Volume 16 Number 8 1988 Nucleic Acids Research

Regulation of *in vitro* translation by double-stranded RNA in mammalian cell mRNA preparations

Greg Pratt, Angela Galpine, Nigel Sharp, Susan Palmer and Michael J.Clemens

Department of Biochemistry, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, UK

Received January 11, 1988; Revised and Accepted February 25, 1988

ABSTRACT

Polyadenylated mRNA has been purified from a variety of human and mouse cell sources. These preparations are actively translated in the wheat germ cell-free system but have only poor ability to stimulate the nuclease-treated reticulocyte lysate. The translation of endogenous and exogenous globin mRNA is strongly inhibited by the poly(A)$^+$RNA preparations in reticulocyte lysates. Both polysomal and non-polysomal RNA have similar effects but poly(A)$^+$RNA is almost 2000-fold more inhibitory than poly(A)$^-$RNA on a weight basis. The inhibition is abolished in the presence a high concentration of poly(I).poly(C). Analysis of endogenous eIF-2 in the lysate reveals that the α subunit becomes extensively phosphorylated in the presence of the inhibitory poly(A)$^+$RNA. Prolonged incubation of lysate with poly(A)$^+$RNA also causes some nucleolytic degradation of polysomal globin mRNA. These characteristics suggest that some eukaryotic cell mRNAs contain regions of double-stranded structure which are sufficiently extensive to activate translational control mechanisms in the reticulocyte lysate.

INTRODUCTION

The ability of double-stranded RNA (dsRNA) to regulate translation in mammalian cells has been recognized for many years. Two pathways have been identified by which such control is exerted, viz. activation of a protein kinase which specifically phosphorylates initiation factor eIF-2, and activation of 2'5' oligoadenylate synthetase which synthesizes pppA2'p5'(A)$_n$ oligomers from ATP (1,2). The former pathway results in loss of ability of eIF-2 to recycle between successive rounds of protein synthesis (3) whereas the production of 2'5' oligoadenylates activates RNase L which degrades mRNA and rRNA (4-6). Both processes result in inhibition of protein synthesis at the translational level.

Both the dsRNA-dependent protein kinase and 2'5' oligo(A) synthetase are constitutively present in reticulocyte lysates (7,8). In addition, in most cell types they are induced by interferon treatment and are believed to be important in the development of an antiviral state in interferon-treated cells

© IRL Press Limited, Oxford, England.

(1,2). There is evidence for activation of both enzymes by the viral dsRNA produced during the replication of some RNA viruses (9,10). It has remained less clear, however, whether these enzymes have a role to play in cells infected with viruses that do not produce dsRNA or in uninfected cells (for example in growth control) and how the enzymes may be activated under such circumstances.

During the course of studies on translation in the reticulocyte lysate of mRNA preparations from a variety of mammalian cells we have observed that protein synthesis declines rapidly in a manner suggestive of inhibition by dsRNA. This paper describes our analysis of this phenomenon and presents evidence that mammalian cellular mRNAs contain regions of double-stranded structure which are able to activate the eIF-2 protein kinase. Such mRNAs thus have the potential to regulate translation at the level of polypeptide chain initiation in cells containing this enzyme.

MATERIALS AND METHODS

Materials

The synthetic dsRNA, polyinosinic acid.polycytidylic acid (poly(I).poly(C)) and cycloheximide were obtained from Sigma Chemical Co. Radiochemicals were from Amersham International. The mouse monoclonal antibody against the α subunit of eIF-2 was purified from ascites fluid from a hybridoma generously provided by Drs K.Scorsone and E.C.Henshaw (University of Rochester Cancer Center, New York). Biotinylated anti-mouse IgG and an avidin/biotinylated peroxidase detection kit were purchased from Vector Laboratories.

Sources of mammalian RNA

Mouse Ehrlich ascites tumour cells were grown in suspension culture as previously described (11). Lymphoblastoid cell lines were all grown in RPMI 1640 medium as previously described for Daudi cells (12). B95-8 (Epstein-Barr virus-transformed marmoset lymphoblasts), DG75 (EBV-negative Burkitt lymphoma) and Ball-1 (acute lymphocytic leukaemia) cells were a gift from Dr J.Arrand (Paterson Laboratories, Manchester). Mouse erythroleukaemia cells (clone M2) (a gift from Dr P.Harrison, Beatson Institute, Glasgow) were grown in Ham's F12 medium with 15% (v/v) horse serum.

