Regulation of protein synthesis in lymphoblastoid cells during inhibition of cell proliferation by human interferons

Michael J. Clemens, Margaret A. McNurlan*, Graham Moore+ and Vivienne J. Tilleray

Cancer Research Campaign Mammalian Protein Synthesis and Interferon Research Group, Department of Biochemistry, St George's Hospital Medical School, London SW17 0RE, England

Received 3 April 1984

Treatment of human lymphoblastoid (Daudi) cells with interferons inhibits cell proliferation in culture within 24 h. The failure of cell growth has been shown to be associated with impaired processing and decreased stability of newly replicated DNA. Because there is a close relationship between DNA replication and protein synthesis we have measured protein synthesis in intact Daudi cells. Protein synthesis declined steadily between 24 and 96 h after interferon treatment to a value which is only 20–30% of the rate in control cells. The enzyme 2′,5′-oligo(A) synthetase is induced but our data do not support a role for the 2′,5′-oligo(A)-activated ribonuclease in the control of translation in this system.

Interferon *Protein synthesis* *DNA replication* *2′,5′-Oligo(A)* *Cell proliferation* *Daudi cell*

1. INTRODUCTION

Among the mammalian cell lines which are sensitive to the cell growth inhibitory effects of interferons, the Daudi line of human lymphoblastoid cells is one of the most amenable to study [1–7]. We are conducting a detailed analysis of the biochemical mechanisms which underlie the inhibition of cell growth and division by human α-interferons in this system and have shown that impairment of cell proliferation is accompanied by changes in DNA replication within 24 h of treatment. Prominent amongst these changes are a strong inhibition of thymidine kinase activity [2,3], a delay in the time required for processing of newly replicated Okazaki fragments to larger intermediates of DNA replication [5], and instability of a proportion of the newly replicated DNA sequences leading to their turnover within a few hours of their synthesis [5]. We have proposed that these molecular changes may be responsible for the inhibition of cell proliferation in response to interferon treatment in this system.

The process of DNA replication in mammalian cells is tightly coupled with that of protein synthesis and requirements for newly synthesised protein for several steps in the assembly of newly replicated DNA have been reported [8–10]. There is little information, however, concerning changes in the rate or pattern of protein synthesis during the response of Daudi cells to interferon treatment. We have therefore investigated the changes in protein synthesis which accompany growth inhibition to determine whether these may be related to the inhibition of DNA replication or the decrease in stability of newly synthesised DNA. Several molecular mechanisms for inhibition of protein synthesis in virus infected, interferon-treated cells have been established. These include induction of 2′,5′-oligo(A) synthetase and the activation of this enzyme by double-stranded RNA in virus-infected

* Present address: The Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB2 9SB, Scotland

+ Present address: Département de Biologie Moléculaire, Institut Pasteur, 25–28 Rue du Docteur Roux, F-75724 Paris Cédex 15, France

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

cells [11,12], the induction of a protein kinase which can phosphorylate the small subunit of initiation factor eIF-2 [13,14], and changes in the ability of cells to methylate newly synthesised mRNA [15]. The majority of these changes occur within the first few hours of interferon treatment but may only take effect in the presence of viral replicative intermediates. In many studies on the effects of interferon on uninfected cells and their extracts little change in the rate of overall protein synthesis has been observed [16–19] although there are exceptions to this finding [20,21]. In this study we have investigated the responses of Daudi cells to prolonged interferon treatment (up to 4 days) and have established some characteristics of the inhibition of translation observed.

2. MATERIALS AND METHODS

2.1. Materials

Human lymphoblastoid cell interferon preparations were kindly provided by Drs K.H. Fantes and M.D. Johnston (Wellcome Research Laboratories, Beckenham, England). They were diluted in RPMI 1640 medium in the presence of serum, to minimise losses due to adsorption of protein. Radiochemicals were from Amersham International or New England Nuclear. 5,6-Dichloro-1-β-D-ribofuranosylbenzimadazole (DRB) was from Calbiochem and poly(I)·poly(C) agarose was from P-L Biochemicals.

