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Modulation of tubulin mRNA levels by interferon in human lymphoblastoid cells

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Blot hybridization with labeled tubulin cDNA showed that treatment of Ramos cells, a human cell line of lymphoblastoid origin, with either α or β interferon (IFN) induced a marked increase in the amount of tubulin mRNA sequences. The level of tubulin mRNA sequences increased rapidly after exposure of cells to IFN-α and reached a maximum after 1 h of treatment, which was four times the control level. Treatment with IFN-β induced a maximal increase after 4 h; the amount of tubulin mRNA sequences was seven times higher than the control level. The mRNA extracted from IFN-treated and nontreated cells was translated *in vitro* in a reticulocyte lysate cell-free system containing [^{35}S]methionine. Electrophoretic analysis of the labeled cell-free products showed an increase in the amount of translatable tubulin mRNA that parallels the time course of induction of tubulin mRNA sequences. Two-dimensional gel electrophoresis of the labeled protein products directed by mRNA indicates that IFN caused a more pronounced increase in the level of α-tubulin than β-tubulin mRNA. Treatment with colchicine, which disrupts the cell microtubules, caused a marked decrease in the tubulin mRNA content. Concomitant treatment of the cells with colchicine and IFN abolished the interferon-dependent induction of tubulin mRNA.

Key words: tubulin mRNA/tubulin cDNA/interferon

Introduction

Among the several regulatory mechanisms induced by interferon (IFN), the antiviral and antimitogenic effects have been thoroughly studied at the molecular level. Interferon has been shown to cause an increase in the activity of three enzymes, EIF-2 protein kinase, (2'-5')-oligoadenylate synthetase E, and phosphodiesterase, which have been correlated with the establishment of the antiviral state. The effect on viral multiplication by these enzymes is primarily due to an inhibition of translation of viral mRNA (Hovanessian *et al.*, 1977; Hovanessian and Kerr, 1978; Kimhi *et al.*, 1979a, 1979b; Revel, 1977; Revel and Groner, 1978; Revel *et al.*, 1980; Schmidt *et al.*, 1978, 1979; Zylberstein *et al.*, 1978). The IFN-dependent increase of these three enzymes was shown to be blocked by inhibitors of transcription, thus suggesting an IFN effect at the corresponding mRNA levels (Baglioni, 1979; Shulman and Revel, 1980). In addition to the elevated activities of these three enzymes, numerous changes in cell membrane components and cell shape were seen in IFN-treated cells (Bourgeade *et al.*, 1981; Chang *et al.*, 1978; Chandabosse *et al.*, 1981; Kohn *et al.*, 1976; Lindahl *et al.*, 1973; Lonai and Steinman, 1977; Pfeffer *et al.*, 1980; Tovy *et* al., 1975). We wondered whether the effect of IFN on cell organelles involves the synthesis of tubulin, the major protein component of microtubules. The present results show that IFN-α or IFN-β induce a large increase in the amount of tubulin mRNA in Ramos cells, a human cell line of lymphoblastoid origin. Colchicine treatment, reported to cause a rapid decline in the amounts of α- and β-tubulin mRNA in several rodent cell lines (Ben-Ze'ev *et al.*, 1979; Cleveland *et al.*, 1981), was found to have a similar effect in the Ramos human cells. The specific induction of increased amounts of tubulin mRNA in the IFN-treated Ramos cells was abolished when these cells were pretreated with colchicine, suggesting two independent regulatory mechanisms for tubulin mRNA expression.

Results

Effects of IFN and colchicine on tubulin and actin mRNA levels

Ramos lymphoblastoid cells (2 x 10^8 cells/assay) were treated with IFN-α and IFN-β at concentrations that cause maximal increase in the synthesis of several other proteins such as HLA antigens (Fellous *et al.*, 1982). At various times after exposure to IFN (1-12 h), poly(A)-containing RNA was extracted and analyzed. To measure the amount of tubulin and actin specific sequences, the mRNA samples were subjected to electrophoresis in formaldehyde-agarose slab gels. The fractionated RNA samples were then transferred by blotting onto a nitrocellulose filter and hybridized with nick-translated [^{32}P]cDNA probes. Figure 1 shows that a single RNA species (of mobility slightly faster than 18S rRNA) hybridized to pT25 cDNA, the tubulin-specific probe. As shown in Figure 1A and B the kinetics of induction of tubulin mRNA sequences as well as their maximal levels were different depending on whether the cells were treated with IFN-α or IFN-β. The amount of tubulin mRNA sequences increased very rapidly after exposure of cells to IFN-α and reached a maximum after 1 h of treatment which was four times the control level. On the other hand, with IFN-β it took 4 h to reach the maximal level but the final increase in tubulin mRNA sequences was much higher, seven times the control value. Following the induction of tubulin mRNA sequences there was a gradual decline in their level, reaching the control value after 6 h of IFN treatment. It should be noted that in these experiments the sum of α- and β-tubulin RNA sequences is scored, because of the great homology between the α- and β-tubulin nucleotide sequences (Krauhs *et al.*, 1981; Ponstingl *et al.*, 1981).