A variety of methods was used to prepare intact RNA from whole cells or cell extracts. Total cellular RNA was made by the LiCl/urea method of Auffray and Rougeon (13). Polysomal RNA and non-polysomal cytoplasmic RNA from Ehrlich cells were prepared by centrifugation of a post-mitochondrial supernatant at 260,000 x g for 2h, followed by extraction of the pellet and

supernatant, respectively, using the same method. Total cytoplasmic RNA from mouse erythroleukaemia cells was prepared by phenol:chloroform extraction of post-nuclear supernatants. RNA preparations were fractionated into poly(A)$^+$ and poly(A)$^-$ populations by oligo(dT)-cellulose chromatography (14). Three cycles of binding and elution (in sterile water) were employed, each involving 3 passes of the flow-through fraction over the column to allow maximum binding of poly(A)$^+$ RNA. SDS was omitted from the last washes to eliminate non-specific inhibitory effects on translation. Globin mRNA was prepared by LiCl/urea extraction of reticulocyte lysate and similarly purified by oligo(dT)-cellulose chromatography.

Cell-free translation systems

Rabbit reticulocyte lysates (native or micrococcal nuclease-treated) and wheat-germ extracts were prepared and incubated using standard, published methods (15). Polysome profiles in the reticulocyte system were determined by sucrose density gradient centrifugation as described previously (5).

Initiation factor eIF-2

The phosphorylation state of endogenous eIF-2 in the reticulocyte lysate was determined by slab gel isoelectric focussing of urea-denatured samples (16), followed by transfer to nitrocellulose and immunoblotting with a monoclonal antibody against the α subunit (17). The blots were developed using a biotinylated second antibody and an avidin/biotinylated peroxidase amplification system with 4-chloronaphthol as a substrate (18). The percent phosphorylation of eIF-2α was quantified by scanning with an LKB 2202 laser densitometer and the data were analysed using the LKB 2190 GELSCAN program.

RESULTS

Polyadenylated RNA from a range of mammalian cells was assayed for its ability to be translated in the two most frequently used heterologous cell-free systems, viz.the wheat germ and nuclease-treated rabbit reticulocyte lysate systems. As an example, the ability of poly(A)$^+$ RNA from Daudi lymphoblastoid cells to stimulate protein synthesis in these systems is shown in Fig.1(a and b). For comparison, the translation of added globin mRNA is also shown. Whereas both the Daudi cell and globin mRNA preparations were translated with linear kinetics in the wheat-germ extract, with the former showing the greater activity at the concentrations used, in the reticulocyte lysate the globin message was much more active than the Daudi cell RNA as a template and translation of the latter ceased after 15 min. Not only was the lymphoblastoid cell mRNA poorly translated in the reticulocyte lysate but it

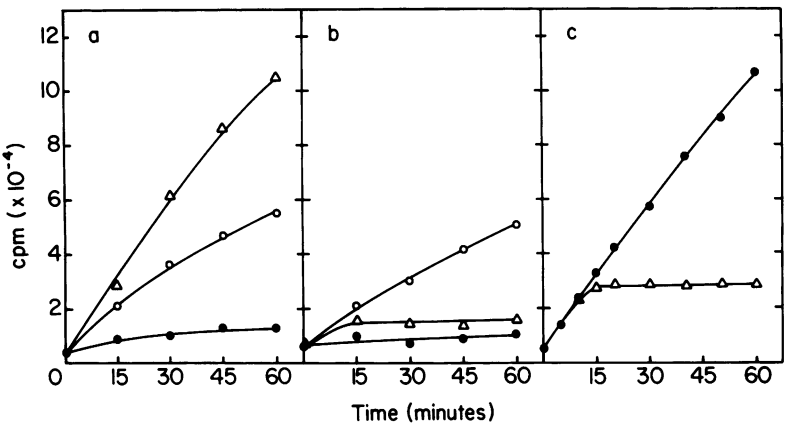

Figure 1. Translation of Daudi cell poly(A)$^+$RNA and globin mRNA in wheat-germ and reticulocyte lysate cell-free systems. Daudi cell total RNA was prepared by the LiCl/urea method and poly(A)$^+$RNA selected by oligo(dT)-cellulose chromatography. Globin mRNA was prepared from rabbit reticulocyte lysate. Translation of these mRNAs was carried out using (a) wheat germ cell-free system, (b) micrococcal nuclease -treated reticulocyte lysate or (c) non-nuclease-treated reticulocyte lysate. ● , no added RNA; ○ , globin mRNA (5 μg/ml); △ , Daudi poly(A)$^+$RNA (10 μg/ml in a and c, 5 μg/ml in b). Protein synthesis was monitored in 5 μl aliquots at the times indicated by the incorporation of [^{35}S]methionine (100 μCi/ml) into hot trichloroacetic acid-insoluble material.

inhibited the translation of the endogenous reticulocyte mRNA in a non-nuclease-treated system (Fig.1c). This also occurred after a consistent lag time of 10-15 min, prior to which translation was normal. These characteristics are not peculiar to Daudi cell mRNA but were observed with RNA preparations from other lymphoblastoid and leukaemic cell lines (both Epstein Barr virus-positive and -negative), mouse Ehrlich ascites tumour cells, mouse erythroleukaemia cells and normal mouse liver. Inhibition of globin synthesis was seen with both endogenous reticulocyte mRNA (Table 1) and exogenous reticulocyte mRNA in the nuclease-treated lysate (Fig.2a) but not the wheat germ system (Fig.2b).

