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Interferon-induced 56,000 Mr protein and its mRNA in human cells: molecular cloning and partial sequence of the cDNA

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ABSTRACT

Treatment of responsive cells by interferons (IFNs) induces within a few hours a rise in the concentration of several proteins and mRNAs. In order to characterize these IFN-induced mRNA species, we have cloned in E. coli the cDNA made from a 17-18S poly(A)$^+$ RNA of human fibroblastoid cells (SV80) treated with IFN-β. We describe here a pBR322 recombinant plasmid (C56) which contains a 400 bp cDNA insert corresponding to a 18S mRNA species newly induced by IFN. The C56 mRNA codes for a 56,000 dalton protein easily detectable by hybridization-translation experiments. The sequence of 66 of the carboxy-terminal amino-acids of the protein can be deduced from the cDNA sequence. IFNs-α, β or γ are able to activate the expression of this gene in human fibroblasts as well as lymphoblastoid cells. The mRNA is not detectable without IFN; it reaches maximum levels (0.1% of the total poly(A)$^+$ RNA) within 4-8 hrs and decreases after 16 hrs.

INTRODUCTION

Interferons (IFNs) are a family of proteins which, after interaction with surface receptors of responsive cells, produce in these cells an antiviral state (1-4), cell-growth inhibition (5,6), or cell-mediated immune responses (7,8). The effects of IFNs are accompanied by changes in the level of specific enzymes (1-4), in surface membrane proteins and lipid composition (9-11), and in the morphology of the cytoskeleton (11). The mechanisms by which IFNs induce these biochemical changes, and the precise role these changes play in the biological function of IFNs, are still under investigation.

Establishment of the antiviral state by IFNs, seems to require the de novo synthesis of cellular mRNAs and proteins, since it is blocked by inhibitors of transcription and translation (12,13) and by other alterations of nuclear functions (14). In the case of the enzyme (2'-5') oligo A synthetase (15), which probably plays an important role in the antiviral and anticellular effects of IFNs (1-4,16,17) and whose activity can be

multiplied by more than a hundred-fold in IFN-treated cells, it could be shown directly that the enzyme increase is mediated by a rise in the level of its specific mRNA (18). The elevation in HLA-A,B,C and β_2-microglobulin antigens on the surface of IFN-treated cells, is also mediated by a marked increase in their mRNAs (19,20). IFNs probably stimulate the expression of a number of other specific mRNAs, since the in vitro translation products of RNA from IFN-treated cells contain several yet unidentified proteins, which appear to be coordinately controlled at the mRNA level by IFNs (21,22). Molecular cloning of the genes corresponding to the various IFN-induced mRNA species, would help to understand how their expression is regulated. Availability of cloned cDNA probes would also allow the direct quantitation of IFN-induced mRNAs and the comparison of the response of specific cells to the various species of IFN.

The biological assay for the (2'-5') oligo A synthetase mRNA by translation in frog oocytes (18) indicated that the 17-18S fractions of human cell RNAs contain the major part of this IFN-induced mRNA activity (23). Other reports suggest that several IFN-induced mRNAs are in the same size range (21). We, therefore, used 17-18S poly(A)$^+$ RNA from IFN-treated human SV80 cells to prepare a cDNA library in E. coli. This library was found to contain about 1% of IFN-induced cDNA clones, one of them harbouring a cDNA for the (2'-5') oligo A synthetase (Merlin, et al. submitted). We describe here another cDNA clone, C56, which contains the partial sequence of a strongly IFN-induced mRNA coding for a 56,000 Mr protein. An IFN-induced protein of this size was observed before by several authors in human fibroblastic cells labeled by ^{35}S-methionine (24-25), or among the in vitro translation products of 16S mRNA from human fibroblasts (26). The C56 mRNA is induced in SV80 cells from undetectable levels to about 0.1% of the poly(A)$^+$ RNA. It is also induced in human lymphoblastoid cells and is regulated by all three types of IFN-α, β and γ.

MATERIALS AND METHODS

Cell cultures and IFNs.

Human SV80 cells (SV40-transformed fibroblastoid cells)(27) were grown to confluent monolayers in Dulbecco's modified Eagle's medium with 10% calf serum in 8% CO_2. Foreskin diploid fibroblasts strain FS11 (28) were grown to confluency in Minimal Eagle's medium, 10% fetal calf serum, in 5% CO_2. Burkitt's lymphoblastoid cell lines Namalva (29), Daudi (30) and Ramos (31) were grown in suspension to 1-1.5x10^6 cells/ml in RPMI 1640 medium, 10% fetal

calf serum in 5% CO_2. All cultures were at 37°C, in the presence of 100 units/ml penicillin and 100 µg streptomycin. Human IFN-β_1 was obtained as described (28) from poly (rI):(rC)-superinduced FS11 fibroblasts cultures (InterYeda Ltd, Israel) and purified (32) to 10^7 units/mg protein. Cell treatment with IFN-β_1 was with 200 units/ml for 12 hours, unless otherwise indicated. Human IFN-α, prepared from Sendai virus-infected chronic myelogenous leukemia cells (Institut Merieux, France) and purified (33) to 10^7 units/mg was a gift of Dr. M. Rubinstein. Human IFN-γ, prepared and purified as described (34) to 10^5 units/mg was a gift from Dr.D. Wallach.

mRNA isolation and analysis.

