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Biochemistry

Two interferon mRNAs in human fibroblasts: *In vitro* translation and *Escherichia coli* cloning studies

[cDNA/pBR322 plasmid/(2′-5′)oligo(A) synthetase]

JEAN WEISSENBACH*, YUTI CHERNAJOVSKY†, MENACHEM ZEEVI†, LESTER SHULMAN†, HERMONA SOREQ‡, URI NIR†, DAVID WALLACH†, MICHEL PERRICAUDET*, PIERRE TIOLLAIS*, AND M. REVEL†

Departments of †Virology and ‡Neurobiology, The Weizmann Institute of Science, Rehovot, Israel; and *Unite de Recombination et Expression Genetique, Institut National de la Santé et de la Recherche Médicale U163, Institut Pasteur, 28 rue du Dr. Roux, Paris, France

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ABSTRACT **Two mRNA species that produce biologically active interferon were isolated from human fibroblasts and studied by size fractionation and cloning in *Escherichia coli* plasmid pBR322. The major fibroblast interferon (Hu IFN-β1) is coded for by the smaller of the two mRNAs, an 11S species, 900 nucleotides long, which in cell-free systems yields a 20,000 M_r protein. The second interferon mRNA species (Hu IFN-β2) is 14S, about 1300 nucleotides long, and codes for another protein of 23,000–26,000 M_r. The two interferon mRNAs do not cross-hybridize. Both are induced by poly(rI·rC), but IFN-β2 mRNA is induced to about 10% in cells by cycloheximide treatment alone whereas under these conditions IFN-β1 is not induced.**

The major interferon produced by human fibroblasts was recently purified and its amino-terminal amino acid sequence determined by Knight *et al.* (1). An interferon mRNA 12S fraction from human fibroblasts was cloned in *Escherichia coli*, and the recombinant DNA nucleotide sequence of the resulting clones was shown to contain the codons corresponding to the structure determined by protein sequencing (2, 3). During the course of similar studies, we observed that human fibroblasts actually contain two interferon mRNAs. As shown here, these two RNAs differ in their size and in their translation products; studies of cDNA recombinant clones in *E. coli* show that the two mRNAs do not readily cross-hybridize. Sehgal and Sagar (4) have also recently succeeded in separating the two interferon mRNAs from human fibroblasts by mercury-agarose gel electrophoresis.

MATERIALS AND METHODS

mRNA Preparation and Translation. Poly(rI·rC) superinduction of human FS11 fibroblasts, preparation of poly(A)$^+$ mRNA, sucrose gradient purification, and mRNA translation in micrococcal nuclease-treated rabbit reticulocyte lysates were described in detail (5). Antibodies to human fibroblast interferon were produced and used for immunoprecipitation of the [^{35}S]methionine-labeled translation products with staphylococcal protein A-Sepharose, as before (5). Interferon mRNA injection into *Xenopus laevis* oocytes was carried out according to Raj and Pitha (6).

Assay of Interferon by Inhibition of Growth of Vesicular Stomatitis Virus (VSV). Incubation medium of oocytes, 40 hr after mRNA injection, was serially diluted in minimal Eagle's medium with 10% fetal calf serum and added to FS11 cultures in a 96-well microtiter plate. VSV was added 20 hr later at 1 plaque-forming unit/cell in medium with 2% serum. At 22 hr after infection, 0.02 ml was diluted 1:15 in sodium carbonate buffer, pH 9.6/0.1% Nonidet P-40 and introduced in the wells of a polyvinyl chloride microtiter plate (Dynatech). After 1 hr at 37°C, the wells were washed with phosphate-buffered saline with 0.02% Tween-20. Antibodies to the G protein of VSV (purified by affinity chromatography on G protein-Sepharose) were added for 1 hr, at 37°C. After washing as above, ^{125}I-labeled protein A (3×10^5 cpm) was added for 1 hr at 37°C. The plate was extensively washed as above and each well was cut out and its radioactivity was measured. A calibration curve with VSV was used to calculate the virus reduction. Observation of VSV cytopathic effect (CPE) can be done on the remaining cultures.