The nature of the inhibitory species in the RNA preparations was investigated by comparing the concentration-dependence for the effects of total cellular RNA, polyadenylated RNA, non-polyadenylated RNA (Fig.3a), and polysomal RNA versus non-polysomal cytoplasmic RNA (Fig.3b). It can be seen that poly(A)$^+$RNA was very much more inhibitory for the reticulocyte lysate (half-maximal effect at ca. 9 ng/ml) than was poly(A)$^-$RNA (half-maximal effect at ca. 16 μg/ml). The latter inhibition may represent a non-specific effect

TABLE 1.
INHIBITION OF ENDOGENOUS GLOBIN SYNTHESIS IN THE RETICULOCYTE LYSATE BY POLY(A)$^+$RNA FROM VARIOUS CELL TYPES.

Experiment	Cellular origin of poly(A)$^+$RNA	Protein synthesis (counts per min x 10^{-4})
I	None added	7.21
	Daudi (EBV +ve Burkitt lymphoma)	2.18
	B95-8 (EBV-transformed lymphoblast)	2.21
	?G 75 (EBV -ve Burkitt lymphoma)	2.54
	Ball-1 (acute lymphocytic leukaemia)	2.37
II	None added	17.41
	Ehrlich ascites (mouse carcinoma)	3.59

Poly(A)$^+$RNAs were prepared from the indicated cell types as described in Figure 1. Reticulocyte lysates were incubated in the presence and absence of these RNA preparations (10 µg/ml) for 60 min and the incorporation of [^{35}S]methionine (100 µCi/ml) into protein determined.

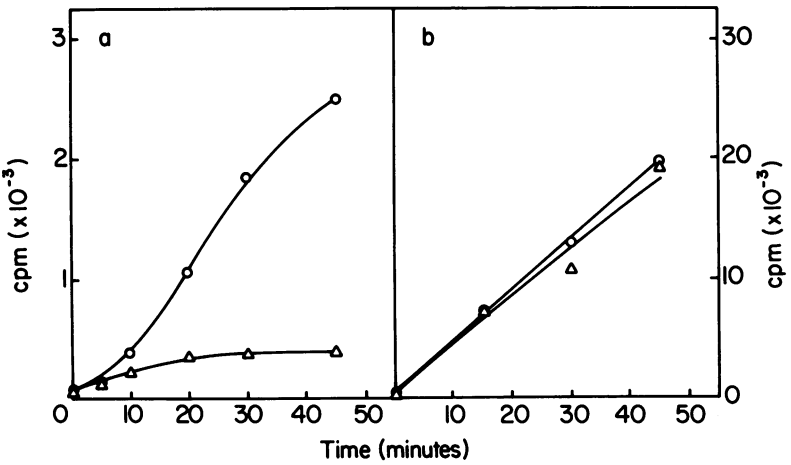

Figure 2. Effect of cytoplasmic poly(A)$^+$RNA on globin mRNA translation in reticulocyte lysate and wheat-germ cell-free systems. Cytoplasmic RNA was prepared from mouse erythroleukaemia cells by phenol:chloroform extraction and poly(A)$^+$RNA was selected by oligo(dT)-cellulose chromatography. Globin mRNA from reticulocytes was translated in (a) a nuclease-treated reticulocyte lysate or (b) a wheat-germ cell-free system in the absence (○) or presence (△) of erythroleukaemia cell poly(A)$^+$RNA (32 µg/ml in a; 13 µg/ml in b). Protein synthesis was measured by the incorporation of (a) [^{14}C]leucine (4 µCi/ml) in 3 µl aliquots or (b) [^{35}S]methionine (100 µCi/ml) in 5 µl aliquots.

