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α-interferon inhibits the expression of heavy chain μ messenger RNA in Daudi cells

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A clone of Daudi cells (Daudi-S) synthesizes the heavy chain of IgM (μ-chain) under routine conditions of cell culture. In the presence of α-interferon, however, synthesis of μ-chain is decreased rapidly at a time when the overall protein synthesis is not modified and the dsRNA-dependent protein kinase and the 2-5A synthetase are induced. This inhibition of μ-chain synthesis seems to be correlated with the antiproliferative action of interferon since it occurs only slightly in another clone of Daudi cells resistant (Daudi-R) to the antiproliferative action of interferon. In these resistant cells, however, the protein kinase and the 2-5A synthetase are induced by interferon. Specific inhibition of μ-chain synthesis in interferon-treated Daudi-S cells is a consequence of decreased steady-state levels of μ-chain mRNA. This effect occurs 4–8 h after addition of interferon in parallel with decreased levels of c-*myc* mRNA and enhanced levels of HLA mRNA. Reduced levels of μ-chain mRNA in interferon-treated Daudi-S cells is not a consequence of its enhanced degradation as shown by actinomycin D chase experiments. Furthermore, nuclear run on experiments rule out an effect on the transcription of μ-chain mRNA. Therefore, the inhibitory mechanism mediated by interferon might be at the level of termination and/or post-transcriptional processing of μ-chain RNA. In contrast, in these same interferon-treated Daudi-S cells, the inhibition of c-*myc* gene expression is due to an enhanced degradation of its mRNA (in accord with other reports). These data indicate that interferon can inhibit gene expression by different mechanisms.

Key words: IgM/inhibition/interferon/RNA

Introduction

Interferons are a family of proteins classified into three main types α, β, γ, each defined by its capacity to induce an antiviral response in cells against different viruses. The different types of interferons also have the capacity to inhibit cell growth and proliferation, regulate cell differentiation, modify the expression of specific oncogenes and activate or inhibit certain components of the immune system (for a review, see Lengyel, 1982).

Attempts by numerous workers to clarify mechanisms responsible for mediating the different effects induced by interferon have not yet come out with definite and precise models. Probably, the mechanisms induced by α-, β- and γ-interferons are not totally identical. For example, data obtained on induced proteins indicate that γ-interferon induces at least 12 proteins, six of which are not induced by α- or β-interferon (Weil *et al.*, 1983). Some differential expression of interferon-inducible genes has also been reported in cells treated with α- and γ-interferon (Rosa *et al.*, 1983; Kelly *et al.*, 1985; Strunk *et al.*, 1985). Besides the induction of some genes (Friedman *et al.*, 1984), α- and β-interferon have been reported to inhibit the expression of the c-*myc* gene, the cellular homologue of the avian myelocytomatosis virus (MC29) oncogene (Jonak and Knight, 1984; Einat *et al.*, 1985; Dani *et al.*, 1985), the c-Ha-*ras* gene, the human cellular homologue of the transforming gene of Harvey murine Sarcoma virus (Samid *et al.*, 1984; Soslau *et al.*, 1984), and *src* gene, the transforming gene of avian sarcoma virus (Lin *et al.*, 1983).

Murine α/β-interferon can inhibit the development of transformed foci in cell cultures transfected with viral and cellular oncogenes (Dubois *et al.*, 1983). In quiescent cultures of Balb/c 3T3 cells, administration of mouse α/β-interferon together with platelet-derived growth factor (PDGF) results in the inhibition of several genes (c-*myc*, c-*fos*, ornithine decarboxylase and β-actin) which are activated by the growth factor (Kimchi *et al.*, 1985).

Here, we describe the specific inhibition of IgM μ-chain synthesis in Daudi Burkitt's lymphoma cells treated with α-interferon. This effect is rapid and correlates with the antiproliferative action of interferon. The inhibition of IgM synthesis by interferon appears to be at the RNA level. Evidence is provided that reduced steady state levels of IgM RNA is not related to enhanced degradation of the mRNA nor to inhibition of initiation of transcription of the gene. These results suggest that the inhibition occurs during the processing of the μ-chain RNA.

Results

Specific inhibition of IgM heavy chain synthesis by α-interferon

During the preparation of monoclonal antibodies specific for the two dsRNA dependent enzymes, 2-5A synthetase and protein kinase, we came across an antibody which specifically immunoprecipitates the heavy chain (μ-chain) of IgM from Daudi cells. Interestingly, the use of this antibody allowed us to observe that the synthesis of the μ-chain of IgM was greatly reduced in Daudi cells after α-interferon treatment (Hovanessian *et al.*, 1986). By kinetic experiment here we show that the inhibition of the μ-chain appears as soon as 3–6 h after the addition of α-interferon (Figure 1). In this experiment, the cells were pulse-labelled for a 3-h period during the 15 h of treatment with interferon and the labelled proteins were analysed by SDS–gel electrophoresis as such or after immunoprecipitation. The inhibition of μ chain synthesis by interferon is specific since the general protein synthesis is not affected. Moreover, the synthesis of the dsRNA-dependent protein kinase (p68 kinase) induced by