Assay of Interferon by (2′-5′)Oligo(A) Synthetase E. Translation products from reticulocyte lysates or from the medium of mRNA-injected oocytes were diluted 1:10 to 1:15 and 0.1 ml was added to a 96-well microtiter plate. After 18 hr at 37°C, cells were lysed with Nonidet P40, and the lysates were adsorbed on poly(rI·rC)-agarose beads, which were then incubated with α-[^{32}P]ATP (0.3 Ci/mmol; 2.5 mM; 1 Ci = 3.7×10^{10} becquerels) as detailed (7). After 20 hr at 30°C, the supernatant was digested by bacterial alkaline phosphatase and the amount of (2′-5′)ApA formed was analyzed by paper electrophoresis at pH 3.5.

Cloning of cDNAs. Procedures established by Rougeon (8) were used. cDNA was prepared from sucrose gradient RNA fractions (4 μg) with reverse transcriptase (RNA-dependent DNA polymerase) from avian myeloblastosis virus (J. Beard), $(dT)_{12-18}$, and 4 mM pyrophosphate (9). Double-stranded cDNA was made with DNA polymerase I (gift of F. Rougeon), digested with S1 nuclease and tailed with dCTP and terminal transferase (10). Plasmid pBR322 DNA linearized with restriction endonuclease *Pst* I was dG-tailed and 50 ng was annealed with 10 ng of dC-tailed double-stranded cDNA. This DNA was used to transform $CaCl_2$-treated *E. coli* DP50, and tetracycline-resistant colonies were grown on L broth agar plates with diaminopimelic acid at 0.1 mg/ml, thymidine at 0.01 mg/ml and tetracycline at 15 μg/ml. Sensitivity to ampicillin at 100 μg/ml was then tested. The biohazard containment conditions were P3 as described by the National Institutes of Health guidelines. Two cDNA probes were prepared in 0.15 ml of solution containing 40 mM Tris·HCl at pH 8.3, 3.3 mM $MgCl_2$, 50 mM KCl, 5 mM dithiothreitol, 20 mM each dATP, dGTP, and dTTP, 100 μCi of [α-^{32}P]dCTP (400 Ci/mmol), boiled calf thymus DNA (digested 2 hr at 37°C with DNase at 5 μg/ml in 10 mM Tris·HCl, pH 7.9/10 mM $MgCl_2$ and extracted with phenol) at 3.3 mg/ml, actinomycin D at 25 μg/ml,

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Abbreviations: VSV, vesicular stomatitis virus; CPE, cytopathic effect; Hu human; IFN, interferon; DBM, diazobenzyloxymethyl.

reverse transcriptase at 250 units/ml, and enriched RNA from either induced or noninduced FS11 cells at 6.5 mg/ml. Each cDNA (10^9 cpm/μg) was extracted with phenol and digested with alkali, and 10^7 cpm was used for replica colony hybridization, 18 hr at 68°C (11).

Selection of mRNA by Hybridization to Plasmid DNA. We used the nitrocellulose techniques of either Riccardi *et al.* (12) or of Harpold *et al.* (13) to hybridize 35 μg of *Eco*RI-digested plasmid DNA to 30 μg of poly(A)$^+$ RNA from induced FS11 or SV80 cells. After elution by boiling 1 min in 0.3 ml of water, 20 μg of rabbit liver tRNA was added before ethanol precipitation. The pellet was washed with 2 M LiCl, then washed with ethanol, dried, and dissolved in 2 μl of water for injection into oocytes.