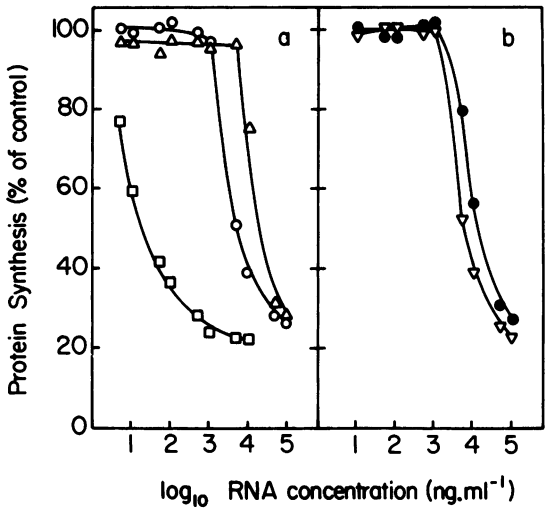

Figure 3. Concentration-dependence for inhibition of reticulocyte protein synthesis by various RNA fractions. Preparations of total cell RNA, polysomal RNA and non-polysomal cytoplasmic RNA were obtained from Ehrlich ascites tumour cells as described in Materials and Methods. Total RNA was fractionated into poly(A)$^+$ and poly(A)$^-$ RNA by oligo(dT)-cellulose chromatography. The indicated concentrations were added to a non-nuclease-treated reticulocyte lysate system and protein synthesis was measured by the incorporation of [^{35}S]methionine after incubation for 45 min. The results are expressed as a % of the incorporation observed in the absence of added RNA. Panel (a): ○ , total cell RNA; □ , poly(A)$^+$RNA; △ , poly(A)$^-$RNA; panel (b): ▽ , polysomal RNA; ● , non-polysomal cytoplasmic RNA.

or (more likely) reflects a small residual contamination of the RNA with poly(A)$^+$ species. Total cellular RNA exhibited a potency intermediate between these extremes. Cytoplasmic polysomal RNA (rRNA plus mRNA) was inhibitory in the reticulocyte lysate to the same extent as total cellular RNA whereas cytoplasmic non-polysomal RNA was about 2.5-fold less potent. These results establish that the inhibitory effects on translation are not due to nuclear RNA species but are caused by polyadenylated RNA present in both polysomes and the non-polysomal fraction of the cytoplasm.

The lag period and "shut-off kinetics" characteristic of the inhibition of reticulocyte protein synthesis by these mammalian cell poly(A)$^+$RNA preparations are reminiscent of the effects of inhibitors which cause the phosphorylation of initiation factor eIF-2 in this system. There are a number of mechanisms by which eIF-2 kinase activity can be stimulated, including activation by low concentrations of dsRNA (19). We examined the possibility

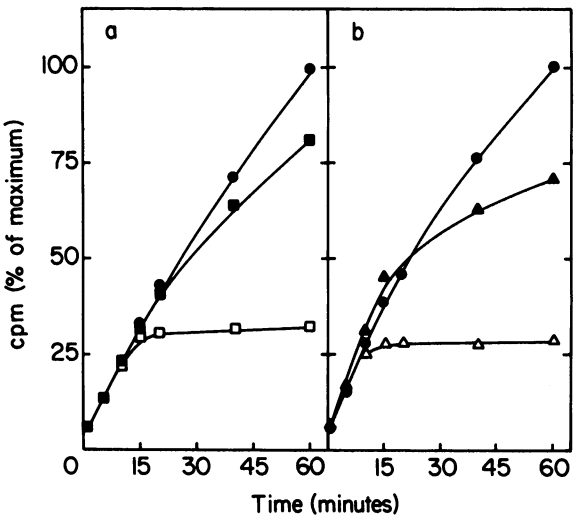

Figure 4. Prevention of poly(A)$^+$RNA-induced inhibition of protein synthesis by a high concentration of poly(I).poly(C). Reticulocyte lysates were incubated in the absence of added RNA (●) or in the presence of: Panel (a) poly(I).poly(C) at 10^{-8} g/ml (□) or 2 x 10^{-5} g/ml (■); Panel (b) Daudi cell poly(A)$^+$RNA (5 μg/ml) alone (△) or with poly(I).poly(C) (2 x 10^{-5} g/ml) (▲). Protein synthesis was monitored by the incorporation of [^{35}S]methionine. 100% represents 1.04 x 10^5 counts per min in (a) and 1.92 x 10^5 counts per min in (b).

that dsRNA in the mRNA preparations was responsible for the inhibition of protein synthesis by attempting to reverse the effect with a high concentration of the synthetic dsRNA poly(I).poly(C). Paradoxically, this agent is able to overcome the inhibitory effects on protein synthesis of low concentrations of itself or other dsRNAs (20,21) and is therefore a very specific diagnostic tool for the presence of dsRNA. As shown in Fig.4, poly(I).poly(C) at 20 μg/ml largely reversed the inhibition caused by itself at 10 ng/ml or by Daudi cell poly(A)$^+$RNA at 5 μg/ml. This suggests that the inhibitory effect of the latter is indeed due to dsRNA structures present in the preparation. Several experiments were carried out to determine whether the inhibitory dsRNA could be separated from the poly(A)$^+$RNA by rigorous fractionation procedures. As illustrated in Table 2, subjecting the RNA preparations to heating and rapid cooling failed to destroy the inhibitory activity. Washing with 3M Na acetate, in order to remove small RNA species (22), also failed to have any effect. RNA prepared from cells by alternative extraction methods had similar properties to those described here (data not shown). We therefore conclude that dsRNA structure(s) are an intrinsic part

TABLE 2.
EFFECT OF VARIOUS TREATMENTS ON THE INHIBITORY PROPERTIES OF DAUDI CELL POLY(A)$^+$RNA.