Total cell RNA was prepared from cultures of 2-4x10^8 cells by the LiCl-urea method (35), and poly(A)$^+$ RNA was obtained as described (19). Electrophoretic analysis was done on 1.3% agarose gels in 6% formaldehyde, followed by blotting onto nitrocellulose and hybridization to nick-translated plasmid DNA as previously described (19). For S_1 analysis, total RNA was prepared from 2-5x10^6 cells by SDS-phenol extraction at 60°C (36), and 25-50 µg RNA were mixed with 10^4 cpm of a 300 bp Hind 3 - Pst 1 fragment of plasmid C56 DNA (fragment I, Fig. 4), ^{32}P-end labeled in the Hind 3 site. The mixtures, in 25-50 µl of 0.4 M NaCl, 40 mM Pipes buffer pH 6.4, 1 mM EDTA, 80% recrystallized formamide, were heated to 65°C for 15 minutes and then to 46°C for 16 hours. After freezing, 200-500 µl of S_1 buffer (0.3 M NaCl, 30 mM Na Acetate buffer pH 4.5, 3 mM ZnCl2) and 600 units/ml S_1 nuclease (PL Biochemicals) were added. The samples were incubated 30 minutes at 37°C, phenol extracted, precipitated with ethanol and analyzed by electrophoresis on 13 cm 6% polyacrylamide gels in 8 M urea (36).

For cloning, the poly(A)$^+$ RNA from SV80 cells treated with IFN-β_1 was size-fractionated by preparative electrophoresis on columns of 1.5% agarose-6M urea in 25 mM Na Citrate buffer pH 3.5 (37). The 17-18S RNA fractions, containing the (2'-5') oligo A synthetase mRNA activity were isolated as detailed elsewhere (Merlin et al., submitted), purified on oligo (dT)-cellulose and used for cDNA synthesis.

cDNA synthesis and cloning in E. coli.

The 17-18S RNA (2 µg) and 2 µg oligo (dT) 12-18 (PL Biochemicals) were heated 1 minute at 90°C in 60 µl water, rapidly cooled to 0°C, supplemented with salts to a final concentration of 50 mM Tris-HCl pH 8.3, 10 mM $MgCl_2$, 75 mM KCl, and left 5 minutes at 42°C before adding 1 mM dithiothreitol 1 mM each dATP, dGTP, dTTP, 0.5 mM dCTP, 20 µCi ^{32}P-α-dCTP (300 Ci/mmol, Amersham), 4 mM Na pyrophosphate and 20 units Reverse Transcriptase

(Dr. J. Beard) in 100 µl final volume. After 45 min at 42°C, 10 mM EDTA and 0.2% SDS were added, and the reaction was extracted with phenol-chloroform, treated with 0.3 N NaOH for 2 hours at 52°C and neutralized. The cDNA was filtered on Sephadex G75, ethanol precipitated and tailed by dATP with Terminal Transferase (Ratliff) (39) to allow priming of the second strand with oligo (dT) (38). The double stranded (ds) cDNA was synthesized with Reverse Transcriptase as above, for 2 hours at 42°C, but without the radioactive nucleotide and pyrophosphate. To insure blunt ends, the ds cDNA was incubated with E. coli DNA polymerase I large fragment (Klenow enzyme; Boehringer) first in 20 mM Tris-HCl pH 8, 75 mM KCl, 5 mM $MgCl_2$, 1 mM DTT for 5 min at 37°C (trimming reaction) and then under the conditions of the filling-in reaction (40). The heaviest ds cDNA fractions separated on a 5-20% sucrose gradient (41) were tailed with dCTP, and annealed with equimolar amounts of Pst 1-cut pBR322 tailed with dGTP. About 7 ng of the vector in 5 µl, were mixed with 100 µl of frozen, CaCl2-treated (42), E. coli MM294 (43), for 30 min at 0°C, then 5 min at 37°C, and after adding 2 ml of LB broth for 2 hours at 37°C. After plating on LB-agar with 10 µg/ml tetracycline, 1.4×10^5 tetracycline-resistant, ampicillin-sensitive, colonies were obtained per µg of recombinant plasmid, versus 3×10^6 colonies per µg pBR322 DNA. Colonies were grown on nitrocellulose filters, fixed and the DNA denatured as described (44). For colony hybridization (44) we used ^{32}P-labeled cDNA probes (2×10^8 cpm/µg), reverse transcribed from 17-18S RNA of IFN-induced SV80 cells or from total poly(A)$^+$ RNA from untreated cells.

Hybridization-translation experiments with cloned cDNA.