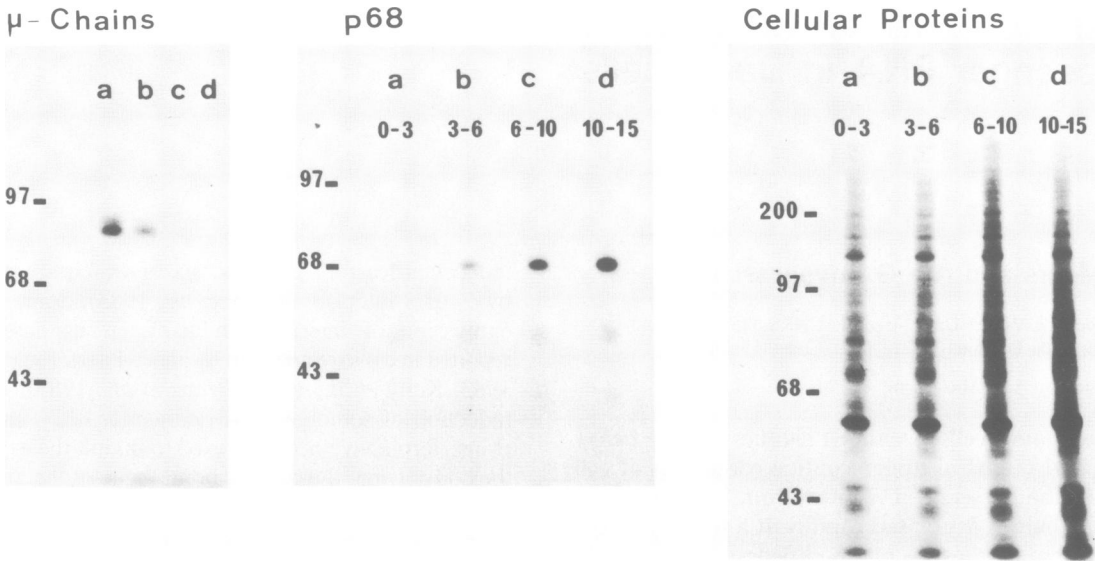

Fig. 1. Specific inhibition of IgM μ-chain by α-interferon. Daudi-S cells were centrifuged and resuspended in labelling medium (1/10 of the normal concentrations in methionine) containing 500 U/ml of α-interferon. The cells were pulse-labelled with 20 μCi/ml of ^{35}S-methionine from 0–3 (**a**), 3–6 (**b**), 6–10 (**c**) and 10–15 (**d**) hours after the addition of interferon. The cell extracts were analysed by SDS–gel electrophoresis either as such (cellular protein) or after immunoprecipitation with mAb 35/2-sepharose (μ-chains) or with mAb 71/10-sepharose (p68 kinase).

interferon (Galabru and Hovanessian, 1987) occurs at a time (6 h) when the synthesis of the μ-chain is severely inhibited.

Action of interferon on the synthesis of μ-chain in Daudi cells sensitive and resistant to the antiproliferative action of α-interferon

We have previously reported that the synthesis of the μ-chain in Daudi cells is tightly dependent on growth conditions of these cells. For example, under normal growth conditions, synthesis of μ-chain is at least 3-fold higher in the G_2/M phase compared to the G_0/G_1 phase of the cell cycle (Hovanessian *et al.*, 1986). Furthermore, incubation of Daudi cells in the absence of serum results in a 60–70% inhibition of cellular protein synthesis and a strong reduction (80–90%) in the synthesis of μ-chain. The μ-chain synthesis resumes rapidly by addition of serum with concomitant stimulation of cell growth.

In order to investigate whether inhibition of μ-chain synthesis is correlated with the antiproliferative action of interferon, we used two clones of Daudi cells sensitive (Daudi-S) and resistant (Daudi-R) to the antiproliferative action of α-interferon. There is a striking difference between the Daudi-S and the Daudi-R cells in their response to the antiproliferative action of α-interferon. While growth inhibition of Daudi-S cells is clearly detectable for as low as 10 U/ml of α-interferon and is complete for 100–1000 U/ml, the growth of Daudi-R cells remains unaffected whatever the concentration of α-interferon used (Figure 2A). The molecular basis for this difference is not known. It is not a difference at the receptor level since α-interferon induces the 2-5A synthetase and the protein kinase with comparable efficiencies in both cells (Figure 2B and C).

To analyse the effect of α-interferon on the synthesis of the μ-chain in Daudi-S and Daudi-R cells, cell extracts from (^{35}S)-methionine labelled, control and interferon-treated cells were purified by affinity chromatography on mAb 35/2-sepharose (Figure 2D). In this experiment, the cells were labelled for a period of time long enough (17 h) to allow the detection of the two forms of the heavy chain: the first-step glycosylated (80 K) and the terminally-glycosylated (84 K) form. [The processing of the 80 K to the 84 K form needs at least 4–5 h (Kubo and Pigeon, 1983; Hovanessian *et al.*, 1986) which accounts for the fact that the 84 K form was not detected in Figure 1 (3 h of pulse-labelling).]

The results (Figure 2D and Table I) show that the μ-chain synthesis was only slightly inhibited (13.5%) by interferon in the Daudi-R cells, in contrast to a strong inhibition (46.5%) in Daudi-S cells. The same (^{35}S) labelled extracts were also purified by the immunoadsorbant 71/10 Sepharose specific for the p68 kinase. As expected, the p68 kinase was induced by interferon in both sensitive and resistant cells (Figure 2E and Table I). Thus, the synthesis of the p68 kinase is enhanced in both types of cells treated with α-interferon whereas strong inhibition of μ-chain synthesis and inhibition of cell growth are only observed in Daudi-S cells. These data favour the suggestion that inhibition of μ-chain synthesis is correlated with the mechanism of anti-growth action of α-interferon in Daudi-S cells. Accordingly, inhibition of μ-chain expression was not observed in cells in which interferon did not exert an antiproliferative action. For example, the synthesis of μ-chain in other Burkitt's lymphoma cell lines such as BJAB, Raji, BL17 and BL57 was not affected by α-interferon whereas these cells respond to interferon by the induction of the 2-5A synthetase and the p68 kinase (data not shown).