Treatment of RNA	Protein synthesis at 15min (counts per min x 10^{-4})	Protein synthesis at 60min (counts per min x 10^{-4})
No RNA added	13.65	40.14
Heated (80°C, 3min) with rapid cooling	7.34	6.23
Not heated	6.55	5.69
No RNA added	3.10	10.66
RNA washed with 3M Na acetate	2.83	4.13

Poly(A)$^+$RNA was prepared from Daudi cells as described in Figure 1. The RNA was subjected to heating and rapid cooling where indicated in order to denature any intermolecular double-stranded structures. In a separate experiment, an RNA preparation, following ethanol precipitation, was washed with 3M Na acetate to remove low molecular weight species. The RNA preparations were assayed for inhibition of endogenous protein synthesis in reticulocyte lysates as described in Table 1.

of one or several cytoplasmic polyadenylated mRNA species or at the very least are tightly associated with such molecules.

We confirmed the involvement of initiation factor eIF-2 in the inhibition of protein synthesis by mammalian polyadenylated RNA by two approaches. Firstly, a purified complex of eIF-2 with the guanine nucleotide exchange factor GEF (eIF-2B) stimulated protein synthesis in the presence of added poly(A)$^+$RNA, whilst having little effect on the uninhibited lysate (data not shown). Secondly, as shown in Fig.5, the endogenous eIF-2 in the lysate became extensively phosphorylated during incubation with Ehrlich cell poly(A)$^+$RNA. Using a one-dimensional isoelectric focussing gel separation of the phosphorylated and unphosphorylated forms of the α subunit of eIF-2, in combination with immunoblotting with a monoclonal antibody against eIF-2 (17), we were able to show that the α subunit became phosphorylated within 5 minutes of incubation with the RNA, coinciding with the beginning of the translational shut-off. Surprisingly, as much as 80-90% of the eIF-2 was phosphorylated after 10 minutes. Treatment of the lysate with alkaline phosphatase at the end of the incubation restored the migration of the subunit on the isoelectric focussing gel to the position seen with uninhibited lysate samples (Fig.5). Phosphorylation of eIF-2α did not occur in the absence of the added poly(A)$^+$RNA (not shown).

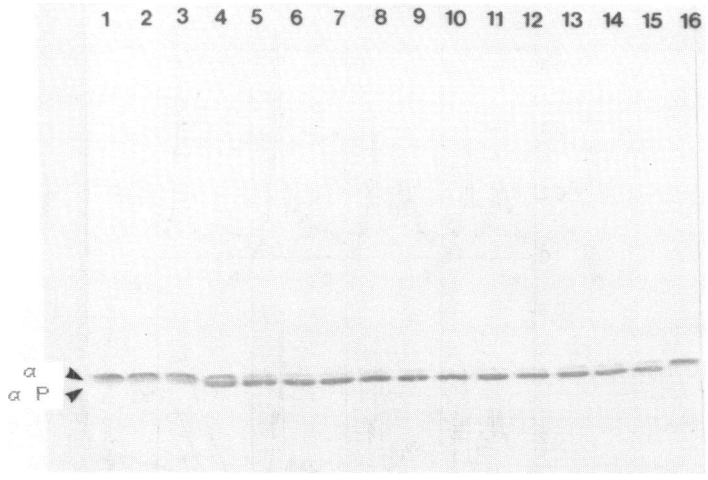

Figure 5. Phosphorylation of endogenous reticulocyte eIF-2 in the presence of poly(A)$^+$ RNA. Incubations were carried out with poly(A)$^+$ RNA from Ehrlich ascites tumour cells (10 μg/ml). 30 μl aliquots were removed at frequent intervals and immediately diluted into 250 μl of gel sample buffer containing 8M urea, 2% (w/v) ampholines, 0.6M 2-mercaptoethanol, 10 mM EDTA and 50 mM NaF. 50 μl samples were subjected to isoelectric focussing on a vertical slab gel, the proteins electrophoretically transferred to nitrocellulose and the phosphorylated and unphosphorylated forms of eIF-2α identified using a monoclonal antibody, as described in Materials and Methods. A sample that had been incubated for 60 min with the RNA was also subjected to treatment with alkaline phosphatase (4 units/ml at 30°C for 45 min) before denaturation in gel sample buffer. Tracks 1-15: Lysate incubated with the RNA for 0,2,4,6,8,10,12,14,16,18,20,30,40,50 and 60 min; track 16: alkaline phosphatase-treated sample. The arrows indicate the unphosphorylated (α) and phosphorylated (αP) forms of eIF-2α which have been separated on the basis of their different isoelectric points.