The amount of tubulin mRNA is markedly decreased when rodent cells are treated with colchicine or nocodazole, drugs that depolymerize the cell microtubules (Ben-Ze'ev *et al.*, 1979; Cleveland *et al.*, 1981). We obtained similar results when we treated with colchicine 3T3 and 3T6 mouse fibroblast and SVPy 3T3 (SV40 and polyoma transformed 3T3 cell lines, unpublished results). It was interesting to examine whether colchicine produces the same effect in a human cell

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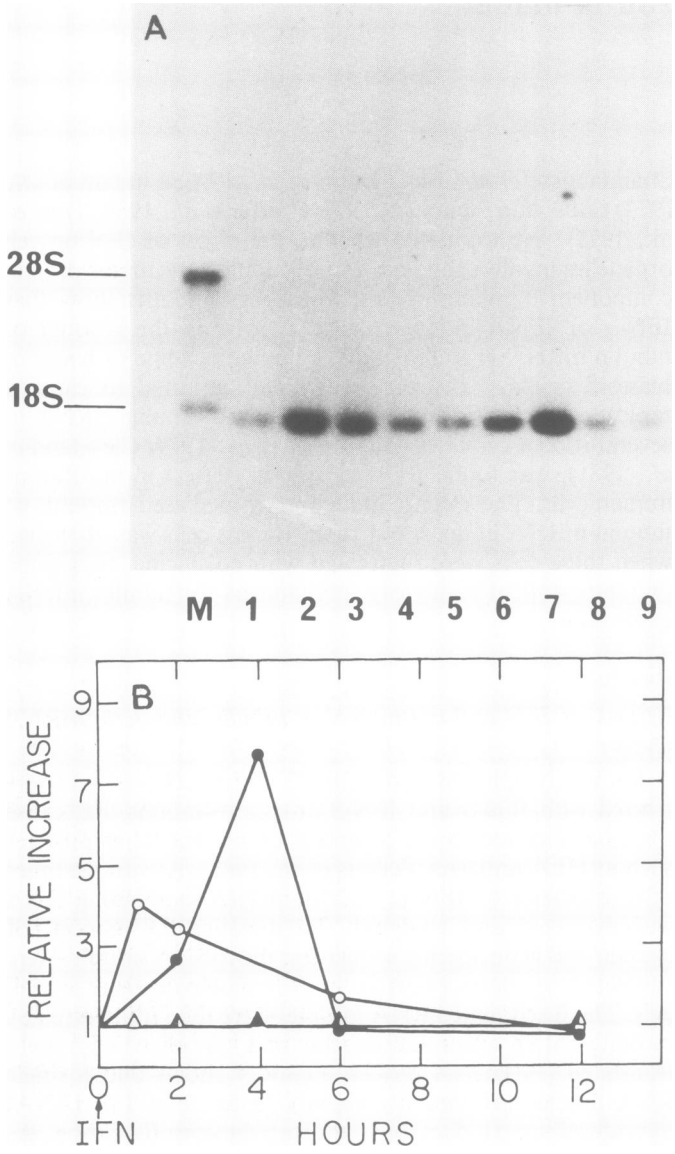