2.2. Cell culture and determination of protein synthesis

Daudi cells were grown in culture and treated with interferons as in [2–5]. Protein synthesis in the intact cells was monitored either by use of a procedure involving labelling with a high concentration of [^{3}H]phenylalanine [22] or, less rigorously, by the incorporation of [^{14}C]leucine or [^{35}S]methionine. In the case of phenylalanine incorporation the specific radioactivity of the precursor in the culture medium and in the intracellular amino acid pool was determined as in [22]. The specific radioactivity of phenylalanine in protein was determined on acid hydrolysates by a similar method. Full details and characterization of this method for use with cultured cells will be described elsewhere (M.A. McNurlan et al., in preparation). Incorporation of methionine into cell protein was determined by trichloroacetic acid precipitation of aliquots of cell suspension as in [2–5].

2.3. Preparation of cell extracts

Cell extracts were prepared by detergent lysis of washed Daudi cells using a modification of a procedure previously described for other types of cultured cells [23]. Lysis was effected with 0.25% Nonidet P40 and postmitochondrial supernatants were prepared by centrifugation of the lysates at $10\,000 \times g$ for 10 min. Supernatants were stored in small aliquots under liquid nitrogen.

2.4. Determination of polysome profiles

The distribution of ribosomes between subunits, monomers and polysomes was determined by sucrose density gradient centrifugation and fractionation as in [24].

2.5. Protein synthesis in cell extracts

Incorporation of [^{35}S]methionine into nascent chains coded by endogenous mRNA in polysomes was monitored in vitro as in [23]. Incorporation was determined by trichloroacetic acid precipitation of protein on filter discs [25]. In this system the majority of incorporation represents the completion of preexisting nascent polypeptide chains.

2.6. Determination of 2′,5′-oligo(A) synthetase activity

Preparation of extracts and assays of the activity of 2′,5′-oligo(A) synthetase were carried out as in [26]. The enzyme was bound to poly(I)·poly(C)–agarose, incubated in vitro with [^{3}H]ATP for 20 h at 30°C and the products chromatographed on DEAE–cellulose. 2′,5′-oligo(A) compounds were eluted with 0.35 M KCl [26]. The activity is expressed as nmol ATP converted into 2′,5′-oligo(A) per mg protein in the original cytoplasmic extracts.

2.7. Incorporation of thymidine into DNA

Thymidine incorporation was determined as in [2–5].

2.8. Determination of protein and RNA in cells

Protein and total cellular RNA were assayed as in [27].

3. RESULTS AND DISCUSSION

The effects of various times of interferon treatment on the rate of protein synthesis in Daudi cells are listed in table 1. As growth inhibition develops, from 24 h onwards, the rate of protein synthesis falls relative to that in exponentially growing control cells such that a 20–30% inhibition is observed after 24 h, increasing to 60–70% by 48 h and greater than 70% thereafter. These rates were determined using a rigorous procedure involving labelling with saturating concentrations of [^{3}H]phenylalanine, a method which has been substantiated in a number of mammalian systems [22]. Table 1 also indicates that there is a fall in the RNA content of the cells (largely a reflection of the number of ribosomes per cell) during the response to interferon treatment. However, the decline in protein synthesis is greater than the decrease in ribosome number.

As in many interferon-treated cell systems, the enzyme 2′,5′-oligo(A) synthetase is induced in Daudi cells within 8–12 h [6,28]. Fig.1 illustrates the time course of induction and shows that no further increase in enzyme activity is observed after 24 h. The level of the enzyme thus does not correlate with the increasing inhibition of protein synthesis observed beyond 24 h. Measurements of 2′,5′-oligo(A) products in Daudi cells have failed to reveal any synthesis of these oligonucleotides following interferon treatment [6]. The lack of involvement of the 2′,5′-(A) system in the effects on protein synthesis in Daudi cells is further suggested by the data in fig.2. If there had been activation of a 2′,5′-(A)-dependent ribonuclease, which degrades mRNAs [29], one would expect to see a more rapid decline in protein synthesis following inhibition of transcription in the cells [30]. However, when all new mRNA synthesis was blocked by the addition of 60 μM DRB to cultures of control and interferon-treated Daudi cells, protein synthesis declined at similar rates in both cases, with a half-life of approx. 7–9 h. This suggests that the stability of the bulk of the mRNA population is the same in control cells and in cells subjected to 48 h of interferon treatment.