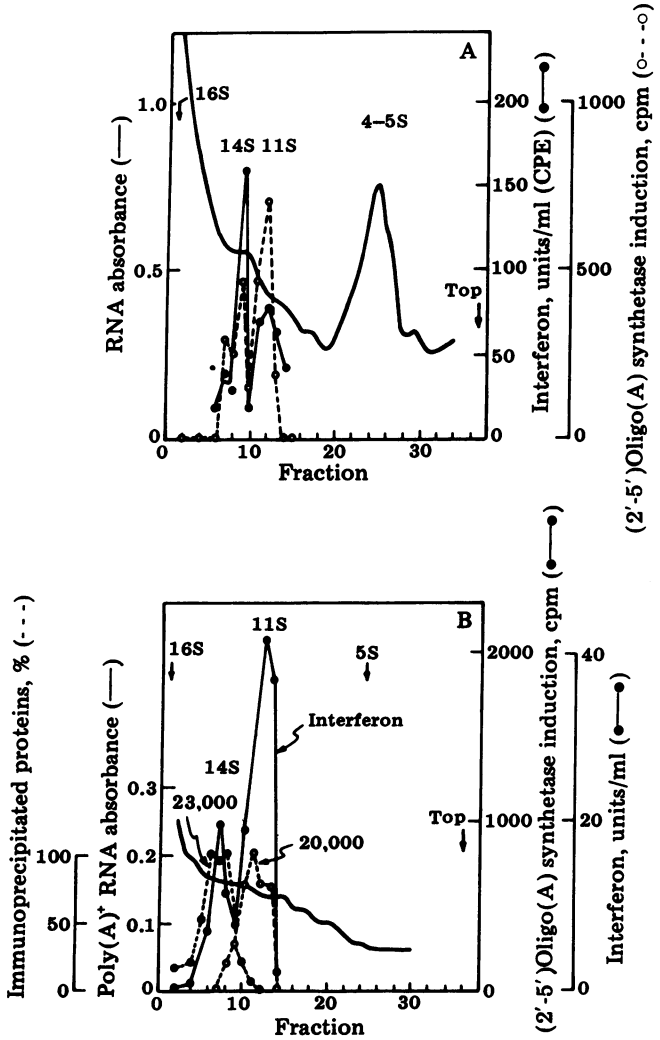

FIG. 1. Separation of two interferon mRNAs from induced FS11 cells. (*A*) RNA (300 μg) heated in formamide was fractionated by sucrose gradient centrifugation (5). *E. coli* RNA was used as markers. Ten oocytes were each injected with 6 μg of RNA and after 40 hr the medium was assayed for inhibition of VSV CPE (●—●), and (using a 1:15 dilution) assayed for (2′-5′)oligo(A) synthetase E induction in FS11 cells (○- - -○). Standard interferon (10 units/ml) gave 4500 cpm of (2′-5′)ApA. A background of 400 cpm was subtracted. (*B*) Same as *A*, but with 50 μg of poly(A)$^+$ RNA. Translation reaction mixtures in reticulocyte lysate, with 0.5 μg of RNA from each sucrose gradient fraction, were diluted 1:12 and added to FS11 cells and (2′-5′)oligo(A) synthetase E induction was measured (●—●). An interferon standard was used to calculate the titer. The translation products were immunoprecipitated as in Fig. 2 and the intensities of the 23,000 (●- - -●) and 20,000 (○- - -○) bands were measured by scanning the autoradiographs. The data are given in percent of the intensity in the peak fraction.

RESULTS

Separation of Two Interferon mRNAs from Human Fibroblasts. Cytoplasmic RNA was isolated from FS11 diploid fibroblasts induced by a 4-hr treatment with poly(rI·rC) at 100 μg/ml and cycloheximide at 50 μg/ml, actinomycin D (2 μg/ml) being added for the last hour. The RNA was fractionated on a sucrose gradient and injected into oocytes, and interferon released in the oocyte medium (14) was assayed. Fig. 1 shows that, both by VSV growth inhibition and by (2′-5′)-oligo(A) synthetase E induction, two peaks of interferon mRNA activity can be separated, one at 14S and one at 11S. The light peak gave more enzyme E induction than did the heavy peak. In Fig. 1*B*, sucrose-gradient fractions of poly(A)$^+$-RNA from similar FS11 cultures were translated in a reticulocyte lysate. The same two peaks of interferon mRNA were again detected.

The [^{35}S]methionine-labeled translation products from the reticulocyte lysate were immunoprecipitated with an antiserum against partially purified interferon (5) and electrophoresed in a polyacrylamide gel with sodium dodecyl sulfate. Two proteins are immunoprecipitated from the translation products of induced mRNA that are absent in those of noninduced mRNA (Fig. 2). These two proteins, were designated 23,000 and 20,000, according to their molecular masses, which in different experiments varied between 23,000 and 26,000 daltons and between 19,000 and 21,000 daltons, respectively. Fig. 1*B* shows that the large, 14S, interferon mRNA directs the synthesis of the 23,000 protein whereas the small, 11S, interferon mRNA directs the synthesis of the 20,000 protein.