An additional effect of dsRNA in the reticulocyte lysate is the activation of the enzyme 2'5'oligo(A) synthetase, resulting in the synthesis of the pppA2'p5'(A)$_n$ series of compounds (8). These are activators of ribonuclease L which degrades mRNA and inhibits protein synthesis (4-6). We therefore investigated whether this pathway is also activated in the presence of the added poly(A)$^+$ RNA. Cleavage of polysomal globin mRNA was measured, as an assay for the endonucleolytic activity of RNase L, by examining polysome profiles from lysates incubated in the presence of cycloheximide to prevent ribosomal run-off (5). Fig.6(a-d) shows that, as expected from the inhibition of initiation, in the absence of cycloheximide reticulocyte polysomes broke down within 7 minutes of incubation with Ehrlich cell polyadenylated RNA. In the presence of the elongation inhibitor the poly(A)$^+$ RNA did not cause much

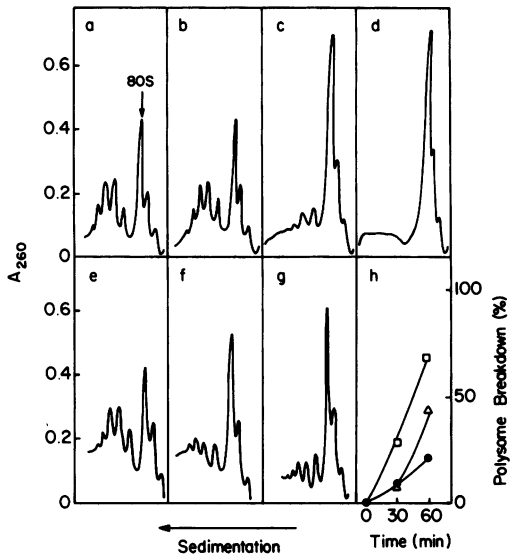

Figure 6. Polysome breakdown caused by $_{+}$poly(A)$^{+}$RNA in the presence and absence of cycloheximide. Reticulocyte lysate was incubated under protein synthesis conditions in the presence of poly(A)$^{+}$RNA from Ehrlich ascites tumour cells (5 µg/ml) for 0, 5, 7 and 10 min (panels a-d). Additional incubations were carried out for 60 min at 30°C in the presence of cycloheximide (100 µg/ml) without added RNA (panel e) or in the presence of poly(A)$^{+}$RNA (panel f) or poly(I).poly(C) (1 µg/ml) (panel g). Polysome profiles were immediately examined by sucrose density gradient centrifugation as described previously (5). The distribution of ribosomes between different size classes of polysomes was calculated for each sample and the % loss of larger polysomes (4-7 ribosomes) plotted as a function of incubation time (panel h). ● , control lysate; △ , plus poly(A)$^{+}$RNA; □ , plus poly(I).poly(C).

cleavage of polysomal mRNA up to 30 minutes but did result in a 44% loss of larger polysomes after 60 minutes (Fig.6f and h). However, the synthetic dsRNA poly(I).poly(C) was more effective, giving 29% loss of polysomes at 30 minutes and 69% loss at 60 minutes (Fig.6g and h). These results suggest that some activation of the 2'5'(A)$_{n}$ synthetase-RNase L pathway occurs in the presence of exogenous poly(A)$^{+}$ RNA but it is a late and relatively small effect and does not contribute significantly to the early shut-off of protein synthesis described here.

DISCUSSION

We have obtained evidence that mRNAs from a variety of mammalian sources, including both normal liver (data not shown) and several transformed cell

lines (Table 1) contain regions of double-stranded structure which can regulate overall rates of translation in vitro. Such regulation occurs largely as a result of activation of the dsRNA-dependent protein kinase which phosphorylates and inactivates the protein synthesis initiation factor eIF-2. There is also some indication that another dsRNA-dependent pathway, viz. the 2'5'oligo(A)$_n$ synthetase-RNase L system, may also be activated by double-stranded regions of mRNAs. Our results confirm some of the data of Baum and Ernst (23) who used HeLa cell poly(A)$^+$ RNA preparations. However our conclusions differ from theirs in that we find polysomal RNA is also inhibitory in the reticulocyte lysate.