Cell surface expression of IgM in interferon-treated Daudi-S and Daudi-R cells

Further evidence that α-interferon affects the expression of μ-chain differently in Daudi-S and Daudi-R cells was provided by direct study of the cell surface IgM in both types of cells, treated with interferon. Daudi-S and Daudi-R were treated with 500 U/ml of interferon, then cell surface IgM was assayed with different dilutions of our mAb 35/2 (anti-μ). Figure 3 shows that in Daudi-S cells there is >50% reduction of cell surface IgM whereas <10% reduction is

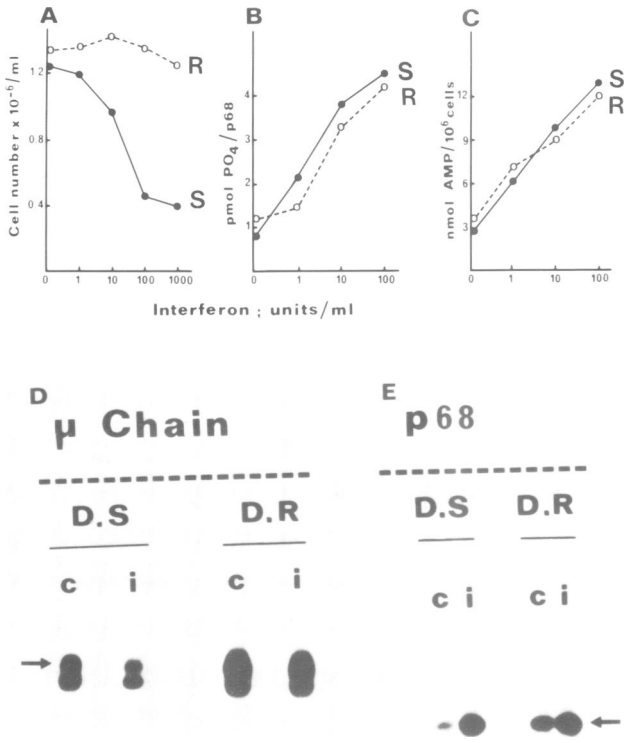

Fig. 2. Specific inhibition of μ-chain synthesis in Daudi cells is correlated with the antiproliferative action of interferon. Daudi cells sensitive (S) or resistant (R) to the antiproliferative action of interferon were seeded at 4×10^5 cells/ml and grown in the absence or in the presence of different concentrations of α-interferon. **A**, The cell number was determined after two days of culture. **B** and **C**, Cell extracts were prepared after 17 h of treatment with interferon and analysed for protein kinase (B) activity (Galabru and Hovanessian, 1985) or for 2-5A synthetase (C) activity (Buffet-Janvresse *et al.*, 1983). (**D**) and (**E**) control cells (lanes c) or cells treated with 500 U/ml of interferon (lanes i) were labelled with ^{35}S-methionine for the time of treatment with interferon (17 h) and the cell extracts were immunoprecipitated with mAb 35/2-Sepharose (D) or with mAb 71/10-sepharose (E) for the purification of μ-chain and p68 kinase, respectively. The labelled proteins were analysed by SDS–gel electrophoresis. An autoradiograph of the dried gel is presented.

Table I. Specific inhibition of μ-chain synthesis in Daudi cells

Cells	μ-chain	p68 kinase
Daudi-S: Control	202	29
IFN	108 (46.5%)[a]	146
Daudi-R: Control	339	72
IFN	293 (13.5%)[a]	139

The autoradiograms shown in Figure 2D and E were scanned by densitometry. The results are expressed in arbitrary units.
[a]The percent reduction in the level of μ-chain.

observed in Daudi-R cells. These results correlate strongly with the data on μ-chain synthesis shown in Figure 2. Once again, the decrease of cell surface IgM in Daudi-S but not in Daudi-R cells correlates with the antiproliferative action of α-interferon in Daudi-S but not in Daudi-R cells.

Reduced steady state levels of μ-chain mRNA in interferon-treated Daudi-S cells

Preliminary results have suggested that the inhibition of μ-chain synthesis in α-interferon-treated Daudi-S cells is probably at its mRNA level (Hovanessian *et al.*, 1986). For this reason we analysed steady state levels of μ-chain mRNAs in control and α-interferon-treated cells. Two RNA species of 2.4 and 2.7 kb are known to be transcribed from the same μ gene by differential splicing at their 3′ end (Rogers *et al.*, 1980). These 2.4 and 2.7 kb species encode for the secreted and the membrane μ-chains, respectively.

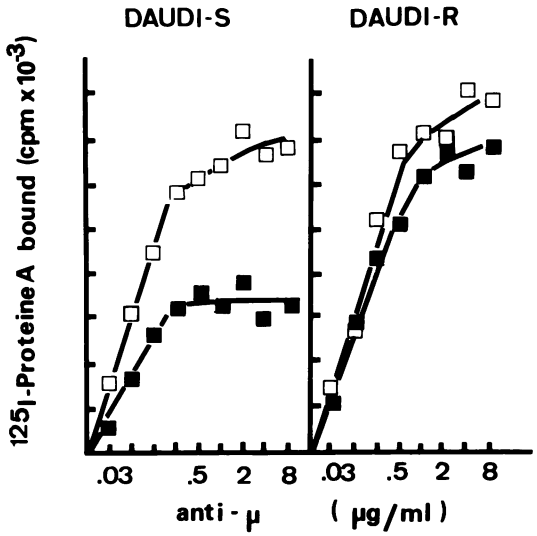

Fig. 3. Specific decrease of surface IgM from Daudi-S cells after interferon treatment. Daudi cells sensitive (Daudi-S) or resistant (Daudi-R) to the antiproliferative action of interferon were incubated in the absence (□) or in the presence of human α-interferon (■) for 72 h. For each time point, the cells were centrifuged, resuspended in culture medium at the concentration of 2×10^6 cells/ml (1 ml for each point) and incubated at 37°C for 30 min in the presence of different concentrations of mAb 35/2 (anti-μ). Fifty μl of ^{125}I-protein A (Amersham) at the concentration of 0.5 μCi/ml was then added and the incubation was continued for another 30 min. The samples were then washed three times in PBS containing 0.1% bovine serum albumin by centrifugation and the radioactivity of the pellet was counted.