The presence of two peaks of interferon mRNA activity is seen as well in the human fibroblastoid cell line SV80 (Fig. 3*B*). Again, the large, 14S, mRNA corresponds to the 23,000 protein, whereas the small, 11S, mRNA corresponds to the 20,000 protein. Further evidence for the existence of two independent interferon mRNAs was obtained from studies with cultures treated by cycloheximide without poly(rI·rC). Under these conditions, the SV80 cells produce about 5% of the interferon and contain up to 10% of the interferon mRNA, as compared to fully induced cells. Immunoprecipitation of the translation

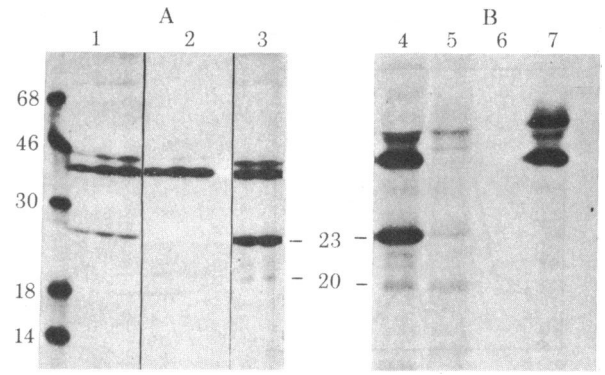

FIG. 2. Immunoprecipitation of the translation products of interferon mRNA. (*A*) Poly(A)$^+$ RNA (0.3 μg from SV80 cultures treated 4 hr with poly(rI·rC) at 100 μg/ml and cycloheximide at 50 μg/ml (actinomycin D, 2 μg/ml, being added for the last hour) was translated in reticulocyte lysates, immunoprecipitated, and electrophoresed on a 15% polyacrylamide gel with sodium dodecyl sulfate (lanes 3). The products of poly(A)$^+$ RNA (0.3–0.5 μg) from SV80 treated only with cycloheximide and actinomycin D are shown in lanes 1, compared to those of poly(A)$^+$ RNA from untreated SV80 cells in lanes 2. Left lane, protein markers, with $M_r \times 10^{-3}$ indicated. (*B*) Translation products of poly(A)$^+$ RNA from fully induced fibroblasts were immunoprecipitated with antibodies to partially purified interferon (lane 4) and to pure β1 interferon (lane 5). Lane 6 shows a reaction without mRNA and lane 7 the products of poly(A)$^+$ RNA from untreated cells, under the conditions of lane 4.

products of poly(A)$^+$ mRNA from cycloheximide-treated cells (Fig. 2, lanes 1) shows that the 23,000 mRNA is induced to about 10–20% of the levels seen in cells treated by both poly(rI·rC) and cycloheximide. On the other hand, the 20,000 mRNA is not induced unless poly(rI·rC) is added (Fig. 2, lanes 3). Fig. 3*A* shows that the cycloheximide-treated cells contain only the heavy interferon mRNA corresponding to the mRNA for the 23,000 protein and not the light interferon mRNA, in line with the absence of 20,000 protein. These experiments confirm the correlation between interferon mRNA's biological activity and the immunoprecipitated translation products and indicate that the induction of the two interferon mRNAs is independently controlled in the cells.

The previous immunoprecipitation experiments were carried out with antiserum prepared against fibroblast interferon only partially purified on carboxymethyl-Sepharose (5). To determine whether both 23,000 and 20,000 proteins correspond to the β interferon whose amino acid sequence was determined by Knight *et al.* (1), we obtained an anti-interferon serum from Y. H. Tan (15), prepared against a pure interferon preparation containing the same sequence. Fig. 2 (lane 5) shows that this antiserum precipitates the 20,000 protein but much less the 23,000 protein, indicating that the 20,000 product of the 11S mRNA is the major fibroblast interferon whose sequence was determined by Knight *et al.* (1). This was further demonstrated by showing that DNAs containing the nucleotide sequence determined by Taniguchi *et al.* (2) hybridize only to the 11S mRNA and not to the 14S mRNA (ref. 4; unpublished data). We conclude, therefore, that the 11S mRNA corresponds to the known fibroblast interferon mRNA (2, 3), and designate this 11S mRNA as Hu IFN-β1 mRNA. To examine in further detail the nature of the 14S mRNA (designated Hu IFN-β2 mRNA), cDNA clones from this mRNA were also isolated.

