Inhibition of polypeptide chain initiation in Daudi cells by interferons

Evidence that activity of initiation factor eIF-2 and availability of mRNA are unimpaired

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The accompanying paper [McNurlan & Clemens (1986) *Biochem. J.* 237, 871–876] shows that the inhibition of proliferation of Daudi cells by human interferons is associated with impairment of the overall rate of protein synthesis. We have examined whether two of the mechanisms which are believed to control translation in interferon-treated virus-infected cells may be responsible for the inhibition of protein synthesis during the antiproliferative response in these uninfected cells. Although the rate of polypeptide chain initiation is lower in interferon-treated Daudi cells, as indicated by the disaggregation of polysomes, there is no significant inhibition of activity of initiation factor eIF-2 or of [40 S·Met-tRNA_i] initiation complex formation in cell extracts. The phosphorylation state of the α subunit of eIF-2 remains unaltered. There is no major decrease in mRNA content as a proportion of total RNA up to 4 days of interferon treatment, as judged by poly(A) content, although the amount of total mRNA/10⁶ cells eventually declines. The mRNA present in extracts from interferon-treated cells remains translatable when added to an mRNA-dependent reticulocyte lysate system. We conclude that neither the interferon-inducible eIF-2 protein kinase pathway nor the 2',5'-oligo(adenylate)–ribonuclease L pathway are responsible for the inhibition of polypeptide chain initiation. Rather, the data suggest impairment at the level of formation of [80 S ribosome·mRNA] initiation complexes.

INTRODUCTION

Daudi cells are a line of transformed human B lymphoblastoid cells, originally derived from a Burkitt lymphoma [1,2]. They contain multiple copies of the Epstein–Barr virus genome but produce very little intact virus under normal growth conditions [2]. Daudi cells are of particular interest in the study of cellular growth regulation since they have undergone a reciprocal translocation of the *c-myc* proto-oncogene from chromosome 8 to the heavy chain immunoglobulin locus on chromosome 14, an event which is believed to contribute to their transformed phenotype [3]. These cells are very sensitive to the growth inhibitory effects of interferons [1,4–7] and have been reported to show a marked loss of *c-myc* mRNA sequences in response to interferon treatment [8–11].

We have established that the inhibition of cell proliferation which occurs when Daudi cells are treated with human interferons is also associated with a significant decrease in the rate of cellular protein synthesis, in a time- and dose-dependent manner [7,12]. This occurs in the absence of any infection of the cells by exogenous viruses.

There have been many studies on the molecular mechanisms responsible for the inhibition of protein synthesis, and thus viral replication, in interferon-treated, virus-infected cells (reviewed in [13]). Two pathways have received particular attention, i.e. induction of a double-stranded RNA-dependent protein kinase which phos-

phorylates and inactivates protein synthesis initiation factor eIF-2 [14], and induction of the enzyme 2',5'-oligo(adenylate) synthetase which polymerizes ATP into 2',5'(A) oligomers [15]. These nucleotides in turn activate a ribonuclease (RNAase L) which degrades viral and cellular mRNA and rRNA [16,17]. In contrast with these detailed studies, little attention has been paid to the mechanism of inhibition of protein synthesis, where it occurs, in uninfected cells exhibiting inhibition of growth in response to interferon treatment. In view of the effect of interferons on protein synthesis in Daudi cells, where the only viral entity present is the endogenous, non-lytic Epstein–Barr virus, we have investigated whether either the eIF-2(α) kinase or the 2',5'-oligo(A) synthetase mechanism may be involved in the control of polypeptide chain initiation, or whether other steps in this process are regulated during inhibition of cellular proliferation.

MATERIALS AND METHODS

General

The interferon preparation used for these experiments was a mixture of Hu-IFN α species obtained from Sendai virus-induced Namalwa cells and was kindly provided by Drs. K. H. Fantes and M. D. Johnston (Wellcome Research Laboratories, Beckenham, Kent, U.K.). It had a specific activity of 7.1×10^7 units/mg of protein. The following procedures were carried out exactly as described previously: growth of Daudi cells in culture

and treatment with interferons [4–7,12]; preparation of cell extracts and analysis of polysomes on sucrose density gradients [12]; determination of protein synthesis in cell extracts *in vitro* [12]; preparation of [³⁵S]Met-tRNA_f [16].