Figure 4 shows that the steady state levels of μ-chain mRNAs (both 2.4 and 2.7 kb species) are significantly reduced (65% of inhibition) by α-interferon in Daudi-S cells. The inhibition is specific since in this same experiment (the same blot), the steady state levels of HLA mRNAs were found to be increased by α-interferon, as it has been reported previously (Fellous *et al.*, 1982; Rosa *et al.*, 1983). The steady state levels of μ-chain mRNAs in Daudi-R cells were also found to be affected by interferon (25% inhibition) treatment in this particular experiment, although much less than in Daudi-S cells. This latter effect of α-interferon in Daudi-R cells was not found to be reproducible in two other independent experiments (data not shown, see also Figure 5). Daudi-S and Daudi-R cells differ in their sensitivity to the anti-proliferative action of interferon but they respond equally well to interferon for the induction of HLA, 2-5A synthetase or p68 kinase, which suggests that interferon triggers several common events in both cells.

In this experiment, we also confirmed that the levels of c-*myc* mRNAs are specifically reduced by α-interferon treatment in Daudi-S cells (48% of inhibition) in accord with previous results (Jonak and Knight, 1984; Einat *et al.*, 1985; Dani *et al.*, 1985). In Daudi-R cells, however, only a slight inhibition of c-*myc* mRNA levels is observed (8%). Our Daudi-R cells seem to respond slightly to the inhibition of

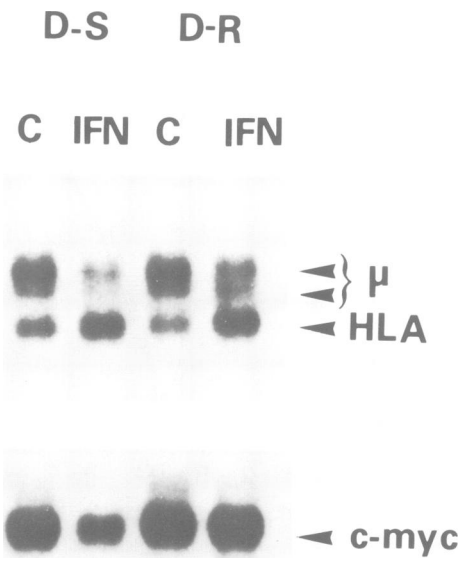

Fig. 4. Northern blot analysis of μ-chain mRNA from control and α-interferon treated Daudi cells. Cytoplasmic RNAs were prepared from control (lanes C) and interferon-treated (lanes IFN; 500 U/ml for 17 h) Daudi-S (D-S) and Daudi-R (D-R) cells. A Northern blot obtained from poly(A)$^+$ RNAs (2.5 μg) run in 1% agarose gel was hybridized at 42°C with c-*myc* exon 3 cDNA probe, dehybridized and rehybridized at 42°C with μ cDNA and HLA_1 cDNA probes.

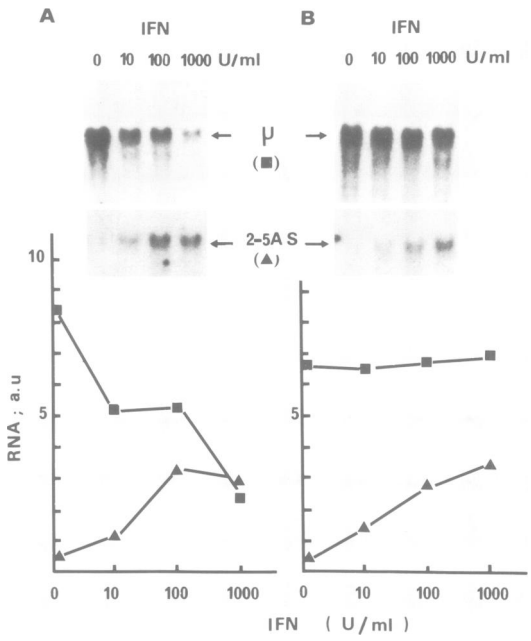

Fig. 5. Dose response of inhibition of μ-chain RNA by α-interferon. Cytoplasmic RNAs were extracted from Daudi-S (**A**) and Daudi-R (**B**) cells after 18 h of treatment with increasing concentrations of α-interferon (IFN). A Northern blot obtained from total RNAs (10 μg) run on 1% agarose gel was hybridized successively with human μ cDNA probe and 2-5A synthetase (2-5AS) probe. Different autoradiograms of the same blot were subjected to densitometry to quantitate the levels of μ-chain and 2-5A synthetase RNAs. The ordinate represents the areas of the RNA peaks which are expressed as arbitrary units (a.u.). A. Daudi-S cells. B. Daudi-R cells.

μ-chain and c-*myc* mRNAs by interferon, probably due to the elevated concentrations of interferon (500 U/ml) used throughout these experiments in order to demonstrate a maximal inhibition in Daudi-S cells. Accordingly, at 100 U/ml of interferon, the steady-state levels of c-*myc* and μ chains mRNAs were not modified in Daudi-R cells.