Fig. 1. Hybridization of tubulin cDNA to size-fractionated RNA from IFN-treated cultures. Poly(A)-containing RNA was extracted from Ramos lymphoblastoid cell cultures treated for various times with IFN-α or IFN-β. 2 μg of each mRNA preparation were subjected to electrophoresis on 1.2% agarose gel and the size-fractionated RNA was blotted onto a nitrocellulose sheet and hybridized to nick-translated [^{32}P]tubulin cDNA clone pT25 (10 x 10^6 c.p.m./blot). The blot was then exposed for 3 days to Agfa Curie X-ray film. **A: lane 2**, no IFN; **lanes 3–6**, IFN-α for 1, 2, 6, and 12 h; **lanes 7–10**, IFN-β for 2, 4, 6, and 12 h. [^{32}P]rRNA was used as a marker **(lane 1)**. **B**: The autoradiogram represented in **A** was scanned and the relative intensity of the band hybridized with [^{32}P]cDNA from tubulin clone pT25 is presented as a function of time of treatment with IFN-α (○—○) and to IFN-β (●—●). The hybridization with pA72 [^{32}P]cDNA from actin clone pA72 with mRNA isolated from the same cells treated with IFN-α (△—△) or IFN-β (▲—▲) is also presented.

line and whether the induction of tubulin mRNA by IFN would be antagonized by colchicine. The experiment described in Figure 2 again shows an increase in tubulin mRNA sequences in Ramos cells treated with IFN-β (lane 2). When the cells were treated with 10^{-5} M colchicine the level of tubulin mRNA decreased markedly. Addition of IFN-β to colchicine-treated cells failed to induce any increase in the level of the tubulin mRNA sequences.

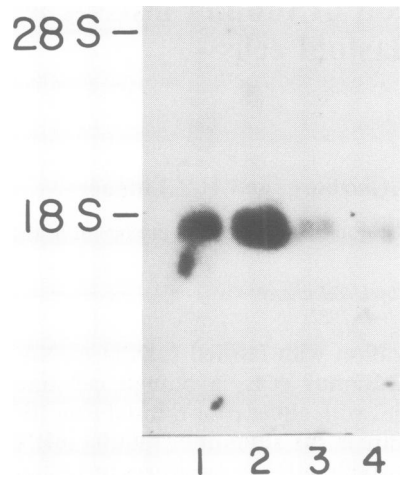

Fig. 2. Effect of IFN and colchicine on the level of tubulin mRNA sequences. Poly(A)-containing RNA was isolated from Ramos cells treated either for 4 h with IFN-β or for 6 h with 10^{-5} M colchicine or with both colchicine and IFN-β. In the latter case, cells were pretreated with colchicine for 2 h prior to addition of IFN-β and incubation was continued for an additional 4 h. After gel electrophoresis, the level of tubulin mRNA sequences was examined by hybridization to pT25 [^{32}P]cDNA as described in Figure 1. **Lane 1**: no IFN; **lane 2**: IFN-β for 4 h; **lane 3**: 10^{-5} M colchicine; **lane 4**: colchicine + IFN-β.

In vitro translation of mRNA isolated from Ramos cells treated with IFN-α or IFN-β

The hybridization data described above show the effect of IFN treatment on the induction of tubulin mRNA sequences. This approach measures the total amount of tubulin mRNA sequences but will not determine the amount of translatable mRNA. Therefore, the biological activity of tubulin mRNA extracted from IFN-treated cells was analyzed for its ability to direct the synthesis of tubulin in a reticulocyte lysate cell-free system. The [^{35}S]methionine-labeled cell-free products were analyzed by electrophoresis on SDS-polyacrylamide slab gels. Figure 3A shows that the amount of synthesized tubulin is significantly higher when directed by mRNA extracted from cells exposed to IFN as compared with mRNA from control cells. The increase in synthesized tubulin is already evident with mRNA isolated from cells after 1 h exposure of the cells to IFN-α (compare lane 2 to lane 1) or after 4 h to IFN-β (lane 6). Neither IFN-α nor IFN-β were found to affect the amount of actin mRNA. Figure 3B shows the calculated ratio of the *in vitro* synthesized tubulin over that of actin as a function of time following IFN treatment. The maximal amount of translatable tubulin mRNA is obtained after exposure for 1 h to IFN-α and after exposure for 4 h to IFN-β. It should be noted that the increase in translatable tubulin mRNA following IFN treatment is lower than that detected by the hybridization method for tubulin mRNA sequences (Figure 1B) although the time course of the induction is very similar.