Table 1

Rates of protein synthesis in Daudi cells during inhibition of cell proliferation by interferons

Time of interferon treatment (days)	Rate of protein synthesis (μg/10^6 cells per h) (±SE)	RNA content (μg RNA/10^6 cells) (±SE) (n = 3–5)
0 (control cells)	3.08 ± 0.08 (26)	11.70 ± 1.00
1	2.34 ± 0.08 (5)	9.96 ± 0.08
2	1.12 ± 0.13 (10)	8.78 ± 0.94
3	0.83 ± 0.05 (12)	8.09 ± 0.12
4	0.74 ± 0.10 (12)	5,21 ± 0.48

Exponentially growing Daudi cells were treated with human α-interferons (5 reference units/ml) and maintained in culture for the times indicated. Rates of protein synthesis were calculated from time courses of incorporation of [^{3}H]phenylalanine (2.5 μCi/ml, 2.5 mM) into protein. Specific radioactivities of phenylalanine in the extracellular and intracellular amino acid pools and in protein were measured as described in section 2. Results are expressed as means ±SE with numbers of determinations given in parentheses

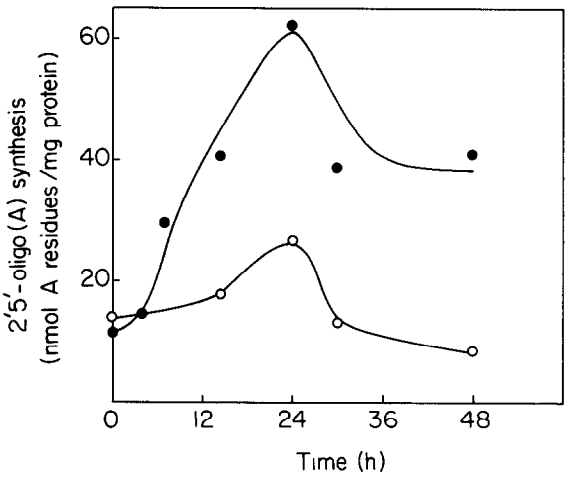

Fig.1. Induction of 2′,5′-oligo(A) synthetase by interferons in Daudi cells. Exponentially growing cells were treated with human α-interferons (5 reference units/ml) and incubated for the times shown (●). Another batch of the same population of cells was maintained in culture in the absence of interferons (○). Cell extracts were prepared by detergent lysis of washed cells and 5 μl of each post-mitochondrial supernatant was incubated with 50 μl poly(I)·poly(C)–agarose for 20 min at 0°C followed by incubation with 1 mM [^{3}H]ATP and chromatography of the products as described in section 2. The conversion of ATP to 2′,5′-oligo(A) products is expressed as nmol adenylate residues polymerized per mg total protein in the cell extracts.