The effect of interferon dose on the reduction of μ-chain mRNA in Daudi-S cells

The reduction in the steady-state levels of μ-chain mRNAs depends on the concentration of α-interferon added to the cells, with 40% of inhibition for 10 U/ml, reaching 70% of inhibition for 1000 U/ml (Figure 5A). This correlates with the dose response experiment that we performed previously at the protein level (Hovanessian *et al.*, 1986). Pronounced inhibition of μ-chain protein and of its mRNA is best observed for concentrations of interferon above 100 U/ml. For this reason, we chose 500 U/ml of interferon throughout all our experiments to observe maximal inhibitory effect of interferon on μ-chain mRNA. This concentration range also correlates with the data on the antiproliferative action of interferon which are significant for 10 U/ml and maximal for concentrations above 100 U/ml (Figure 2A). The Northern blot used to study the dose response of μ-chain mRNA to interferon was also used to analyse the steady state levels of the 2-5A synthetase mRNAs (1.8 kb species) to serve as an internal control. The levels of 2-5A synthetase mRNAs were found to be increased by interferon treatment as expected (Figure 5A).

In a similar dose response experiment with Daudi-R cells treated with α-interferon, the steady state levels of μ-chain mRNA were not affected even at 1000 U/ml of interferon. However, these Daudi-R cells responded as Daudi-S cells for the induction of 2-5A synthetase mRNA (Figure 5B). This 1.8 kb mRNA encodes a 46 kd 2-5A synthetase induced by interferon (Benech *et al.*, 1985; Saunders *et al.*, 1985).

In accordance with the kinetics of inhibition of μ-chain protein synthesis in α-interferon-treated Daudi-S cells (Figure 2), the steady state levels of μ-chain mRNAs were found to be greatly reduced at 8 h following treatment with interferon. At 17 h post-treatment with interferon the level of μ-chain mRNAs was at least 10-fold less than that detected in untreated cells. In this same experiment, the increased levels of HLA mRNAs, observed 17 h after α-interferon treatment, indicated once again that the reduction of μ-chain mRNAs is specific (Figure 6).

Effect of interferon on the half life of μ chain mRNA

We next examined whether the reduction in the levels of the μ-chain mRNAs after α-interferon treatment could be attributed to an enhanced degradation of these mRNAs as was reported to be the case for c-*myc* (Dani *et al.*, 1985). For this purpose, control and interferon-treated Daudi cells were incubated with actinomycin D for various times. Northern blots were then prepared from poly(A)$^+$ RNAs and hybridized with μ-chain and c-*myc* probes as well as HLA probe, for an internal calibration. Autoradiograms obtained by different exposures were used for quantitation. Enhanced degradation of c-*myc* RNAs after interferon treatment was observed (Figure 7). In contrast, the half life of μ-chain mRNA (3 h) was found to be identical in control and interferon-treated cells (Figure 7). The reduction of μ-chain RNA by α-interferon treatment therefore cannot be attributed to an enhanced degradation of poly(A)$^+$ RNAs,

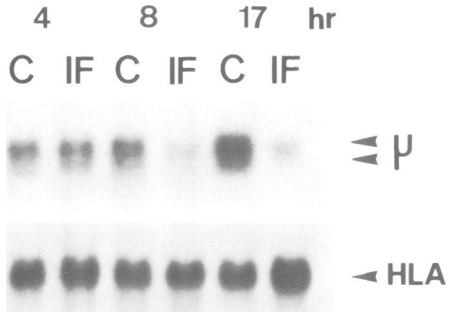

Fig. 6. Kinetics of inhibition of μ chain RNA by α-interferon. Cytoplasmic RNAs were extracted from control (**lanes C**) and α-interferon-treated (**lanes IF**) Daudi-S cells after 4, 8 or 17 h of treatment. A Northern blot obtained from poly(A)$^+$ RNAs (3 μg) run on 1% agarose gel was hybridized successively with μ cDNA probe and HLA_1 cDNA probe.

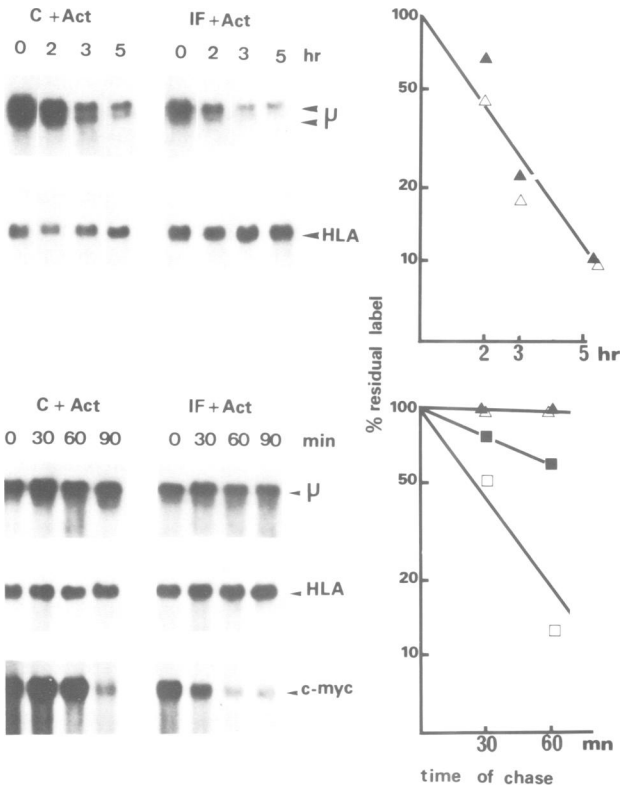

Fig. 7. Actinomycin D chase of μ chain from control and α-interferon-treated Daudi-cells. Control (C) and α-interferon-treated (IF; 500 U/ml for 10 h) Daudi-S cells were incubated with 5 μg of actinomycin D (Act) per ml for the times indicated. Northern blots obtained from poly(A)$^+$ RNAs (3 μg each) run on 1% agarose gels were hybridized with μ, HLA_1 and c-*myc* probes. Autoradiograph of the blot (after different exposures) was quantitated by densitometric scanning and normalized with HLA_1 RNA (on the right). Symbols stand for μ-chain mRNA control (▲) and interferon-treated (△) cells; c-*myc* mRNA from control (■) and interferon-treated (□) cells.

and appears to be different from the action of α-interferon on c-*myc* RNAs.