The labeled cell-free products were also analyzed by two-dimensional gel electrophoresis, which allows better identification of tubulin and the separation of the α- and β-tubulin subunits. In control Ramos cultures not exposed to IFN the amount of [^{35}S]methionine incorporated into α-tubulin is lower than that in β-tubulin (Figure 4A). The lower level of labeling of the α-subunit is in part due to its lower methionine content as compared with the β-subunit (Krauhs *et al.*, 1981; Ponstingl *et al.*, 1981). After treatment with IFN-β there is a gradual increase in the relative amount of labeled

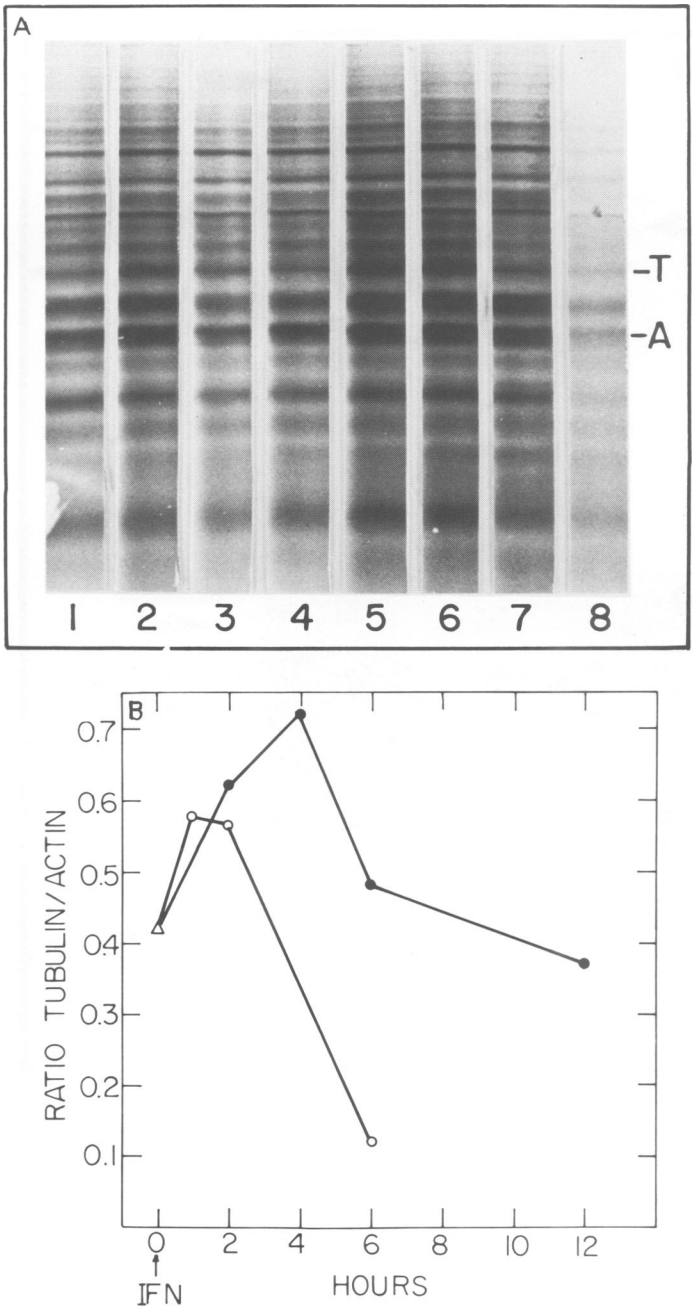

Fig. 3. SDS-polyacrylamide slab gel electrophoresis of cell-free translation products directed by mRNA isolated from IFN-treated cells. **A**: Poly(A)-containing RNA was isolated from Ramos cells treated for various periods of time with IFN-α or IFN-β, and translated in the reticulocyte lysate cell-free system. The [^{35}S]methionine-labeled translation products were subjected to SDS-polyacrylamide slab gel electrophoresis. The gel contained 10% acrylamide and 0.8% bis-acrylamide, and was 12 cm long. After Coomassie brilliant blue staining, the gel was dried and exposed to Agfa Curie X-ray film. **Lane 1**: no IFN; **lanes 2, 3, and 4**: exposure to IFN-α for 1, 2, and 6 h; **lanes 5, 6, 7, and 8**: exposure to IFN-β for 2, 4, 6, and 12 h; **Lane 9**: cell-free products of rat brain poly(A)-containing mRNA. **B**: The autoradiogram obtained in **A** was scanned and the ratio of tubulin to actin was calculated and is presented as a function of time of treatment with IFN-α (○—○) and IFN-β (●—●).