The exact nature of the inhibitory molecule(s) in poly(A)$^+$ RNA preparations from different mammalian cell types remains to be established. The characteristics of the effects described in the present work distinguish the inhibitory activity from that of previously described small RNA molecules such as reticulocyte 3S RNA (24), the iRNA of chick embryo muscle (25), nuclear RNAs from adenovirus-infected cells (26) or poly(A) (27-29). None of these agents causes phosphorylation of eIF-2. Our results are more consistent with a role for the secondary structure of mRNAs themselves in the regulation of initiation. The possibility of such regulation through localized activation of an eIF-2 kinase has been demonstrated by Baglioni's group using mRNA to which a double-stranded region has been covalently attached (30). The naturally occurring equivalents of this might be poly(A)$^+$ RNA species containing stretches of oligo(U) of sufficient length to form stable intramolecular double-stranded structures with part of the poly(A) tail (31-33). Studies with synthetic polymers indicate that a length of 30-80 base pairs is required for activation of the dsRNA-dependent eIF-2 kinase (20,34). We have shown that ca. 9 ng/ml of poly(A)$^+$ RNA is sufficient to cause half-maximal inhibition. Pure double-stranded reovirus or bacteriophage RNA is active at a minimum concentration of 0.1 ng/ml (35). On this basis the data in Fig.3 would therefore suggest that approximately 1% by weight of poly(A)$^+$ RNA exists in the form of double-stranded regions of sufficient length to activate the kinase. Although this estimate is subject to many uncertainties it is entirely plausible since it has been reported that nearly 20% of poly(A)$^+$ mRNAs in HeLa cells also contain some sort of oligo(U) sequence (32,33). The chromatographic behaviour of poly(A)$^+$ RNA on cellulose columns (35) also suggests that a similar percentage of mRNA contains a substantial proportion of secondary structure. Examples of oligo(U)-containing messages include those for human β-actin (36), human c-myc

(37) and eukaryotic protein synthesis initiation factors (38,39). For an mRNA size of 2 kilobases, the mean length of an oligo(U) tract that occurs in 1 molecule in 5 and constitutes 0.5% by weight of the total mRNA population would be 50 nucleotides.

The presence of dsRNA in nuclear RNA (40,41) or viral RNA (42) from a variety of sources is well documented; the present data exclude either of these classes of RNA as the sole source of the dsRNA identified and suggest that at least a portion of normal cellular cytoplasmic mRNA also contains such structures.

Although dsRNA can activate both the eIF-2 kinase and the 2'5'oligo(A)$_n$ synthetase pathways in reticulocyte lysates (43) we have shown here that the naturally occurring cellular RNAs exert their effects primarily via the former mechanism. The relative importance of the 2 pathways, and the extent of the dsRNA-mediated inhibition of protein synthesis, has been shown to vary between different lysates and also between different types of dsRNA activators (34). It is therefore not possible to predict which mechanism may be more important in vivo as this may vary with cell type.

The implications of these results for the physiological control of protein synthesis remain to be assessed. We have identified a potential class of natural activators of the dsRNA-dependent enzymes previously implicated in the antiviral and cell growth-regulatory effects of the interferons. Our data suggest that activation of the eIF-2 protein kinase and 2'5'oligo(A)$_n$ synthetase in the cytoplasm does not necessarily require acute infection of cells by viruses that replicate via dsRNA intermediates. Thus these enzymes may be functional in the control of translation in uninfected as well as virus-infected cells (44). However, it remains to be determined whether double-stranded regions of cellular mRNAs can indeed activate the eIF-2 kinase or 2'5'oligo (A)$_n$ synthetase in intact cells and, if so, how high rates of protein synthesis can be maintained, when necessary, in the presence of such RNA species.

ACKNOWLEDGEMENTS

We thank Miss V.J.Tilleray for skilled technical assistance and Dr E.C.Henshaw for a gift of the anti-eIF-2 monoclonal antibody. This work was supported by grants from the Cancer Research Campaign, the Leukaemia Research Fund and the Gunnar Nilsson Cancer Research Trust. G.P. and S.P. held studentships from the Medical Research Council and N.S. was the holder of a CASE award from the Science and Engineering Research Council.