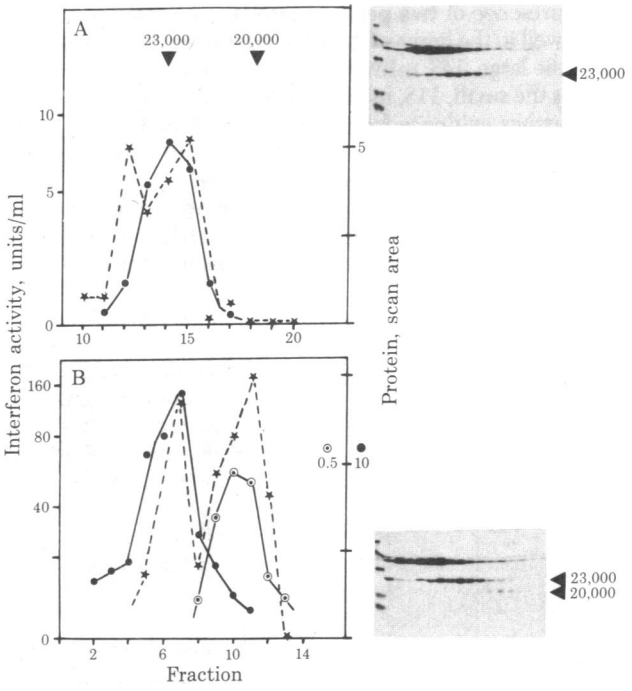

FIG. 3. Interferon mRNAs from SV80 cells. (*A*) Poly(A)$^+$ RNA (230 μg) from SV80 cultures treated 4 hr with cycloheximide at 50 μg/ml (actinomycin D at 2 μg/ml for the last hour) was fractionated by sucrose gradient centrifugation (5). Ten oocytes were each injected with 0.4 μg of RNA and after 40 hr the medium was assayed on FS11 cells for interferon synthesis by reduction of VSV growth, using the radioimmunoassay (★---★). Translation products of 0.24 μg of RNA in reticulocyte lysates were immunoprecipitated (gel on right) and the intensity of the 23,000 band was determined by scanning the autoradiograph (●—●). (*B*) Poly(A)$^+$ RNA (170 μg) from SV80 cultures treated as in *A*, but with the addition of poly(rI·rC) at 100 μg/ml, was analyzed as above. Immunoprecipitation (right) shows both 23,000 (●—●) and 20,000 (⊙—⊙). Note the different ordinates in *A* and *B*.

Cloning in *E. coli* of the 14S Fibroblast Interferon mRNA. Interferon mRNA from the 14S peak from induced FS11 cells was used to prepare double-stranded cDNA, which was introduced in the *Pst* I site of pBR322 for cloning. A total of 3000 ampicillin-sensitive tetracycline-resistant transformant clones were obtained. The bacterial colonies were hybridized *in situ* (11) with [^{32}P]cDNA prepared from the 14S RNA fraction to either induced or untreated FS11 cultures. About 25 different colonies that hybridized to the "induced cDNA" but not the "noninduced cDNA" were identified. The DNA insert from the plasmid of one such clone, A341, was excised with *Pst* I, labeled by nick-translation (16), and used for *in situ* colony hybridization. By this method and restriction mapping, 12 clones were identified as containing various fragments of the IFN-β2 mRNA sequence.

Recombinant plasmid DNA from clone A341 was linearized by *Eco*RI and immobilized on small nitrocellulose filters. Poly(A)$^+$ RNA from induced FS11 cells was hybridized to the A341 DNA, eluted by boiling for 1 min, and translated in reticulocyte lysates. Fig. 4*B* shows that only one protein is coded for by A341-selected RNA (compare slots 2–4 to slot 1 without added RNA). This protein comigrates with the 23,000 protein immunoprecipitated by anti-interferon (compare lane 7 to lanes 6 and 8). The translation product of A341-selected RNA is itself immunoprecipitated by anti-interferon (lane 9) and again comigrates with the immunoprecipitated 23,000 protein produced by poly(A)$^+$RNA from induced FS11 cells (lane 10). Fig. 4*A* shows that A341-selected RNA produces interferon activity as determined by (2′-5′)oligo(A) synthetase induction. The biological activity obtained was proportional to the amount of 23,000 protein produced (Fig. 4*B*, lanes 1–4).