Interferon action on the transcription of the μ gene

Daudi cells were incubated with α-interferon for different periods of time and the transcription rate of the gene for the μ-chain was analysed in the isolated nuclei using two probes, one complementary to an early region of the gene (J) and

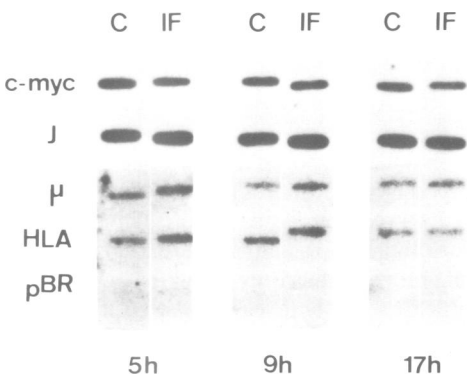

Fig. 8. Slot blot assay of nuclear transcripts from μ chain gene after interferon treatment. Daudi cells were incubated as such (control; **lanes C**) or treated with 500 U/ml of α-interferon (**lanes IF**) for 5, 9 or 17 h. *In vitro* ^{32}P-labelled RNAs from isolated nuclei were analysed for hybridization with different plasmids bound on Hybond-N membrane filters. pBR322 was used as non-specific DNA to measure background levels. The following inserts: c-*myc*, HLA_1, J and μ were of 1.6 kb, 525 bp, 6.5 kd and 1.2 kb respectively. For hybridization, each set of filters (1 cm^2 each) was placed in 2 ml of hybridization buffer, in the wells of a 6-well plate (Costar). Each plate was sealed in plastic bags.

the other complementary to exons 3 and 4 of the constant region. The transcription of the μ-chain gene was not inhibited after 5, 9 or 17 h of treatment with α-interferon and in fact a slight increase was observed. The transcription of HLA gene which was analysed in parallel as a positive control was found to be increased by α-interferon treatment as has already been reported (Dani *et al.*, 1985). In addition, we found that the transcription rate of c-*myc* was not affected by α-interferon treatment in our Daudi cells, in accordance with other previous reports (Knight *et al.*, 1985; Dani *et al.*, 1985).

Discussion

The synthesis of the heavy chain (μ) of IgM is reduced significantly in α-interferon-treated Daudi cells. This reduction is specific since it occurs at a time when the overall protein synthesis is normal and the dsRNA-dependent protein kinase and 2-5A synthetase are induced. Previously, we have presented evidence to indicate that neither the processing of the μ-chain nor its turn-over are affected in α-interferon-treated cells. On the other hand, here we show that the decreased steady state levels of μ-chain mRNAs in α-interferon-treated Daudi cells are the cause of its decreased synthesis. The precise mechanism by which the expression of μ-chain mRNAs becomes down regulated by α-interferon is not yet clear. Our results indicate that this down regulation is not a consequence of a mechanism of an enhanced degradation of μ-chain poly(A)$^+$ RNAs, as it is the case for c-*myc* mRNAs in our Daudi-S cloned cells. Furthermore, nuclear run-on experiments indicate that the transcription of μ-chain gene is not inhibited by α-interferon treatment.

Having no effect on the initiation of transcription nor on the stability of poly(A)$^+$ RNA, it is possible that α-interferon inhibits an intermediate event during RNA processing, such as polyadenylation of μ mRNAs and/or its splicing. The RNAs encoding for the membrane (μm) and the secreted (μs) forms of the μ-chain are transcribed from the same gene and processed by alternative splicing. The polyadenylation

of μm and μs RNAs is a complex mechanism which depends on the relative positions and strengths of two poly(A) sites as well as on transcription termination. The control in the formation of μm or μs RNAs has been related in part to a change in the amounts of a common factor used with different affinities by both poly(A) sites (Galli *et al.*, 1987). It might be possible that interferon treatment of Daudi cells leads to an inactivation of this factor(s) involved in poly(A) recognition. In addition to this, there might be an effect at the splicing process, thus preventing the cleavage of the pre μ-chain mRNAs. In both cases, the result would be the observed decrease in poly(A)$^+$ μ-chain RNA populations.

Interferon inhibits the expression of the cellular oncogene c-*myc* in Daudi cells. The mechanism of this inhibition has been reported to be either at the level of the initiation of transcription (Einat *et al.*, 1985) or at a post-transcriptional level (Knight *et al.*, 1985; Dani *et al.*, 1985). In the latter case, it was suggested that interferon inhibits c-*myc* expression through an enhanced degradation of c-*myc* mRNAs by the 2-5A dependent nuclease since c-*myc* mRNAs may form a stable stem-loop structure capable to activate the 2-5A system (Wreschner *et al.*, 1981; Saito *et al.*, 1983; Dani *et al.*, 1985). Here we also present evidence for an enhanced degradation of c-*myc* poly(A)$^+$ RNA rather than inhibition of transcription in our clone of Daudi-S cells after treatment with α-interferon. Interestingly, in this same clone of Daudi cells, the down regulation of μ-chain RNAs by interferon is not due to its enhanced degradation. Thus, interferon regulates the expression of c-*myc* and μ-chain RNAs by two different mechanisms. However, both of these inhibitory mechanisms seem to be correlated with the antiproliferative action of interferon.