Recombinant plasmid DNAs, purified by CsCl-ethidium bromide centrifugation (45), were cut with Eco Rl, and 5-30 µg were dissolved in 20 µl of Tris-HCl pH 7.5, 1 mM EDTA, heated for 10 min to 100°C, quickly cooled and spotted in 1 µl aliquots on 4 mm diameter discs of nitrocellulose (Schleicher and Schüll BA 85). The filters were blotted three times for 1 minute on Whatman 3MM paper wetted first with 0.5 N NaOH, 1.5 M NaCl, then with 1.5 M Tris-HCl pH 7.5 in 2 x SSC (0.6 M NaCl, 30 mM Na Citrate) and finally with 2 x SSC. After drying 1 hour at room temperature, the filters were baked for 2 hours at 80°C in vacuo (B. Paterson, private communication). Prior to hybridization, the filters were incubated for 2 hours at 37°C in sterile siliconized glass vials of 6 mm diameter, with 50% recrystallized formamide, 20 mM Pipes buffer pH 6.4, 0.75 M NaCl and 1 mM EDTA (Buffer A). For hybridization, several filters were incubated together with poly(A)$^+$ RNA from SV80 cells using 10 µg RNA per filter in 30 µl Buffer A, for 16-24

hours at 37°C. The filters were transferred to a 50 ml sterile plastic tube and washed (46) by Vortex agitation in 1ml/per filter of each prewarmed solution: first, once for 5 min at 37°C in Buffer A, then without incubation once in Buffer A at 37°C, then once in 20 mM Tris-HCl pH 7.5, 0.15 M NaCl, 1 mM EDTA, 0.5% SDS (Buffer B) at room temperature, and 3 times in Buffer B at 52°C, finally in 10 mM Tris-HCl pH 7.5, 1 mM EDTA (Buffer C) 4 times at 52°C. Each filter was transferred to a siliconized Eppendorf microtube and washed with 1 ml Buffer C at 52°C. RNA elution from the filter was done at 96°C for 2 minutes with 0.3 ml Buffer C containing 40 µg/ml rabbit liver tRNA, and after quick cooling the RNA was ethanol precipitated twice and dissolved in 2 µl water. Each selected RNA (1 µl) was added to a 12.5 µl translation reaction containing a rabbit reticulocyte lysate treated with micrococcal nuclease, and protein synthesis was carried out as previously described (28). The translation products were analyzed by electrophoresis on a SDS-12% polyacrylamide gel (47), followed by autoradiography on Agfa Curix films.

DNA restriction mapping and sequencing.

Purified plasmid DNA (45) was cut with restriction enzymes (Biolabs or Boehringer) under the conditions prescribed by the manufacturer. DNA fragments were separated by electrophoresis on 1-2% agarose (Seakem) slab gels. DNA sequencing was done by the chemical degradation method of Maxam and Gilbert (48).

RESULTS

Isolation of cDNA clones from IFN-induced human mRNA.

The 17-18S poly(A)$^+$ RNA fractions from IFN-treated SV80 cells, which contain the (2'-5') oligo A synthetase mRNA (18,23), and probably several other IFN-induced mRNAs (21,26), was used to prepare ds cDNA, which was inserted in the Pst 1 site of pBR322. After cloning in *E. coli* MM294, the bacterial colonies were screened by differential hybridization. Two replicas of the clones grown on nitrocellulose filters were hybridized either to ^{32}P-cDNA from 17-18S poly(A)$^+$ RNA of IFN-induced cells, or to ^{32}P-cDNA from total poly(A)$^+$ RNA of non-induced cells. This procedure was chosen because it decreases the risk to miss induced cDNA clones corresponding to partially induced sequences, although at the price of detecting false positive colonies. Out of 3500 clones screened, about 40% hybridized strongly to the "induced" probe, and 8% gave a clear differential signal, hybridizing preferentially or uniquely to the induced probe (Fig. 1). The final screening was done on this latter group of clones by hybridizing the

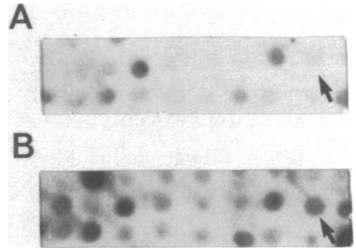

FIGURE 1. Differential hybridization of cDNA recombinant clones. Colonies were hybridized on nitrocellulose filters with ^{32}P-labeled cDNA probes prepared from 17-18S poly(A)$^+$ RNA of IFN-treated SV80 cells (B), or from total poly(A)$^+$ RNA of non-induced cells (A). 2x10^5 cpm of probe were used per filter. Exposure was for 24 h at -70°C with intensifying screen. The arrows indicate clones which hybridized only with the probe made from induced mRNA.

nick-translated plasmid DNAs to RNA blots of IFN-induced and of control cell RNA. In a typical experiment, one clone out of 6 corresponded to an IFN-induced RNA, indicating that the cDNA library as a whole contains 1-2% of IFN-induced sequences.

One clone designated C56 showed a particularly strong differential hybridization. The C56 cDNA labeled by nick-translation was hybridized to nitrocellulose blots of poly(A)$^+$ RNA fractionated by electrophoresis under denaturing conditions. Figure 2 shows that the C56 cDNA hybridizes to a unique band of 18S RNA present in IFN-induced RNA, but completely absent from control cell RNA, even after prolonged exposure of the autoradiography. The same experiment shows that a HLA-A,B,C cDNA probe (50), labeled to the same specific activity, gives a clear signal with non-induced RNA. In the case of HLA-A,B,C RNA, IFN treatment produces a quantitative increase in the level of the mRNA (19), but not an absolute induction as for the C56 mRNA. In comparison, tubulin cDNA (51) hybridized with both induced and non-induced control cell RNA, with almost the same efficiency (Fig. 2). The C56 cDNA appears, therefore, to represent an RNA species that is most strongly regulated by IFN treatment.