Assays of 40 S initiation complex formation

The ability of cell extracts to form 40 S initiation complexes was assayed by incubation in 100 μ l reaction volumes with [³⁵S]Met-tRNA_f (1100 Ci/mmol; 4.5×10^6 c.p.m./ml) for 2 min at 30 °C under the same ionic conditions as were used for protein synthesis. These were: 25 mM Hepes/K⁺ (pH 7.6), 114 mM-KCl, 0.9 mM-magnesium acetate, 6 mM-2-mercaptoethanol, 0.5 mM-spermidine, 5 mM-creatine phosphate, 180 μ g of creatine kinase/ml, 1 mM-ATP and 0.25 mM-GTP. The cell extract constituted 60% of the incubation volume. The inhibitor of polypeptide chain elongation, emetine, was present at 1 mM to prevent movement of [40 S·Met-tRNA_f] complexes into polysomes [18]. Under these conditions negligible amounts of radioactivity are associated with any fraction larger than native 40 S subunits (results not shown). The reaction was stopped by rapid cooling on ice and the samples were analysed by centrifugation for 2.75 h at 234000 *g* on 20–50% sucrose gradients containing 0.1 mM-sodium cacodylate, pH 6.6, 100 mM-KCl and 2 mM-magnesium acetate. After fractionation and precipitation with cetyltrimethylammonium bromide in the presence of carrier RNA and sodium acetate buffer, pH 5.0 [19], the fractions were filtered on glass fibre discs and the radioactivity was determined by liquid-scintillation counting.

Assays of [eIF-2·GTP·Met-tRNA_f] ternary complex formation

Postribosomal supernatants were prepared by centrifugation of cell extracts for 3 h at 106000 *g*. Under the salt conditions in these extracts (118 mM-KCl) the majority of the eIF-2 is soluble and remains in the supernatant [20]. The ability of this eIF-2 to form ternary complexes was assayed by incubation in 50 μ l reaction volumes at 30 °C in the presence of 20 mM Hepes/K⁺ (pH 7.6), 100 mM-KCl, 1 mM-magnesium acetate, 0.5 mM-dithiothreitol, 0.2 mM-EDTA, 1 mM-ATP, 0.25 mM-GTP, 3 mM-phosphoenolpyruvate, 18 μ g of pyruvate kinase/ml and [³⁵S]Met-tRNA_f (1100 Ci/mmol; 4.5×10^6 c.p.m./ml). To stop the reaction the incubations were diluted into 0.3 ml of cold wash buffer (20 mM-Hepes/K⁺, pH 7.6, 100 mM-KCl, 2 mM-magnesium acetate) and immediately filtered on cellulose nitrate discs [21]. The discs were washed three times, dried and the eIF-2-associated radioactivity was determined by liquid-scintillation counting. Ternary complex formation under these conditions was a linear function of both the amount of postribosomal supernatant added and the time of incubation (up to at least 20 min). A standard incubation time of 10 min was used in most experiments to ensure that eIF-2 activity, rather than availability of [³⁵S]Met-tRNA_f, was the limiting factor.

Analysis of the phosphorylation state of eIF-2

Cells were washed three times in the cold in 10 mM-Hepes (pH 7.6)/140 mM-KCl and approx. 4.5×10^6 cells were then lysed in 0.5 ml of isoelectric focussing buffer [9.8 M-urea, 2% (v/v) Nonidet P-40, 2% (w/v) pH 3.5–10 ampholines (LKB), 1% (v/v) 2-mercapto-

ethanol, 50 mM-NaF]. The lysates were briefly centrifuged in a Beckman Microfuge and the supernatants were stored at –70 °C.

