska-Stewart, M. & Stewart, W. E. (1977) *J. Gen. Virol.* **37**, 629–633