Characterization of the protein product of IFN-induced C56 mRNA.

The mRNAs selected by hybridization to C56 cDNA filters (as described in Methods), were translated in a rabbit reticulocyte *in vitro* system and the [^{35}S]-methionine-labeled products were analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 3). A predominant 56,000 Mr protein is translated from C56-selected mRNA (Fig. 3A, lane 5, and B, lane 3), but not from RNA

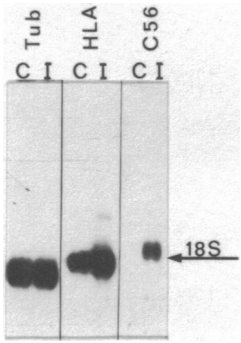

FIGURE 2. Characterization by hybridization to RNA blots of recombinant plasmid clone C56, harbouring cDNA for an IFN-induced mRNA. Poly(A)$^+$ RNA from IFN-treated SV80 cells (I) or from non-treated cells (C), 7 µg, were electrophoresed on agarose gels and after blotting to nitrocellulose were hybridized to nick-translated [^{32}P]-plasmid DNA of either the C56 clone, a human HLA cDNA clone (50) or a rat tubulin cDNA clone (49). Exposure was for 48 h. Position of radioactive 18S ribosomal RNA marker is indicated.

eluted off the pBR322 DNA filters (Fig. 3A, lane 2, and B lane 2) or other unrelated plasmid DNAs. The background of proteins translated from non-specifically adsorbed RNAs was low, and the identification of the 56,000 Mr protein as the translation product of the mRNA selected by the C56 cDNA clone

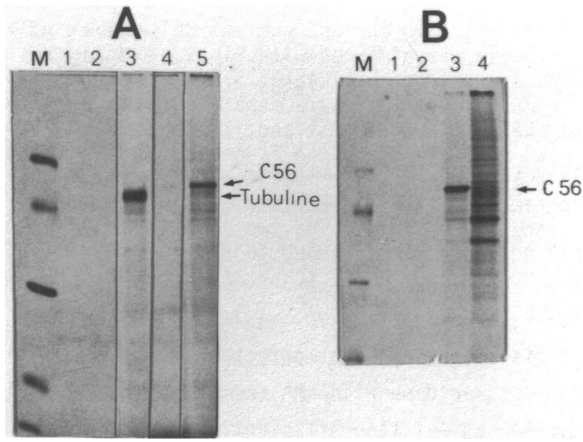

FIGURE 3. Translation products of mRNA selected on plasmid clone C56 DNA. Plasmid DNAs immobilized on nitrocellular filters were used to select mRNA from SV80 cells, either IFN-treated or non-treated by IFN. The mRNA was translated in reticulocyte lysates with ^{35}S-methionine (28) and the reactions analyzed on SDS-polyacrylamide gels. Two different experiments are shown. A: lane 1 - no mRNA, lane 2 - pBR322 selected IFN-treated mRNA (30 µg DNA/filter),lanes 3-4 - non-treated RNA selected, respectively, by tubulin cDNA (6 µg/filter) and C56 DNA (30 µg DNA/filter), lane 5 - C56 DNA selected IFN-treated mRNA. B: lane 1 - no mRNA, lane 2 - pBR322 selected IFN-treated mRNA, lane 3 - C56 cDNA - selected IFN-treated mRNA, lane 4 - total IFN-treated poly(A)$^+$ mRNA, lane M - ^{14}C-labeled (New England Nuclear) protein molecular weight markers. 69,000 (albumin), 46,000 (ovalbumine), 30,000 (carbonic anhydrase), 18,300 (lactoglobulin), 12,300 (cytochrome C).