For isoelectric focusing analysis, 1.5 mm thick vertical polyacrylamide slab gels containing 9.2 M-urea and a mixture of ampholines were prepared and run as described in [22]. After prefocusing of the gels, 50 μ l samples of whole cell lysates were applied and focusing was performed at 3 W (constant power) for 15 h, followed by 900 V (constant voltage) for 45 min. The proteins were electrophoretically transferred to 0.45 μ m nitrocellulose paper (Millipore) for 3 h at 200 mA (constant current) in the presence of 0.7% (v/v) acetic acid. The phosphorylated and unphosphorylated forms of the α subunit of eIF-2, which have different isoelectric points [22], were identified by binding a purified monoclonal anti-eIF-2 α antibody (a generous gift from K. Clarke and E. C. Henshaw, University of Rochester, NY, U.S.A.). The primary antibody was then located by binding biotinylated anti-(mouse IgG) followed by an avidin-biotinylated peroxidase reagent (Vector Laboratories, Burlingame, CA, U.S.A.). A colour reaction using 4-chloro-naphthol and H₂O₂ as substrates gave purple bands on the white background of the nitrocellulose paper which were quantified by scanning in an LKB laser densitometer.

Preparation of reticulocyte lysate and postribosomal supernatant

Rabbit reticulocyte lysate was prepared as described [23] and treated with micrococcal nuclease to make translation in this system dependent on added mRNA [24]. Postribosomal supernatant was prepared from non-nuclease-treated lysate by centrifugation for 170 min at 165000 *g*.

Isolation of total RNA

Total RNA was extracted from approx. $(5-15) \times 10^7$ cells by the LiCl/urea method of Auffray & Rougeon [25], using at least 0.7 ml of 3 M-LiCl/6 M-urea per 10^7 cells. The yield from control cells was about 30–50 μ g of RNA/ 10^7 cells, corresponding to a recovery of total cellular RNA of 30–45% [12]. The RNA was undegraded, as judged by ethidium bromide staining of the 18 S and 28 S rRNA bands on agarose gels and by translation of the poly(A)⁺ RNA in cell-free protein synthesizing systems (M. J. Clemens, A. Johnstone & G. Pratt, unpublished work).

Determination of poly(A)⁺ RNA content in total RNA

The polyadenylated mRNA content of total RNA was estimated by measurement of the amount of poly(A) hybridizable to [³H]poly(U) [26]. A sample (10–50 μ g) of each RNA preparation was incubated with [³H]poly(U) (7×10^4 d.p.m.; 20–72 Ci/mmol) (Amersham International) for 1 h at 37 °C in the presence of 3 mM-Tris/HCl (pH 7.0), 0.45 M-NaCl, 45 mM-sodium citrate and 50% (v/v) deionized formamide in a volume of 0.3 ml. A series of poly(A) standards (5–100 ng) was incubated in parallel under the same conditions. The extent of hybridization was assessed by treating the incubations with heat-treated (100 °C, 15 min) ribonuclease A (3.8 μ g/ml) and DNase I (15 μ g/ml) for 2 h at 30 °C to degrade all single-stranded polynucleotides. The remaining acid-insoluble radioactivity was precipitated by adding 5 ml of 10% (w/v) trichloroacetic acid in the

Fig. 1. Effect of interferon treatment on the proportion of ribosomes in polysomes

Cells were incubated with or without interferons (5 units/ml) for the times indicated and cell extracts were prepared. Polysome profiles were determined by sucrose density gradient centrifugation and the proportion of ribosomes in polysomes was quantified by measuring the areas under the monomer/subunit and polysome regions of the gradients. Typical profiles are shown in Fig. 2. The results of two separate experiments are illustrated here. The bar at the left of the Figure indicates the range of values for control cells growing exponentially.

presence of 1 mg of uridine and 1 mg of carrier yeast RNA. Precipitates were filtered on glass fibre discs, washed with 10% trichloroacetic acid/0.02% (w/v) uridine and the radioactivity was determined by liquid-scintillation counting. Hybridization of poly(U) was a linear function of the amount of RNA present and all data fell within the range of the poly(A) standards assayed. Total RNA from control cells had a poly(A) content of 2.39 ± 0.18 ng/ μ g of RNA.

RESULTS

The decline in the proportion of ribosomes in polysomes which occurs progressively with increasing time of interferon treatment is illustrated in Fig. 1. Although Daudi cells always have a substantial fraction of ribosomes in the form of inactive 80 S particles even during exponential growth (Fig. 2a), this fraction increases as the protein synthesis rate is inhibited after interferon treatment. This suggests a progressive block in one or more steps in the initiation of protein synthesis.