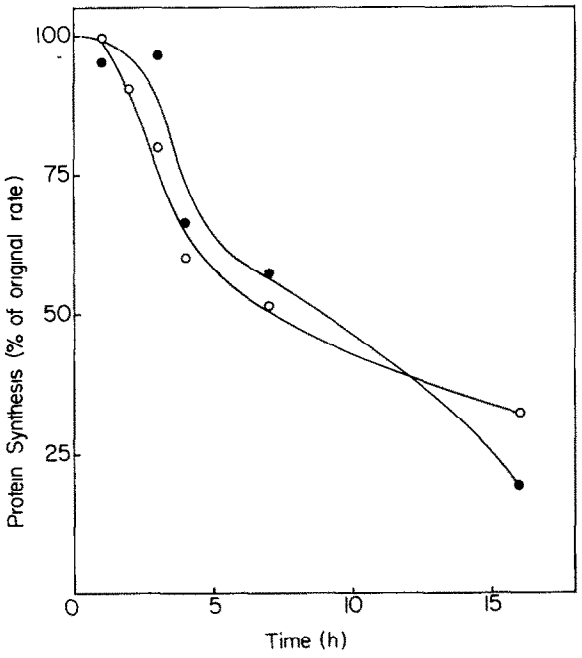

Fig.2. Rate of decline of protein synthesis in control and interferon-treated Daudi cells in the absence of new mRNA synthesis. Cells incubated with (●) or without (○) interferons (5 reference units/ml) for 48 h were treated with 60 μM DRB. Protein synthesis was then monitored at the times indicated by the incorporation of [^{3}H]leucine (10 μCi/ml, 0.38 mM, 30 min at 37°C). Duplicate 0.3-ml aliquots of cell suspensions were diluted into 5 ml phosphate-buffered saline, the cells were washed by centrifugation and the protein in the pellets was precipitated with 5% (w/v) trichloroacetic acid for determination of acid-insoluble radioactivity. The means of 2 separate experiments are shown. The results are expressed as % of the incorporation obtained before addition of DRB.

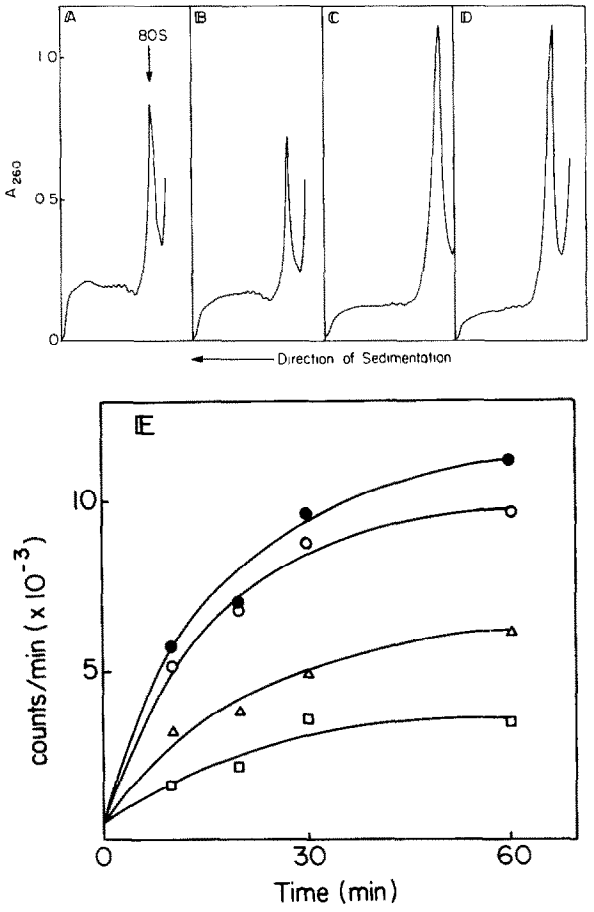

Fig.3. Disaggregation of polysomes and inhibition of in vitro protein synthesis by inteferon treatment. Exponentially growing Daudi cells were untreated (A) or treated with interferons (5 units/ml) for 1 day (B), 2 days (C) and 4 days (D). Cell extracts were prepared as described in section 2. Distributions of ribosomes in polysomes were determined by centrifugation through 20–50% sucrose gradients [24] followed by continuous measurement of A_{260} in a recording spectrophotometer. (E) Corresponding levels of protein synthesis achieved on incubation of equal A_{260} amounts of cell extracts with [^{35}S]methionine (60 μCi/ml) in vitro under conditions of protein synthesis: (●) control, (○) 1-day treated, (▲) 2-day treated, (□) 4-day treated. Radioactivity in protein in 20-μl samples is shown.

The inhibition of protein synthesis in interferon-treated Daudi cells is associated with a decrease in the proportion of ribosomes in polysomes, as measured both by direct quantitation of polysome size distribution on sucrose density gradients (fig.3A–D) and by the synthesis of nascent polypeptide chains by Daudi cell extracts in vitro (fig.3E). These results suggest inhibition of translation at the level of polypeptide chain initiation. This has been confirmed in our laboratory by analysis of the ability of cell extracts to form initiation complexes (not shown). Overall our results suggest an interferon-induced impairment in the activity of one or more components involved in the initiation process, rather than a decreased stability of mRNA, in Daudi cells.