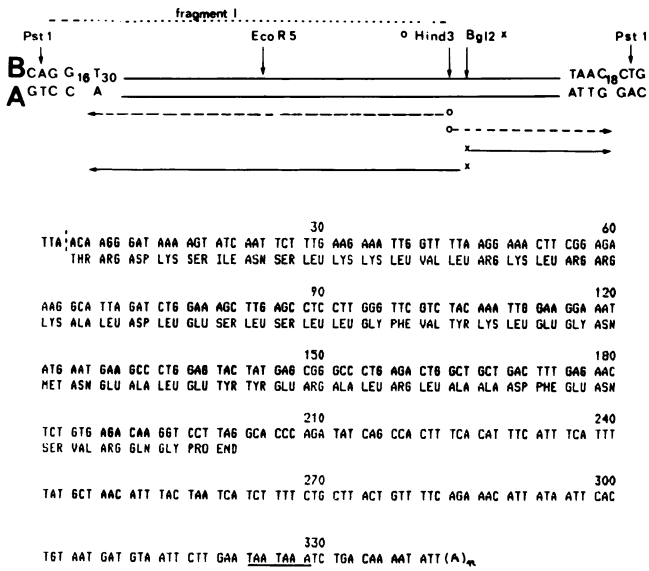

Figure 4. Partial restriction map and nucleotide sequence of the C56 450 bp insert. The C56 plasmid was digested with Hind 3, end-labeled with α-[^{32}P]-dCTP by the DNA polymerase I-large fragment(Klenow enzyme, Boehringer) and the Hind 3 - Pst 1 fragments were separated on a 1% agarose gel. In order to sequence the complementary strand, the plasmid was 5'-labeled at the Bgl 2 site with γ-[^{32}P]-ATP by the T_4-polynucleotide kinase (Biolabs) and the Bgl 2 - Pst 1 fragments were isolated. Sequencing was made by the Maxam and Gilbert technique (48). Sequence of coding strand A (right to left) is shown in the lower panel. The two first thymidylic residues of the sequence of strand A probably correspond to the AT tail as indicated in the upper diagram.

is unambiguous. This protein was not detectable when the hybridization-translation experiment was done with RNA from control cells (Fig. 3A, lane 4), although tubulin mRNA was selected efficiently by tubulin cDNA filters from the same control RNA preparations (Fig. 3A, lane 3). These experiments confirm that the C56 mRNA is induced by IFN and that is is actively translated. The size of the C56 mRNA (about 2,000 nucleotides) is large enough to encode a 56,000 Mr protein.

Sequence of the C56 450 bp cDNA insert.

Both strands of the cDNA insert were sequenced by the method of Maxam and Gilbert (48), using the 3' and 5' end labeled restriction fragments shown in Fig. 4. All the restriction sites which could be deduced from the nucleotide sequence, were verified to be present. Taking into account the

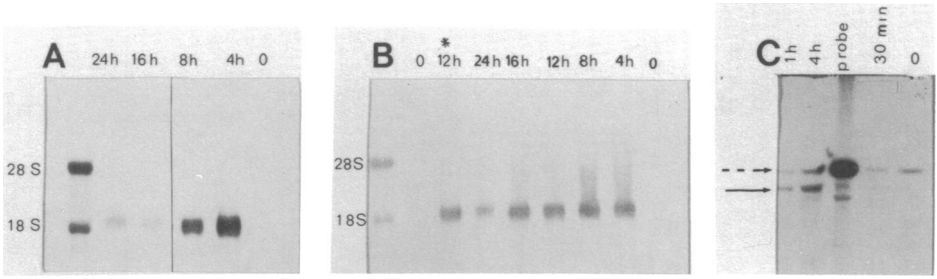

FIGURE 5. Time course of the induction of C56 mRNA by IFN.
A). Poly(A)$^+$ RNA 7 µg from Namalva cells treated with IFN-α 1000 U/ml for the indicated times were electrophoresed on agarose gels and, after blotting, were hybridized with nick-translated [^{32}P]-C56 plasmid DNA.
B). Poly(A)$^+$ RNA,7 µg from SV80 cells treated with 200 U/ml IFN-β for the indicated times. The asterisk indicates a RNA sample from cells treated with IFB-β_1 purified on monoclonal antibody column ($2x10^8$ U/mg)(52).
C). Poly(A)$^+$ RNA, 1 µg, from SV80 cells treated as in (B) was hybridized in liquid with 3' end-labelled fragment I of C56 DNA (see Fig. 4). The hybrids were treated with S_1-nuclease and analyzed on denaturing gels (see Methods). the mRNA-hybridized probe (——>) is shorter than the self-reassociated probe (---->).

AT and GC tails, the actual insert sequence was 340 bp long (Fig. 4). An open reading frame of 195 nucleotides was found on strand A, from position 4 to 198 in Fig. 4 (the two thymidylic residues in position 1 and 2 being possibly still part of the AT tails). The open frame is terminated by the stop codon UAG, which is followed 121 nucleotides downstream by the hexanucleotide AAUAAA, considered as a signal for polyadenylation (51) and actually located 20 nucleotides before the poly(A) tail. When end-labeled fragments of the cDNA insert were hybridized in liquid with IFN-induced mRNA, only the fragments labeled in strand B could be protected from S_1 nuclease digestion, confirming that the A strand corresponds to the mRNA strand (not shown). The sequence allows to deduce 65 aminoacids of the presumed carboxy-terminal end of the 56,000 Mr protein, and indicates the presence of a region rich in basic aminoacids from nucleotides 31 to 63 of the C56 cDNA clone.

Time course of C56 mRNA induction by IFN.