The activity of initiation factor eIF-2 is often rate-limiting for protein synthesis under a number of conditions where translation is inhibited in eukaryotic cells [27,28]. Since this factor can be inactivated by phosphorylation by an interferon-inducible, double-stranded RNA-dependent protein kinase [14], we examined whether eIF-2 functions are impaired in

interferon-treated Daudi cells. In these experiments protein synthesis was inhibited either by a 24 h treatment with a moderately high concentration of interferons (50 units/ml) or by a prolonged treatment (up to 4 days) with lower concentrations (5–10 units/ml). Two kinds of assay were used to determine the activity of eIF-2 in cell extracts. As illustrated in Figs. 2(b) and 2(c), the formation of $[40\text{ S} \cdot \text{Met-tRNA}_i]$ initiation complexes may be determined by labelling cell extracts with exogenous $[^{35}\text{S}]\text{Met-tRNA}_i$. This reaction requires eIF-2 and GTP. The formation of labelled 40 S initiation complexes in extracts from control cells and cells treated with Hu-IFN- α (50 units/ml for 24 h) showed no significant difference, even though the number of polysomes (Fig. 2a) and the protein synthetic activity of the extracts (results not shown) were lowered by interferon treatment. A similar conclusion was reached when longer times of interferon treatment were used (Table 1). Overall, the results from five separate experiments where protein synthesis was clearly inhibited gave a mean value of 40 S initiation complex formation, expressed as a percentage of controls, of $83.9 \pm 11.2\%$ (mean $\pm$ S.E.M.). This suggests little if any inhibition at this step in initiation. In the second type of assay the formation of ternary complexes between eIF-2, Met-tRNA_i and GTP was measured using postribosomal supernatants from pairs of interferon-treated and control cell extracts. Fig. 3 shows that the formation of these complexes is a linear function of the amount of supernatant added and that no period of interferon treatment up to 4 days inhibits this reaction. Indeed, there is a small increase in ternary complex formation, possibly reflecting a redistribution of eIF-2 from the ribosomes into the supernatant when the rate of protein synthesis declines in interferon-treated cells. Thus, in neither of the above assays does the activity of initiation factor eIF-2 correlate with the progressive decline in protein synthesis which occurs in intact interferon-treated Daudi cells [7].

We have also failed to observe any change in the phosphorylation state of the α subunit of eIF-2 (Fig. 4). This was measured by separating the phosphorylated and unphosphorylated forms of eIF-2 α on isoelectric focusing gels of whole cell extracts [22] and quantifying the relative amounts by immunoblotting with a monoclonal antibody against this subunit. In both control and interferon-treated cells as much as 50% of the initiation factor appears to be in the phosphorylated state. This suggests that a high proportion of the eIF-2 in Daudi cells may be inactive, but the significance of this remains to be determined.

An alternative mechanism by which protein synthesis can be controlled in interferon-treated cells which are subsequently infected with viruses is by the degradation of mRNA. This process is catalysed by the 2',5'-oligo(A)-dependent ribonuclease L [16,17]. Such a mechanism can inhibit polypeptide chain initiation by limiting the availability of intact mRNA for translation. We have therefore determined both the quantity [as measured by poly(A) content] (Fig. 5) and the translatability of total mRNA in extracts from Daudi cells (Fig. 6) at various times during the interferon-induced growth inhibition. We have found only a small change in the poly(A) content per unit of total cellular RNA after 1–4 days of treatment. Control cell total RNA contained 2.39 ± 0.18 ng of poly(A)/ μ g of RNA and after 1, 2, 3

Fig. 2. Polysome profiles and formation of [40 S·Met-tRNA_i] initiation complexes in extracts from control and interferon-treated cells

Cells were incubated with or without interferons (50 units/ml) for 24 h and extracts were prepared. Panel (a) shows the polysome profiles of the control (—) and interferon-treated (---) extracts on sucrose density gradients. Sedimentation was from right to left. The position of 80 S monomeric ribosomes is indicated. Panels (b) and (c) show the formation of [40 S·Met-tRNA_i] initiation complexes by the control (b) and interferon-treated (c) extracts after a 2 min incubation with [³⁵S]Met-tRNA_i as described in the Materials and methods section. The positions of 40 S, 60 S and 80 S ribosomes on the sucrose gradients are indicated.