REFERENCES

1. Farrell,P.J., Sen,G.C., Dubois,M.F., Ratner,L., Slattery,E. and Lengyel,P. (1978) Proc.Natl.Acad.Sci.USA **75**, 5893-5897.
2. Clemens,M.J. and McNurlan,M.A. (1985) Biochem.J. **226**, 345-360.
3. Pain,V.M. (1986) Biochem.J. **235**, 625-637.
4. Wreschner,D.H., James,T.C., Silverman,R.H. and Kerr,I.M. (1981) Nucl.Acids Res. **9**, 1571-1582.
5. Clemens,M.J. and Williams,B.R.G. (1978) Cell **13**, 565-572.
6. Slattery,E., Ghosh,N., Samanta,H. and Lengyel,P. (1979) Proc.Natl.Acad. Sci.USA **76**, 4778-4782.
7. Levin,D.H., Petryshyn,R. and London,I.M. (1980) Proc.Natl.Acad.Sci.USA **77**, 832-836.
8. Hovanessian,A.G. and Kerr,I.M. (1978) Eur.J.Biochem. **84**, 149-159.
9. Samuel,C.E., Duncan,R., Knutson,G.S. and Hershey,J.W.B. (1984) J.Biol. Chem. **259**, 13451-13457.
10. Williams,B.R.G., Golgher,R.R., Brown,R.E., Gilbert,C.S. and Kerr,I.M. (1979) Nature **282**, 582-586.
11. van Venrooij,W.J.W., Henshaw,E.C. and Hirsch,C.A. (1970) J.Biol.Chem. **245**, 5947-5953.
12. Gewert,D.R., Moore,G. and Clemens,M.J. (1983) Biochem.J. **214**, 983-990.
13. Auffray,C. and Rougeon,F. (1979) Eur.J.Biochem. **107**, 303-314.
14. Clemens,M.J. (1984) In Hames,B.D. and Higgins,S.J. (eds), Transcription and Translation: A Practical Approach, IRL Press, Oxford, pp.211-230.
15. Clemens,M.J. (1984) In Hames,B.D. and Higgins,S.J. (eds), Transcription and Translation: A Practical Approach, IRL Press, Oxford, pp.231-270.
16. Wong,S-T., Mastropaolo,W. and Henshaw,E.C. (1982) J.Biol.Chem. **257**, 5231-5236.
17. Scorsone,K.A., Panniers,R., Rowlands,A.G. and Henshaw,E.C. (1987) J.Biol. Chem. **262**, 14538-14543.
18. Hawkes,R., Niday,E. and Gordon,J. (1982) Anal.Biochem. **119**, 142-147.
19. Farrell,P.J., Balkow,K., Hunt,T., Jackson,R.J. and Trachsel,H. (1977) Cell **11**, 187-200.
20. Hunter,T., Hunt,T., Jackson,R.J. and Robertson,H.D. (1974) J.Biol.Chem. **250**, 409-417.
21. Clemens,M.J., Safer,B., Merrick,W.C., Anderson,W.F. and London,I.M. (1975) Proc.Natl.Acad.Sci.USA **72**, 1286-1290.
22. Shore,G.C. and Tata,J.R. (1977) J.Cell Biol. **72**, 726-743.
23. Baum,E.Z. and Ernst,V.G. (1983) Biochem.Biophys.Res.Commun. **114**, 41-49.
24. Dionne,C.A., Stearns,G.B., Kramer,G. and Hardesty,B. (1982) J.Biol.Chem. **257**, 12372-12379.
25. Sarkar,S., Mukherjee,A.K. and Guha,C. (1981) J.Biol.Chem. **256**, 5077-5086.
26. Sarma,M.H., Beach,T.A. and Chatterjee,N.K. (1978) Biochem.Biophys.Res. Commun. **82**, 384-391.
27. Jacobson,A. and Favreau,M. (1983) Nucl.Acids Res. **11**, 6353-6368.
28. Lemay,G. and Millward,S. (1986) Arch.Biochem.Biophys. **249**, 191-198.
29. Bablanian,R. and Banerjee,A.K. (1986) Proc.Natl.Acad.Sci.USA **83**, 1290-1294.
30. De Benedetti,A. and Baglioni,C. (1984) Nature **311**, 79-81.
31. Nichols,J.L. and Pryhitka,B. (1982) Biochem.Biophys.Res.Commun. **107**, 989-997.
32. Kulkosky,J.W., Wood,W.M. and Edmonds,M. (1985) Biochemistry **24**, 3678-3686.
33. Wood,W.M., Wallace,J.C. and Edmonds,M. (1985) Biochemistry **24**, 3686-3693.
34. Content,J., Lebleu,B. and De Clercq,E. (1978) Biochemistry **17**, 88-94.
35. Robertson,H.D. and Hunter,T. (1975) J.Biol.Chem. **250**, 418-425.
36. Ponte,P., Ng,S.Y., Engel,J., Gunning,P. and Kedes,L. (1984) Nucl.Acids Res. **12**, 1687-1696.
37. Colby,W., Chen,E., Smith,D. and Lennson,A. (1983) Nature **301**, 722-725.

38. Nielsen,P.J., McMaster,G.K. and Trachsel,H. (1985) Nucl.Acids Res. **13**, 6867-6880.
39. Rychlik,W., Domier,L.L., Gardner,P.R., Hellmann,G.M. and Rhoads,R.E. (1987) Proc.Natl.Acad.Sci.USA **84**, 945-949.
40. Bases,R. and Kaplan,B.H. (1973) Biochim.Biophys.Acta **312**, 574-580.
41. Torelli,U., Cadossi,R., Torelli,G., Ferrari,St. and Ferrari,Se. (1975) FEBS Lett. **56**, 96-100.
42. Ehrenfeld,E. and Hunt,T. (1971) Proc.Natl.Acad.Sci.USA **68**, 1075-1078.
43. Williams,B.R.G., Gilbert,C.S. and Kerr,I.M. (1979) Nucl.Acids Res. **6**, 1335-1350.
44. Petryshyn,R., Chen,J-J. and London,I.M. (1984) J.Biol.Chem. **259**, 14736-14742.