Human cell lines were analysed at different times after exposure to IFN, for the presence of RNA hybridizing on electrophoretic blots to C56 cDNA. In the fibroblastoid cell line SV80, IFN-β causes a rapid induction of C56 mRNA, from undetectable levels at time zero to a maximum level at 4 hours (Fig. 5B). The C56 mRNA decreases again in these cells from 16 to 24 hours after exposure to IFN. A preparation of IFN-β_1 purified to over $2.0x10^8$ U/mg

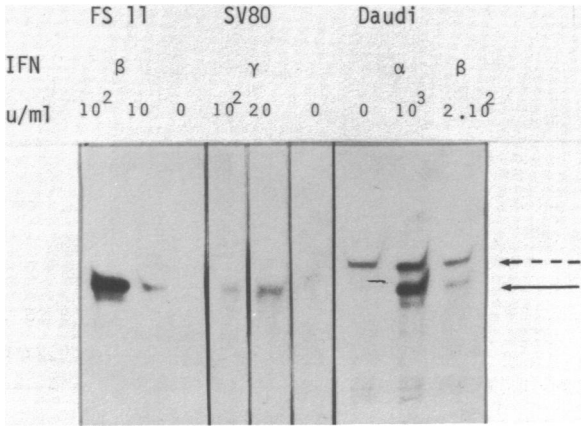

Figure 6. C56-mRNA in various human cell-lines treated with IFN-α,β, or γ. Cells ($5x10^6$) were treated for 12 hours with indicated concentrations of IFNs. Total cell RNA was extracted, hybridized to fragment I of C56 DNA and analyzed by S_1-nuclease as described in Methods and Fig. 4C. (———>) mRNA-hybridized probe; (----->) self-reassociated probe.

protein by affinity chromatography on an anti-IFN-β_1 monoclonal antibody column (52), induced the C56 mRNA in SV80 cells as efficiently as the cruder Blue-Sepharose-purified (32) preparation (Fig. 5B, asterisk). A similar rapid induction of C56 mRNA was found in human lymphoblastoid Namalva cells treated with IFN-α (Fig. 5A). Maximum C56 mRNA levels were again seen at 4 hours, followed by a decrease from 8 to 16 hours after IFN treatment.

To analyze in more details the early induction period, hybridization of poly(A)$^+$ RNA from SV80 cells to the C56 cDNA insert was analysed by the S_1 nuclease procedure (36). C56 RNA was detected at 1 hour after treatment with 200 U/ml IFN-β (Fig. 5C), but not yet at 30 minutes. A large increase was seen from 1 to 4 hours after IFN treatment. From this S_1 analysis, we could also calculate that at 4 or 12 hours after IFN, the C56 mRNA represents 0.1% of the total mRNA of SV80 cells. This level appears to be higher than that of the (2'-5') oligo A synthetase mRNA, which also accumulated more slowly than the C56 mRNA, reaching a maximum level between 8 and 12 hours of IFN treatment (Merlin et al, submitted).

Induction of C56 mRNA by IFN-α, β and γ in various cells.

We used several combinations of human cells and IFN species to examine whether the C56 mRNA induction was a general phenomenon. The experiment in Fig. 6 shows that IFN-β induces the C56 mRNA in human diploid fibroblasts FS11. A dose-dependent response was observed: 10 U/ml of IFN-β (antiviral units measured on FS11 cells) were sufficient to give a detectable level of

C56 RNA but 100 U/ml gave a much higher response. The same dose-dependence was found for SV80 cells (not shown). We next examined the ability of type II IFN-γ to induce the C56 mRNA. SV80 cells were treated for 12 hours with 20 or 100 U/ml of IFN-γ (antiviral units measured on Wish cells). A clear induction of the C56 mRNA was detected (Fig. 6), but the induction was not dose-dependent. Induction of C56 RNA was, however, not seen at the very low IFN-γ concentrations which we found previously (34) to induce the HLA mRNAs (results not shown).

Human lymphoblastoid Daudi cells are known to be more sensitive to the anti-growth effect of IFN than most other cells (53). We, therefore, examined the induction of the C56 mRNA in Daudi cells. Figure 6 shows that both IFN-α and IFN-β induced the C56 mRNA in these cells. The level of C56 mRNA induced by 200 U/ml of IFN-β in Daudi cells was, however, not higher than in fibroblasts FS11 (Fig. 6) or in SV80 cells (Fig. 5).

DISCUSSION

A cDNA library was prepared from 17-18S poly(A)$^+$ RNA from human fibroblastoid SV80 cells, treated for 12 hours with 200 U/ml IFN-β_1 and this library was screened for the presence of cDNA clones corresponding to IFN-induced sequences. One of the recombinant plasmid, C56, contains part of the sequence of an 18S mRNA species, undetectable in control cells, and whose level increases to 0.1% of the total poly(A)$^+$ RNA after 4 hours of IFN treatment. The major translation product of the mRNA selected by the cloned C56 cDNA, is a 56,000 Mr protein. Sequencing of the 400 bp cDNA insert allows to predict 66 aminoacids of the carboxyterminal end of the protein. The C56 mRNA is induced in various human cells, including lymphoblastoid and fibroblastic cell lines. It is induced by all three species of human IFN, α, β and γ. A nearly homogenous preparation of IFN-β_1, purified on a monoclonal antibody column, was shown to induce the C56 mRNA. The induction of this mRNA appears, therefore, as a specific and general characteristic of IFN action.