Table 1. 40 S initiation complex formation and protein synthesis in extracts from control and long-term interferon-treated cells

Cells were incubated with or without interferons (5 units/ml) for up to 4 days and extracts were prepared. The formation of [40 S·Met-tRNA_i] initiation complexes and the protein synthetic activity of the extracts were measured in separate incubations, using equal A_{260} amounts of extracts, as described in the Materials and methods section. Radioactivity in [³⁵S]Met-tRNA_i bound to native 40 S ribosomal subunits was calculated from the sum of the values in the peak fractions, after subtraction of the appropriate baseline values (see the shaded areas in Figs. 2b and 2c). Incorporation of [³⁵S]methionine (80 μ Ci/ml) into protein in 20 μ l samples was determined after 30 min of incubation and has been corrected for zero-time values. The values in parentheses indicate the percentage of the appropriate control values.

Extract	Time of treatment (days)	$10^{-3} \times 40$ S initiation complexes (c.p.m.)	$10^{-3} \times$ Protein synthesis (c.p.m.)
Control	—	2.32	66.2
Interferon-treated	2	2.00 (86%)	35.7 (54%)
Control	—	2.24	42.7
Interferon-treated	4	3.09 (138%)	16.6 (39%)

and 4 days of interferon treatment the values were 2.55 ± 0.10 , 2.10 ± 0.08 , 2.00 ± 0.25 and 1.92 ± 0.08 ng/ μ g of RNA respectively. Since the bulk of cellular RNA is rRNA, these results indicate that there is only a minor decrease in the ratio of mRNA to ribosomes. This is not sufficient to account for the decline in initiation of protein synthesis after interferon treatment indicated by the loss of polysomes (Fig. 1). However, we have noted previously that the content of total RNA per cell declines with interferon treatment [12] and this is reflected in the lower yield of poly(A)⁺ RNA obtained after exposure to high doses or for prolonged times (Figs. 5b and 5c). Thus, there is a decrease in the absolute amount of mRNA per cell which, together with the loss of ribosomes, will contribute to the overall decline in protein synthesis per cell. Nevertheless, the latter precedes the loss of RNA and, in most conditions, is of a greater magnitude. Fig. 6(b) shows that the mRNA in the interferon-treated cell extracts remains functionally intact and potentially available for translation, since it is utilized by the messenger-dependent reticulocyte lysate system equally as well as mRNA from control cell extracts. Again this can be contrasted with the relative inability of the interferon-treated cells to translate this mRNA, as reflected in the polysome run-off activity of the extracts (Fig. 6a).

We have tested the possibility that translation in

Fig. 3. Formation of [eIF-2 · GTP · Met-tRNA_f] ternary complexes in postribosomal supernatants from cells treated with interferons for different times

Cells were incubated with or without interferons (5 units/ml) for up to 4 days and extracts were prepared. Ternary complex formation in the postribosomal supernatants from these extracts was assayed as described in the Materials and methods section, using up to 20 μ l of sample (expressed here as A_{260} units of extract) in 50 μ l incubations. The incubation time was 10 min. The results are shown as radioactivity in Met-tRNA_f retained on cellulose nitrate filters. ●, Control cell extracts; ○, interferon-treated cell extracts.

Fig. 4. Separation of phosphorylated and unphosphorylated forms of eIF-2 by isoelectric focusing

Cells were incubated with or without interferons (5 units/ml) for 3 days, then rapidly washed and lysed in urea- and ampholine-containing buffer as described in the Materials and methods section. Samples (50 μ l) of cell extracts (tracks 1 and 2) were subjected to isoelectric focusing on a vertical slab gel and the proteins were transferred electrophoretically to nitrocellulose. The α subunit of eIF-2 was identified by using a monoclonal antibody in combination with a biotinylated second antibody and an avidin-biotinylated peroxidase reagent. The positions of the phosphorylated and unphosphorylated α subunit are indicated. These were determined from the migration of purified eIF-2. Also shown are the effects of incubating a postmitochondrial extract from Daudi cells for 30 min at 30 °C, either in the presence of 10 mM-EDTA (to inhibit protein kinases and allow endogenous phosphatases to dephosphorylate eIF-2) (track 3), or in the presence of purified reticulocyte haem-controlled repressor (20 units/ml) which is an eIF-2 α -specific protein kinase (track 4).