Our results are relevant to our previous observations concerning DNA replication during growth inhibition of Daudi cells by interferons [3–5]. As

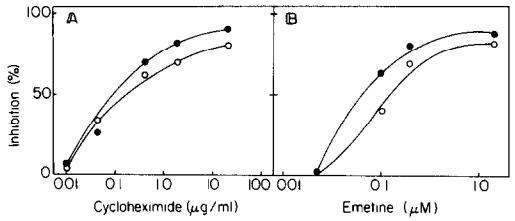

Fig.4. Relationship between the rate of protein synthesis and incorporation of [^{3}H]thymidine into DNA in Daudi cells. Exponentially growing cells were incubated with the indicated concentrations of (A) cycloheximide or (B) emetine for 30 min and then labelled with [^{3}H]thymidine (2 μCi/ml) or [^{14}C]leucine (2 μCi/ml) for 60 min. Incorporation of radioactivity into DNA (○) or protein (●) was determined by trichloroacetic acid precipitation of cell suspensions and inhibition is expressed relative to the incorporation obtained in the absence of the inhibitors.

fig.4 illustrates, the rate of DNA synthesis is closely linked with that of protein synthesis as judged by the sensitivities of the two processes to the translational inhibitors cycloheximide and emetine. The basis for this relationship has not been established but it may reflect a continuous requirement for synthesis of histones and other chromatin-associated proteins during DNA replication [8–10]. In view of the present observations concerning protein synthesis during interferon-induced growth inhibition, it may be that one or more proteins essential for DNA replication are synthesised at an insufficient rate to support the normal replication of the cellular genome, thus leading to defects in the initiation, processing or stability of newly replicated DNA.

It is presently unclear whether the synthesis of all cellular proteins is inhibited to an equal extent following interferon treatment. In view of the apparent inhibition of polypeptide chain initiation, it is possible that the translation of those mRNAs with the lowest affinities for limiting amounts of pre-initiation complexes would be preferentially inhibited. Conversely, those messages with high efficiencies of initiation may continue to be translated at near-normal rates [31]. These considerations may explain the apparently greater effect of interferon treatment on protein synthesis and on the stabilty of newly replicated DNA than on the rate of DNA polymerisation per se. [5]. Differential inhibition of synthesis of different proteins could also provide an explanation for the effects of interferon treatment on cell differentiation and on the expression of specific genes in a variety of cell systems [32,33].

In conclusion, we have shown that protein synthesis is strongly inhibited at a rather late stage in the response of Daudi cells to human interferon treatment and have presented evidence suggesting that changes in mRNA stability [and by implication the 2′,5′-(A) system] do not constitute a major mechanism by which this is achieved. The inhibition of polypeptide chain initiation may reflect changes in the activity of initiation factor eIF-2, which can be phosphorylated by an interferon-induced protein kinase [13,14], but this effect appears to require 24–48 h of interferon treatment before it is manifested. This late response may explain the previous failure of others to demonstrate substantial inhibition of protein synthesis in uninfected, interferon-treated cells in culture.

ACKNOWLEDGEMENTS

M.J.C. holds a Career Development Award from the Cancer Research Campaign and this work is supported by a grant from this source. G.M. was supported by a Science and Engineering Research Council Research Studentship. We thank Barbara Bashford for the drawings and Vivienne Marvell for preparation of this manuscript.