The important question is whether the 56,000 Mr protein product of the C56 mRNA is present in IFN-treated cells. Rubin and Gupta (24,25) have described the induction of a 56,000 Mr protein by IFN-α, β and γ, which is maximally synthesized in human fibroblasts 4-5 hours after IFN treatment. Although the translation product of the C56 mRNA could be processed *in vivo* to a protein of a different size, it is tempting to identify the C56 mRNA as the template for the *in vivo* labeled 56,000 Mr protein. Preliminary results

actually show that the in vitro translation product of mRNA selected on the C56 cDNA and the in vivo labeled 56,000 Mr protein of SV80 cells, migrate to the same position upon 2-dimensional gel electrophoresis (to be published). The possible function of the 56,000 Mr protein has, unfortunately, not been identified. In extracts of chicken fibroblasts, a protein of similar size was reported to copurify with the (2'-5') oligo A synthetase activity (54). When, however, we injected the mRNA selected on C56 cDNA to xenopus oocytes, under conditions where the (2'-5') oligo A synthetase mRNA is translated into active enzyme (18), no synthetase activity was observed. Furthermore, the C56 cDNA does not appear to share common sequences with the cDNA clone of the (2'-5') oligo A synthetase mRNA that we recently characterized (Merlin et al, submitted). The C56 mRNA appears, therefore, unrelated to the IFN-induced (2'-5') oligo A synthetase. The size of the 56,000 Mr protein is close to that of tubulins, and tubulin mRNA was reported to be increased somewhat by IFN treatment (55). No sequence homology or cross-hybridization was, however, found with tubulin cDNA (49) in our experiments. The identity and function of the C56 mRNA and protein, which are strongly induced by IFNs, remains to be elucidated. Isolation of the human gene coding for the C56 mRNA and its expression in heterologous cells could be the method of choice to identify the protein and study its activity.

Recently, Samanta et al. (56) reported the isolation of a cDNA clone containing part of a 17S mRNA sequence induced by IFN in mouse ascites cells, and which appears to code for a 56,000 Mr protein. The relation of this mouse mRNA to the human C56 mRNA described here will be interesting to investigate. The C56 mRNA starts to accumulate within 1 hour after exposure of human cells to IFN, and reaches a maximum in 4 hours. At later times, the level of C56 mRNA decreases again even if the cells are continuously exposed to IFN. The IFN-induced mouse 17S mRNA (56) also decreases after 12 hours of IFN treatment. This decrease could result from a reduction in the synthesis of the mRNA or from an increased degradation. It is interesting to note that Colonno and Pang (26) found that the association of IFN-induced mRNAs with polysomes decreases in human cells from 8 to 24 hours after IFN treatment.

The C56 mRNA is not detectable in various human cells not treated with IFN. Its strong increase shortly after IFN addition could be used to monitor the response of human tissues to IFN administered in vivo by various routes. The availability of the cloned C56 cDNA probe should allow to develop a rapid hybridization test for this purpose.

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REFERENCES

1. Revel, M. (1979) in Interferons I, Gresser, I. Ed., pp.101-163 Academic Press, New York
2. Baglioni, C. (1979) Cell 17, 255-264
3. Lengyel, P. (1982) in Ann. Review of Biochemistry 52 252-282
4. Sen, G. (1982) in Progress in Nucleic Acid Research and Molecular Biology, Cohn, W.E. Ed., Vol. 27, pp. 105-156 Academic Press, New York
5. Gresser, I. and Tovey, M. (1978) Biochim.Biophys.Acta 516 231-247
6. Taylor-Papadimitriou, J. (1980) in Interferon II, Gresser, I. Ed., pp. 13-46 Academic Press, New York
7. Bloom, B., Schneck, J., Rager-Zisman, B., Quan, P.C., Neighbour, A., Minato, N., Reid, L. and Rosen, O. (1982) in Interferons, UCLA Symposium on Molecular and Cellular Biology, Merigan, T. and Friedman, R. Eds., Vol. XXV, pp. 269-278 Academic Press, New York
8. DeMaeyer, E. and DeMaeyer-Guignard, J. (1981) CRC Crit. Rev.Immunol. 2, 167-188
9. Fellous, M., Kamoun, M., Gresser, I. and Bono, R. (1979) Eur.J.Immunol. 9, 446-452
10. Chandrabose, K., Cuatrecasas, P. and Pottathil, R. (1981) Biochem. Biophys. Res.Commun. 96, 661-668
11. Tamm, I., Wang, E., Landsberger, F. and Pfeffer, L. (1982) in Interferons, UCLA Symposia on Molecular and Cellular Biology, Merigan, T. and Friedman, R. Eds., Vol. XXV, pp. 159-179 Academic Press, New York
12. Taylor, J. (1964) Biochem. Biophys. Res.Commun. 14, 447-451
13. Stewart, W. (1979) The Interferon System pp. 196-222 (Springer-Verlag)
14. Young, C., Pringle, C. and Follett, E. (1975) J.Virol. 15, 428-441
15. Hovanessian, A., Brown, R. and Kerr, I. (1977) Nature 268, 537-540
16. Nilsen, T. and Baglioni, C. (1979) Proc.Natl.Acad.Sci. USA 76, 2600-2604
17. Kimchi, A., Shure, H. and Revel, M. (1979) Nature 282, 849-851
18. Shulman, L. and Revel, M. (1980) Nature 287, 98-100
19. Fellous, M., Nir, U., Wallach, D., Merlin, G., Rubinstein, M. and Revel, M. (1982) Proc.Natl.Acad.Sci. USA 79, 3082-3086
20. Rosa, F., Fellous, M., Dron, M., Tovey, M. and Revel, M. (1982) Immunogenetics, in press
21. Colonno, R. (1981) Proc.Natl.Acad.Sci. USA 78, 4763-4766