control and interferon-treated Daudi cell extracts may be equalized by the addition of reticulocyte postribosomal supernatant, which has a stimulatory effect on many cell-free systems [29] (Fig. 7). Because there are fewer polysomes in the interferon-treated extract there is less endogenous protein synthesis than in the control. Little if any of this is due to re-initiation *in vitro* (results not shown). The reticulocyte fraction stimulates protein synthesis in both extracts, as a result of new initiation, but does not eliminate the difference in activity between the extracts. This suggests that the activity of some component is the interferon-treated system, which is not provided by the reticulocyte supernatant, is still limiting for protein synthesis under these conditions.

DISCUSSION

The principal conclusion to be drawn from these results is that two of the major mechanisms for inhibition of protein synthesis in interferon-treated, virus-infected cells [i.e. double-stranded RNA-dependent phosphorylation of eIF-2 and 2',5'-oligo(A)-dependent degradation of mRNA] are not responsible for the impairment of polypeptide chain initiation during growth inhibition of Daudi cells. Nevertheless it is clear from the present data and from our previous results [7,12] that there is an appreciable decline in the rate of initiation, for which a molecular mechanism must be sought. The magnitude of this decline, together with the fall in the number of ribosomes [12] and the amount of mRNA per cell (Fig. 5), is sufficient to account for the overall extent of protein synthesis inhibition.

The binding of initiator Met-tRNA_f to eIF-2 and the transfer of [eIF-2 · GTP · Met-tRNA_f] complexes to native

40 S ribosomal subunits are not inhibited significantly in interferon-treated Daudi cells. Similarly, the amount and availability for translation of total mRNA are relatively unimpaired, except after prolonged treatment or exposure to high concentrations of interferons. We have not observed any decline in mRNA content up to 48 h after treatment with 10 units of interferons/ml (Fig. 5), in spite of maximal induction of 2',5'-oligo(adenylate) synthetase within 24 h [12]. This is consistent with our previous observation that overall mRNA turnover was not accelerated in Daudi cells exhibiting impaired growth and protein synthesis under these conditions [12]. These data do not, of course, rule out changes in the concentration or utilization for protein synthesis of individual mRNAs, such as those for the proto-oncogene *c-myc* [8–11], the products of which may be critical for the maintenance of normal rates of protein synthesis, DNA replication or proliferation in these tumour cells. Indeed *c-myc* mRNA has been shown to be specifically destabilized in Daudi cells within a few hours of interferon treatment, resulting in a rapid decline in its steady state concentration [11]. We also cannot exclude the possibility that the eventual decrease in cellular mRNA (and ribosome) content may be due to 2',5'-oligo(A)-dependent activation of RNAase L, but RNA turnover and activity of this enzyme have not been studied during this phase of the growth inhibition.

Our data concerning the absence of any general activation of the double-stranded-RNA-dependent eIF-2 kinase or 2',5'-oligo(A) synthetase systems in interferon-treated Daudi cells are in agreement with the conclusions of Silverman *et al.* [30]. This group found that induction of both enzymes occurred equally well in Daudi cells that were sensitive or resistant to growth inhibition by interferons and was therefore not sufficient to cause the inhibition. They did not examine rates of protein synthesis in these cells but it seems likely that these would show the same relative sensitivity as cell growth.

The presence of near normal amounts of potentially translatable total mRNA, relative to ribosomes, together with unimpaired ability to form [40 S·Met-tRNA_i]

Fig. 5. Quantification of poly(A)⁺ RNA in control and interferon-treated cells and comparison with the rate of total protein synthesis

Cells were incubated with or without interferons either for 1–4 days at 10 units/ml or, in a separate experiment, for 24 h at 3–30 units/ml. Relative rates of total protein synthesis in the intact cells were measured by incorporation of [³⁵S]methionine (7.5 μCi/ml) into acid-insoluble material after a 1 h incubation at 37 °C. Total RNA was extracted from each batch of cells and the poly(A) content was assessed by hybridization with [³H]poly(U) as described in the Materials and methods section. Panel (a) illustrates typical curves for poly(U) hybridization to increasing amounts of RNA from control cells (●) and cells treated with interferons (10 units/ml) for 1 day (○) and 2 days (△). Panels (b) and (c) show the yields of poly(A) obtained per 10⁶ cells (□) (means ± s.e.m. for triplicate determinations) in comparison with the protein synthetic activity of the cells from which the RNAs were prepared (■) (means ± the range of duplicate determinations); (b) interferon time course; (c) interferon dose curve. Note the variability between the experiments in the response of protein synthesis at 24 h to a low dose of interferons.