REFERENCES

[1] Hilfenhaus, J., Damm, H. and Johanssen, R. (1977) Arch. Virol. 54, 271–277.
[2] Gewert, D.R., Shah, S. and Clemens, M.J. (1981) Eur. J. Biochem. 116, 487–492.
[3] Gewert, D.R., Moore, G. and Clemens, M.J. (1983) Biochem. J. 214, 983–990.
[4] Gewert, D.R., Moore, G., Tilleray, V.J. and Clemens, M.J. (1984) Eur. J. Biochem., in press.
[5] Moore, G., Gewert, D.R. and Clemens, M.J. (1984) Eur. J. Biochem., in press.
[6] Silverman, R.H., Watling, D., Balkwill, F.R., Trowsdale, J. and Kerr, I.M. (1982) Eur. J. Biochem. 126, 333–341.
[7] Mogensen, K.E. and Bandu, M.-T. (1983) Eur. J. Biochem. 134, 355–364.
[8] Tseng, B.Y. and Goulian, M. (1975) J. Mol. Biol. 99, 317–337.

[9] Seale, R.L. and Simpson, R.T. (1975) J. Mol. Biol. 94, 479–501.
[10] De Pamphilis, M.L. and Wassarman, P.M. (1980) Annu. Rev. Biochem. 49, 627–666.
[11] Hovanessian, A.G. and Kerr, I.M. (1979) Eur. J. Biochem. 93, 515–526.
[12] Williams, B.R.G., Golgher, R.R., Brown, R.E., Gilbert, C.S. and Kerr, I.M. (1979) Nature 282, 582–586.
[13] Zilberstein, A., Kimchi, A., Schmidt, A. and Revel, M. (1978) Proc. Natl. Acad. Sci. USA 75, 4734–4738.
[14] Sen, G.C., Taira, H. and Lengyel, P. (1978) J. Biol. Chem. 253, 5915–5921.
[15] De Ferra, F. and Baglioni, C. (1983) J. Biol. Chem. 258, 2118–2121.
[16] Merigan, T.C., Winget, C.A. and Dixon, C.B. (1965) J. Mol. Biol. 13, 679–691.
[17] Kerr, I.M. (1971) J. Virol. 7, 448–459.
[18] Falcoff, E., Falcoff, R., Lebleu, B. and Revel, M. (1972) Nat. New Biol. 240, 145–147.
[19] Gupta, S.L., Sopori, M.L. and Lengyel, P. (1973) Biochem. Biophys. Res. Commun. 54, 777–783.
[20] Brouty-Boyé, D., Macieira-Coelho, A., Fiszman, M. and Gresser, I. (1973) Int. J. Cancer 12, 250–258.
[21] Fuse, A. and Kuwata, T. (1976) J. Gen. Virol. 33, 17–24.
[22] Garlick, P.J., McNurlan, M.A. and Preedy, V.R. (1980) Biochem. J. 192, 719–723.
[23] Pain, V.M., Lewis, J.A., Huvos, P., Henshaw, E.C. and Clemens, M.J. (1980) J. Biol. Chem. 255, 1486–1491.
[24] Austin, S.A., Pain, V.M., Lewis, J.A. and Clemens, M.J. (1982) Eur. J. Biochem. 122, 519–526.
[25] Mans, R.J. and Novelli, G.D. (1961) Arch. Biochem. Biophys. 94, 48–53.
[26] Lewis, J.A., Mengheri, E. and Esteban, M. (1983) Proc. Natl. Acad. Sci. USA 80, 26–30.
[27] Munro, H.N. and Fleck, A. (1969) in: Mammalian Protein Metabolism (Munro, H.N. ed.) vol. 3, pp. 423–525, Academic Press, New York.
[28] Koliais, S.I. and Kortsaris, A. (1982) J. Gen. Virol. 58, 191–193.
[29] Clemens, M.J. and Williams, B.R.G. (1978) Cell 13, 565–572.
[30] Nilsen, T.W., Maroney, P.A. and Baglioni, C. (1983) Mol. Cell. Biol. 3, 64–69.
[31] Nuss, D.L. and Koch, G. (1976) J. Mol. Biol. 102, 601–612.
[32] Fisher, P.B., Mufson, R.A. and Weinstein, I.B. (1981) Biochem. Biophys. Res. Commun. 100, 823–830.
[33] Lough, J., Keay, S., Sabran, J.L. and Grossberg, S.E. (1982) Biochem. Biophys. Res. Commun. 109, 92–99.