22. Farrell, P., Broeze, R . and Lengyel, P. (1979) Nature 279, 523-525
23. Revel, M., Kimchi, A., Shulman, L. Wolf, D., Merlin, G., Schmidt, A. and Friedman, M. (1981) Miami Winter Symposium, Cellular responses to molecular modulators, Mozes, L., Schultz, J., Scott, W. and Weiner, A. Eds., Vol. 18, pp.361-384 Academic Press, New York.
24. Rubin, B. and Gupta, S.(1980) J. of Virology 34, 446-454
25. Rubin, B. and Gupta, S. (1980) Proc.Natl.Acad.Sci. USA 77, 5928-5932
26. Colonno, R. and Pang, R. (1982) J.Biol.Chem. 257, 9234-9237
27. Todaro, J. (1966) Science 153, 1252-1257
28. Weissenbach, J., Zeevi, M., Landau, T. and Revel, M. (1979) Eur. J. Biochem. 98, 1-8
29. Klein, G., Bombas, L. and Gothoskar, B. (1972) Int.J.Cancer 10, 44-57
30. Klein, E., Klein, G., Nadkarri, J.G., Nadkarri, J.J., Wigzell, H. and Clifford, P. (1968) Cancer Res. 28, 1300-1310
31. Klein, G., Terasaki, A., Billung, R., Honig, R., Tondal, M., Westmas, A. and Clements, G. (1976) Int. J. Cancer 18, 639-644
32. Knight, E., Jr. and Fahey, D. (1981) J.Biol.Chem. 256,3609-3611
33. Rubinstein, M., Levy, W., Moschera, J., Lai, C., Hershberg, R., Bartlett, R. and Petska, S. (1981) Arch.Biochem.Biophys. 210, 307-318
34. Wallach, D., Fellous, M. and Revel, M. (1982) Nature 299, 833-836
35. Auffray, C. and Rougeon, F. (1980) Eur.J.Biochem. 107, 303-314
36. Maroteaux, L., Kahana, H., Mory, Y., Groner, Y. and Revel, M. (1983) EMBO, J. in press.
37. Rosen, J.M., Woo, S., Holder, J. ,Means, A. and O'Malley B.W. (1975) Biochemistry 14, 69-78
38. Land, H., Grez, M., Hauser, H., Lindermaier, W. and Schutz, G. (1981) Nucl.Ac. Res. 9, 2251-2267
39. Roychudury, R. and Wu, R. (1980) Methods in Enzymology 65, pp.43-62
40. Buell, G., Wickens, M., Payvar, F. and Schimke, R. (1978) J.Biol.Chem. 253, 2483-2495
41. Atger, M., Perricaudet, M., Tiollais, P. and Milgrom, E. (1980) Biochem. Biophys. Res. Comm. 93, 1082-1088
42. Morrison, D. (1979) Methods in Enzymology 68, 326-331
43. Backman, K., Ptashne, M. and Gilbert, W. (1976) Proc.Natl.Acad.Sci. USA 73, 4174-4178
44. Mory, Y., Chernajovsky, Y., Feinstein, S., Chen, L., Nir, U., Weissenbach, J., Malpiece, Y., Tiollais, P., Marks, D., Ladner, M., Colby, C. and Revel, M. (1981) Eur.J.Biochem. 120, 197-202
45. Clevell, D. and Helinsky, D. (1969) Proc.Natl.Acad.Sci. USA 62,1159-1166
46. Parnes, J., Velan, B., Felsenfeld, A., Ramanathan, L., Ferrini, U., Appella, E. and Seidman, T. (1981) Proc.Natl.Acad.Sci. USA 78, 2253-2257.
47. Laemli, U. (1970) Nature(London) 227, 680-685
48. Maxam, A. and Gilbert, W. (1977) Proc.Natl.Acad.Sci. USA 74, 560-564
49. Ginzburg, I., Behar, L., Givol, D. and Littauer, U.Z. (1980) Nucl.Ac. Res. 9, 2691-2697
50. Ploegh, H., Orr, H. and Strominger, J. (1980) Proc.Natl.Acad.Sci. USA 77, 6081-6085
51. Proudfoot, N. and Brownlee, G. (1976) Nature 263, 211-214
52. Novick, D., Eshhar, Z., Gigi, O., Marks, Z., Revel, M. and Rubinstein, M. (1983) J. Gen. Virol. in press.
53. Adams, A., Strander, H. and Cantell, K. (1975) J. Gen. Virol. 28, 207-212
54. Ball, L. (1979) Virology 94, 282-296
55. Fellous, A., Ginzburg, I. and Littauer, U. (1982) EMBO.J. 1, 835-839
56. Samanta, H., Dougherty, J., Brawner, M., Schmidt, H. and Lengyel, P. (1982) in Interferons, UCLA Symposium on Molecular and Cellular Biology, Merigan, T. and Friedman, R, Eds. Vol. XXV, pp. 59-72, Academic Press, New York.