Fig. 6. Translation of mRNA in extracts from control and interferon-treated cells and in the nuclease-treated reticulocyte lysate system

Cells were treated with or without interferons (5 units/ml) for 4 days and extracts were prepared. Panel (a) shows the protein synthetic activity of the extracts, measured by the incorporation of [³⁵S]methionine (80 μCi/ml) into acid-insoluble material in 20 μl samples taken at the times indicated: ●, control cell extract; ○, interferon-treated cell extract. Panel (b) shows the translation of the mRNA in the same extracts when various amounts were added to a nuclease-treated reticulocyte lysate system. The indicated amounts of control cell extract (●) or interferon-treated cell extract (○) were added to 180 μl incubations and time-courses of methionine incorporation were determined. The data shown represent the mean rate of incorporation of [³⁵S]methionine (13 μCi/ml) into protein in 20 μl samples over the first 20 min of incubation, after subtraction of the values for lysate without added extract.

Fig. 7. Stimulation of protein synthesis in control and interferon-treated cell extracts by addition of reticulocyte post-ribosomal supernatant

Extracts (30 μl) from control or interferon-treated cells (5 units/ml, 2 days) were incubated in 150 μl final volume in the presence (-----) and absence (—) of 60 μl of reticulocyte postribosomal supernatant. Incorporation of [³⁵S]methionine (100 μCi/ml) into protein was measured in 20 μl samples at the indicated times: ●, control cell extract; ○, interferon-treated cell extract.

pre-initiation complexes following interferon treatment suggests that the most likely defect in initiation in this system must lie in the formation of active 80 S ribosomal·mRNA complexes. This involves a complicated series of events which require initiation factors eIF-3, 4A, 4B and 4F for mRNA binding and eIF-5 for the 60 S subunit joining reaction [27,28]. Our present data cannot distinguish between defects in any of these factors. Whatever is missing is apparently not provided by the stimulatory reticulocyte supernatant; this may rule out eIF-3, which is reported to be present in this fraction [31]. Alternatively, a proportion of the total mRNA in the interferon-treated cells may become sequestered in an intact but untranslatable form, but if this is the case it must become translatable again when Daudi cell extracts are added to the nuclease-treated reticulocyte lysate system (Fig. 6).

In many respects the behaviour of the protein synthesizing system of interferon-treated, growth-inhibited Daudi cells is like that of cells deprived of serum growth factors [32]. In this case too, the cellular mRNA appears to remain stable but untranslated, suggesting that a critical component other than the mRNA itself becomes limiting. Little is yet known of the early events which lead to cell growth inhibition following interferon treatment, but mechanisms such as diminished expression of growth factor receptors, or failure of intracellular pathways to respond to the binding of crucial growth factors to these receptors, are attractive possibilities. It is also not possible to distinguish cause from effect in

considering the relationship between inhibition of cell proliferation and inhibition of protein synthesis. Clearly a certain minimum rate of protein synthesis is necessary for a cell to grow during the cell cycle, but this will not necessarily ensure that DNA replication and cell division will occur. It seems more likely that the impairment of Daudi cell proliferation following interferon treatment will prove to be a consequence of a pleiotypic response involving failure to express specific genes required for DNA replication and/or maturation of chromatin [33,34], together with the loss of the ability of the cells to maintain a rate of protein synthesis sufficient for accumulation of an adequate protein mass.

We are grateful to Dr. E. C. Henshaw and Dr. K. Clarke (University of Rochester Cancer Center, Rochester, NY, U.S.A.) for gifts of anti-eIF-2 α monoclonal antibody and for advice on the analysis of eIF-2 by isoelectric focusing. This research was supported by grants from the Cancer Research Campaign and the Gunnar Nilsson Cancer Research Trust. M.J.C. is a CRC Career Development Award holder.

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Received 23 December 1985/12 March 1986; accepted 14 April 1986