Several results reported previously have suggested that c-*myc* plays an important role in cell proliferation and may participate directly in the replication of the DNA (Heikkila *et al.*, 1987; Iguchi-Ariga *et al.*, 1987). Accordingly, expression of c-*myc* in interferon-treated Daudi cells is inhibited after 2–3 hours of treatment with interferon (Knight *et al.*, 1985). The inhibition of μ-chain expression in Daudi cells by interferon takes place between 4 and 8 h of treatment with interferon, the delay of which, compared to the early inhibition on c-*myc* RNA, can be attributed to longer half-life of μ poly(A)$^+$ RNA (2–3 h) compared to that of c-*myc* poly(A)$^+$ RNA (30–60 min). Hence both inhibitory mechanisms involved for c-*myc* and μ-chain RNA accumulation are early events in the antiproliferative action of interferon. However, at this stage it is not possible to determine whether the inhibitory effect on the μ-chain RNA is a consequence of down regulation of c-*myc* RNA.

Recently, Exley *et al.* have reported the differentiation of interferon-treated Daudi cells with enhanced immunoglobulin synthesis (Exley *et al.*, 1987). In our Daudi-S cells, however, we were not able to detect any synthesis of IgD or IgG following interferon treatment, although IgM synthesis was reduced significantly. Moreover, we found that the inhibition of IgM was reversible as its synthesis resumes 24 h after removal of interferon from the cells (unpublished data). The differences between these observations might be simply due to the different Daudi cell preparations used in these experiments. Our Daudi-S cells have been originated from a cloned cell in contrast to the Daudi cells of Exley *et al.* The wider significance of the inhibitory action of interferon on the expression of μ-chain is not yet clear. Preliminary studies on primary B lymphocyte cultures have shown that interferon exerts only a small inhibitory effect on the synthesis of μ-chain by these mitogen-stimulated lymphocytes (unpublished results). Other reports have suggested that the inhibition of immunoglobulin synthesis by cultured lymphocytes is dependent on the concentration of interferon (Peters *et al.*, 1986) and also might be variable depending on whether interferon is added before, during or after addition of the mitogen (Härfast *et al.*, 1981; Peters *et al.*, 1986; O'Gorman *et al.*, 1987).

By the use of a clone of Daudi cells resistant (Daudi-R) to the antiproliferative effect of interferon, we could illustrate that the mechanism of inhibition of μ-chain expression might be correlated with the mechanism of antiproliferative action of interferon. The expression of μ-chain is not affected in Daudi-R cells whereas both the 2-5A synthetase and the p68 kinase are induced. Cytofluorimetric analysis indicated that interferon-treated Daudi-R cells do not show any modification in their cell cycle which is almost identical to that observed in untreated cells. However, interferon-treated Daudi-S cells (sensitive to the antiproliferative action of interferon) show an increase in the proportion of cells in G_0/G_1 phase at the expense of S and G_2/M phases of their cell cycle (Hovanessian *et al.*, 1986). In view of this, interferon-treated Daudi-S and Daudi-R cells should provide an efficient model for investigating the molecular mechanisms responsible for the specific down regulation of a cellular gene. Here we have provided evidence to suggest that this down regulation is probably at the level of terminal processing of new transcripts and their maturation: at steps such as polyadenylation and/or splicing. The observation that inhibition of μ-chain expression and inhibition of cell growth are closely related suggests that determination of the precise mechanism of down regulation of μ-chain expression might shed some light on the mechanism of antiproliferative action of interferon.

Materials and methods

Cells

Daudi cells were grown in suspension in RPMI-1640 medium containing 10^{-5} M 2-mercaptoethanol and 10% fetal calf serum. Interferon-resistant Daudi cells (provided by I.Kerr, ICRF, London) were prepared by culturing Daudi cells in medium containing 1000 U/ml of human α-interferon. After 3 months, the cells which survived the inhibitory effect of interferon were cloned in agarose (Montagnier and Gruest, 1979) and then cultured in the absence of interferon. One of the cloned cells was further cultured in the presence of α-interferon (1000 U/ml) for 2 months and cloned once again in agarose. One of these clones was used in experiments discussed here. These cells are referred to as Daudi-R cells. α-Interferon-sensitive Daudi (Daudi-S) cells were propagated from cells cloned in agarose.

Cell extracts

For the preparation of cell extracts, Daudi cells were first washed with phosphate buffered saline before addition of lysis buffer: 10 mM Tris–HCl, pH 7.6, 25 mM KCl, 200 mM NaCl, 1 mM EDTA, 10 mM 2-mercaptoethanol, 1% triton X-100 (v/v), 1000 U/ml aprotinin, 0.2 mM PMSF and 10% glycerol (v/v). Cell extracts were centrifuged at 1500 *g* and then at 12 000 *g*. The supernatants after 12 000 *g* centrifugation were stored at −80°C. For radioactive labelling, Daudi cells in the absence or presence of α-interferon were incubated in RPMI-1640 culture medium containing only 5–10% of the normal level of methionine (1.5 μg/ml) and ^{35}S-methionine (12.5 μCi/ml; 400–500 Ci/mmol). Cell extracts were prepared as above. Human α-interferon from Namalwa cells (provided by Hayashibara) was purified in the laboratory by a monoclonal antibody column (Meurs *et al.*, 1983).