α-tubulin as compared with β-tubulin (Figure 4B). The maximal labelling is observed after 4 h of exposure to IFN-β (Figure 4C). After 12 h of IFN-β treatment there is a sharp decline in the amount of translatable mRNA coding for α-tubulin. These results suggest that the genes coding for α- and β-tubulin are regulated differently and independently in IFN-treated cells. In addition, we have observed a gradual induction of mRNA coding for a new protein with an apparent mol. wt. of 70 K (indicated by a bar in Figure 4).

The pattern of proteins labeled *in vivo* was also examined. Cultured Ramos cells were treated with IFN-α or IFN-β and labeled with [^{35}S]methionine for the last 20 min of the incubation period. The labeled proteins were extracted and analyzed by polyacrylamide gel electrophoresis. In these experiments a marked increase of the 70 K protein was also observed but under these conditions the effect on tubulin labeling was not very significant (data not shown). These findings could be due to the relatively large pre-existing pool of tubulin or to the conditions of the labeling experiments.

Discussion

The present studies show that treating lymphoblastoid Ramos cells of human origin with either IFN-α or IFN-β induces a marked increase in the amount of tubulin mRNA sequences. The IFN effect could arise from either enhanced transcription of tubulin genes or from an increase in the stability of the tubulin mRNA chains. A parallel induction of mRNA coding for HLA-A,B,C antigens in the same human cell line was previously shown (Fellous *et al.*, 1982). Contrary to the increase of both tubulin and HLA mRNA sequences, the amount of actin mRNA was not affected either by IFN-α or by IFN-β. The increase in tubulin mRNA is short lived and declines rapidly after 6 h of IFN treatment. This may explain the rather small effect of IFN on the *in vivo* labeling of tubulin.

The biological activity of tubulin mRNA extracted from IFN-treated cells was analyzed by its ability to direct the synthesis of tubulin in the reticulocyte lysate cell-free system. It appears that the maximal increase in translatable tubulin mRNA parallels the time course of induction of tubulin RNA sequences. The extent of induction of translatable tubulin mRNA was less pronounced than that for the induction of tubulin mRNA sequences, detected by hybridization with the tubulin cDNA probe. A similar difference in the two assays was also observed for HLA-A,B,C mRNAs in the same cells (Fellous *et al.*, 1982). Two-dimensional gel analysis indicated that the increase in translatable mRNA, following IFN treatment, is more pronounced for α-tubulin than β-tubulin mRNA chains and may indicate intrinsic differences between the corresponding genes. The 70 K protein, the induction of which is shown both in translatable mRNA and by the labeling of cells *in vivo*, corresponds probably to the 67–69 K protein described previously (Gupta *et al.*, 1979). The function of this protein which accumulates throughout the period of IFN treatment is unknown.

It has been suggested that the expression of tubulin is modulated by the state of assembly by microtubules (Ben-Ze'ev *et al.*, 1979; Cleveland *et al.*, 1981). Following colchicine treatment the amount of free tubulin subunits increases while tubulin-specific mRNA sequences decline and are lost from the cytoplasm with a half-life of <2 h (Cleveland *et al.*, 1981). Our data extend the colchicine effect to human cell lines; moreover they show that the colchicine-induced decline in tubulin mRNA is not reversed by IFN treatment. It could be argued that the opposing effects of IFN and colchicine on the levels of tubulin mRNA result from an effect on a common regulatory site. However, our results show that the simultaneous addition of colchicine and IFN