Immunoprecipitations

The mAb LSH 35/2 is of IgG2a class antibody. It is capable of immunoprecipitating IgM but it does not recognize the denatured antigen in an immunoblot assay. It was preferentially used after coupling to sepharose (mAb 35/2 sepharose) for the purification of ^{35}S-methionine labelled μ chains. Preparation of the immunoaffinity column containing mAb LSH 35/2 specific for μ-chains was according to a method described previously (Galabru and Hovanessian, 1985). The binding of μ-chains to the monoclonal antibody column (mAb-35/2-sepharose) was in buffer I: 20 mM Tris−HCl, pH 7.6, 50 mM KCl, 400 mM NaCl, 1 mM EDTA, 5 mM 2-mercaptoethanol, 1% triton X-100, 0.2 mM PMSF, 100 Units/ml aprotinin and 20% glycerol before heating (95°C, 5 min) in 2-fold concentrated electrophoresis sample buffer. Samples were then analysed by polyacrylamide slab gel electrophoresis. Purification of the p68 kinase was carried out by affinity chromatography using mAb 71/10-Sepharose specific for the kinase (Galabru and Hovanessian, 1987).

RNA isolation

Total cytoplasmic RNA was isolated according to the procedure of Favaloro described in the CSH manual (Maniatis *et al.*, 1982) with the following modification: after centrifugation on sucrose-lysis buffer, the RNAs from the cytoplasmic layer were directly extracted once with hot (60°C) phenol/chloroform. The aqueous phase was then recovered by centrifugation (8000 *g*) and the RNAs were precipitated with ethanol. The poly(A)$^+$ RNAs were selected by oligo(dT)-cellulose chromatography (Aviv and Leder, 1972). RNAs were separated by electrophoresis in 1% agarose gels containing 50 mM Hepes, pH 7.8, 1 mM EDTA and 2 M formaldehyde and transferred to nitrocellulose (Schleicher and Schuell; BA 95; 0.45 μm pore diameter). The RNAs were then covalently linked to the membrane by baking in a vacuum oven for 2 h at 80°C.

Hybridization

The hybridizations were performed as described by Thomas (1980). The probes were prepared with the nick-translation kit of Amersham.

Preparation of nuclei from Daudi cells

For each point, nuclei from 4×10^8 cells were prepared by washing the cell pellet twice with phosphate buffered saline and once with homogenization buffer (HB: 10 mM Tris, pH 7.6, 10 mM NaCl, 10 mM $MgCl_2$). After centrifugation at 1000 *g*, the supernatant was carefully removed and the cells were resuspended in 2 ml of HB. One ml of 1% NP40 in HB was then added to lyse the cells (for 1 min) and the suspension was rapidly diluted with 20 ml of HB. After centrifugation at 1000 *g* for 5 min, the pellet was resuspended in 600 μl of freezing buffer (50 mM Tris, pH 8.3; 40% (v/v) glycerol, 10 mM $MgCl_2$, 0.1 mM EDTA) divided in aliquots and quickly frozen in liquid nitrogen.

Transcription in isolated nuclei

Two hundred μl of nuclei (1.2×10^8) were incubated at 30°C for 30 min with 100 μl of solution A (2 mM of ATP, CTP and GTP, 0.2 mM of UTP, 0.1 mM of S-adenosylmethionine), 80 μl of solution B (0.6 M of KCl, 5 mM of $Mg(OAc)_2$, 12.5 mM dithiothreitol) and 20 μl (200 μCi) of ^{32}P-UTP (3000 Ci/mmol; Amersham) in a total volume of 400 μl. The ^{32}P-RNAs were then extracted as described (Linial *et al.*, 1985).

Hybridization of ^{32}P-RNAs

For binding to membrane filters (Hybond-N; Amersham), plasmids were linearized by restriction enzyme digestion and the DNA was denatured by incubation with 0.2 M NaOH for 30 min at 4°C followed by neutralization with free acid Hepes from a 1.2 M stock solution. The DNA was applied to the membrane using a Schleicher and Schuell slot blot apparatus. Five μg of DNA were applied per slot and the wells were further washed with 6 × SSC. The DNA was then linked covalently to the membrane by 5 min exposure under UV light. The filters were first incubated in 50% formamide, 0.2% polyvinylpyrrolidone, 0.2% Ficoll, 0.5% SDS. After 6 h at 42°C, the filters were hybridized with ^{32}P-RNAs in the same buffer. After hybridization at 42°C for 48 h, the filters were washed at 42°C in 2 × SSC for 10 min, then incubated with 20 μg/ml of ribonuclease A in 2 × SSC for 30 min at 37°C, 0.5% SDS for 60 min and at 50°C in 0.5 × SSC, 0.5% SDS for 3 times 5 min. The filters were exposed to Kodak X-AR5 X ray films.

DNA probes

Plasmid pACYC184 containing the *Eco*RI segment (1 kb) of exon 3 and 4 from the human constant region of IgM (Rabbitts *et al.*, 1981) was provided by T.Rabbitts (MRC Cambridge). Plasmid pUC8 containing a *Bam*HI−*Hin*dIII segment (6.5 kb) in the J region of human IgM was provided by E.Lundgren (University Umea). Plasmid pBR322 containing the *Cla*I segment (1.6 kb) from the third exon of human c-*myc* was a gift from D.Stehelin (Institut Pasteur, Lille). Plasmid pBR322 containing the *Pst*1 segment (525 bp) from HLA1 (Ploegh *et al.*, 1980) was provided by M.Fellous (Institut Pasteur, Paris). An *Eco*RI segment (1 kb) encoding the 46 kd 2-5A synthetase was provided by B.Williams (Saunders *et al.*, 1985).

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