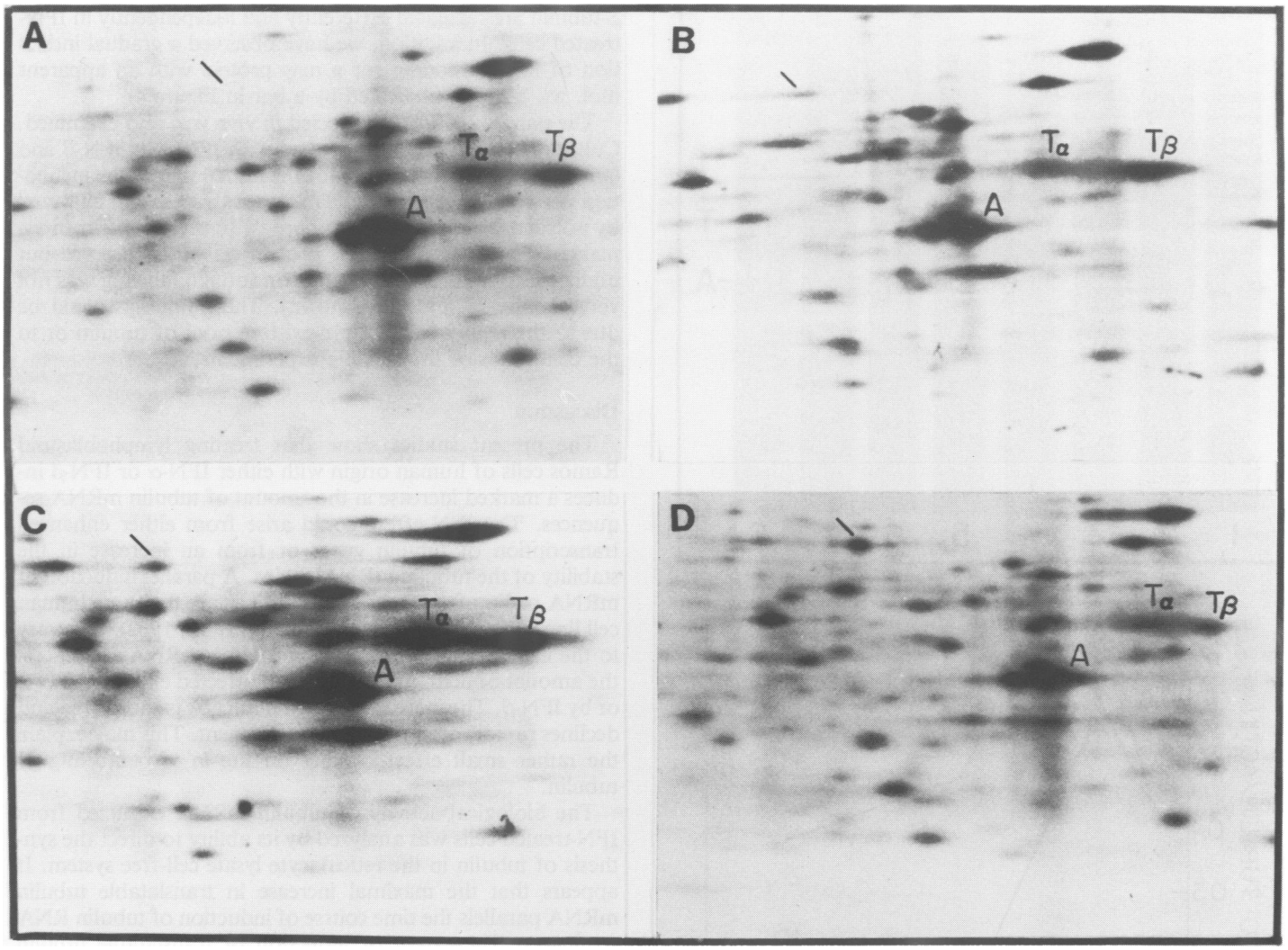

Fig. 4. Two-dimensional gel electrophoresis of cell-free translation products directed by mRNA isolated from cells treated with IFN-β. The [^{35}S]methionine-labeled cell-free products obtained in the experiment described in Figure 3 were analyzed by two-dimensional gel electrophoresis according to O'Farrel (1975). Tα and Tβ are α- and β-tubulin respectively, A is actin and the 70 K protein induced by IFN-β is indicated by the bar. **A**: no IFN; **B, C, D:** exposure to IFN-β for 2, 4, and 12 h, respectively.

does not result in an intermediate level of tubulin mRNA expected from the addition of each agent alone. An alternative hypothesis would suggest two different regulatory sites for colchicine and IFN action. This proposal is also in accord with the more pronounced effect of IFN-β on α-tubulin mRNA chains (Cleveland *et al.*, 1981). The finding that IFN affects differently the levels of α- and β-tubulin mRNAs and the observation that the two mRNA species are coded by different genes, suggests that they may be regulated independently (Cleveland *et al.*, 1980).

Materials and methods

Cells and IFNs

The Ramos human lymphoblastoid cell line was derived from a Burkitt lymphoma (Klein *et al.*, 1976). Cells were grown to the stationary phase (2 x 10^6 cells/ml) in RPMI 1640 medium containing 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, at 37°C. Human leucocyte-interferon (IFN-α), prepared from Sendai virus-infected chronic myelogenic leukemic cells, was obtained from Institut Merieux, Lyon, France (Rubinstein *et al.* 1981). Human IFN-β was prepared from poly(rI):(rC) superinduced FS11 fibroblasts at InterYeda, Rehovot, Israel (Knight and Fahey, 1981; Weissenbach *et al.*, 1979). In all the experiments a pure preparation of IFN-α was used which was purified by repeated h.p.l.c. (Rubinstein *et al.*, 1981). Its specific activity was 5 x 10^8 units/mg protein on human cells; it was pure on SDS-polyacrylamide gel electrophoresis. Fibroblast IFN-β was purified (Knight and Fahey, 1981) to $>10^8$ units/mg protein. Cells were treated with IFN-α and IFN-β at 700 U/ml and 200 U/ml, respectively.

Analysis of tubulin mRNA levels

Messenger RNA was isolated from 2 x 10^8 cells as previously reported (Fellous *et al.*, 1982). The poly(A)-containing RNA was fractionated by electrophoresis in 1.2% formaldehyde-agarose slab gels. RNA samples of 2–5 μg were heated to 60°C for 10 min in a total volume of 20 μl containing 50% formamide, 6% formaldehyde, 20 mM morpholinopropanesulfonic acid, pH 7.0; 5 mM Na acetate, and 1 mM EDTA. After quick cooling, 6 μl of 50% glycerol containing 0.2% bromophenol blue was added and the samples were run at 250 V until the dye marker migrated 13 cm from the origin. Following electrophoresis, the gel was soaked for 30 min in 20 x SSC (1 x SSC contains 0.15 M NaCl, 0.015 M Na citrate) and blotted during 12–15 h with 10 x SSC onto nitrocellulose filters presoaked in 10 x SSC for 20 min at room temperature. The filters were baked for 4–6 h at 80°C and then prehybridized overnight at 42°C with 5 ml of 50% formamide, 5 x SSC, 0.1% Ficoll, 0.1% bovine serum albumin, 0.1% polyvinylpyrolidone, 0.1% sodium pyrophosphate, 0.1% SDS, and 250 μg/ml sonicated and denatured salmon sperm DNA. Hybridization was carried out for 20 h at 42°C under the same conditions but with 10^6 c.p.m./ml of [^{32}P]nick-translated pT25 α-tubulin cDNA probe and pA72 β-actin probe (Ginzburg *et al.*, 1980; Nudel *et al.*, 1982). After hybridization, the blots were washed with 0.1 x SSC and 0.1% SDS first at room temperature and then twice for 15 min each at 50°C, dried and exposed to Agfa Curix RP X-ray films with intensifying screens at −70°C. Quantitative determinations of the amount of hybridized mRNA

were performed by scanning the autoradiograms at 560 nm with a Gilford 2400 S spectrophotometer.

In vitro protein synthesis

Poly(A)-containing RNA was translated in a nuclease-treated rabbit reticulocyte lysate cell-free system containing ^{35}S-labeled methionine (Pelham and Jackson, 1976). The cell-free products were analyzed by SDS-polyacrylamide slab gel electrophoresis (Laemmli, 1970), or by two-dimensional gel electrophoresis (O'Farrel, 1975).

In vivo incorporation of [^{35}S]methionine into proteins

Ramos cells were preincubated for 3 h in methionine-free medium prior to the addition of IFN. IFN-α (700 U/ml) or IFN-β (200 U/ml) were added to the medium for the different times. [^{35}S]methionine (2000 Ci/mM) was added (25 μCi/ml) to the medium for the last 20 min of incubation. The labeled cells were then collected by centrifugation at 1200 *g* for 7 min, washed with phosphate buffered saline and lysed in a lysis buffer containing 10 mM Tris-HCl, pH 7.3; 1.5 mM $MgCl_2$ and 0.5% Nonidet P40. Cell suspensions were kept on ice for 30 min and the cell lysate was centrifuged for 5 min at 12 000 *g* in an Eppendorf centrifuge and the supernatant collected. The amount of radioactivity incorporated into proteins was determined by precipitation in trichloroacetic acid and the synthesized proteins were analyzed on SDS-polyacrylamide gels.

Treatment of cells with colchicine

Colchicine at a concentration of 10^{-5} M (Sigma) was added to the cell medium for a total period of incubation of 6 h.

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