RNA selected by hybridization to A341 DNA or to E474,

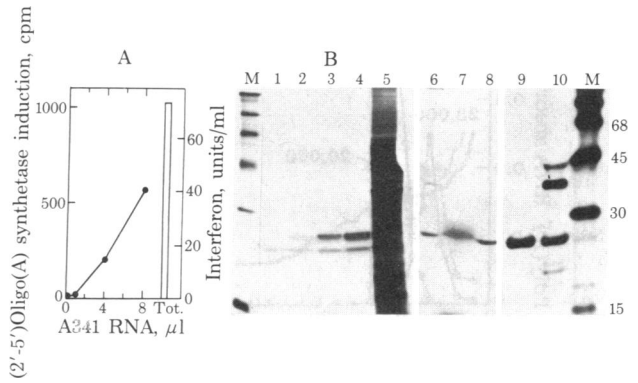

FIG. 4. mRNA selection with DNA of plasmid A341 (Hu IFN-β2 cDNA). (*A*) Poly(A)$^+$ RNA (30 μg) from induced FS11 cells was hybridized (12) to A341 DNA and recovered in 30 μl. One, 4, and 8 μl were translated in reticulocyte lysates (50 μl) and, after 1:7 dilution, 0.1 ml was assayed for (2′-5′)oligo(A) synthetase E induction in FS11 cells. A standard interferon was used to calculate the titer of interferon produced by 0.2 μg of total poly(A)$^+$ RNA. A background of 215 cpm for untreated cells was subtracted. (*B*) Sodium dodecyl sulfate gel electrophoresis. Lanes M, markers, with $M_r \times 10^{-3}$ indicated on right. The same translation reactions as in *A* were analyzed in lanes 2, 3, and 4, respectively (10 μl per slot). Lane 1 is without RNA and lane 5 with 0.2 μg of total poly(A)$^+$ RNA. Total translation products of A341-selected RNA (lane 7) were compared to its immunoprecipitation products (lanes 8 and 9) and to immunoprecipitated products of total poly(A)$^+$ RNA (lanes 6 and 10). Lanes 6, 7, and 8 are from an experiment with less poly(A)$^+$ mRNA than in lanes 9 and 10.

Table 1. Antiviral activity produced by β2 interferon mRNA

Addition	VSV yield by radioimmunoassay, cpm		log virus yield reduction*	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Medium (diluted 1:10) from oocytes injected with:				
RNA hybridized to A341 DNA	1245	3410	0.94	0.37
E474 DNA	830	1145	1.09	0.94
RNA hybridized to unrelated plasmid	4895	5240	0.12	0.04
Water	—	5445	—	0.04
Standard interferon, 3 units/ml	—	190	—	1.1
Virus control	6100	5685	—	—

mRNA was selected in Exp. 1 as described by Ricciardi *et al.* (12) and in Exp. 2 as described by Harpold *et al.* (13).

* A 0.3 log reduction corresponds to 1 unit of interferon activity or 10 units/ml of undiluted oocyte medium.

another recombinant plasmid DNA from the IFN-β2 group, was injected into *Xenopus* oocytes. The oocyte medium was diluted and added to FS11 cell cultures that were infected 18 hours later with VSV. The inhibition of VSV multiplication was measured by inhibition of the viral CPE and by radioimmunoassay of VSV. Table 1 shows that the RNA selected on the two different plasmids of the IFN-β2 group produced antiviral activity. This activity was neutralized by anti-interferon serum (not shown).

Nick-translated A341 plasmid DNA was hybridized to mRNAs from FS11 or SV80 cells fractionated by electrophoresis on agarose gels and transferred to diazobenzyloxymethyl (DBM) paper according to Alwine *et al.* (17). Fig. 5 shows that A341 DNA gives a major hybridization band to an RNA that is found in cells induced to produce interferon but not in uninduced cells. The size of this RNA was determined in several experiments to be 1270 ± 70 nucleotides. A slight hybridization to a smaller RNA (750–800 nucleotides) is also detected. Controls with uncloned cDNA show hybridization to both induced and uninduced RNA. Using DNA fragments excised from A341 by *Pst* I, we carried out a R_0t curve analysis of the hybridization reaction with total poly(A)$^+$ RNA from induced [4 hr poly(rI·rC) with cycloheximide] and noninduced cells (Fig. 5*B*). From these data, we calculate that A341 DNA corresponds to mRNA sequences present at 0.1–0.2% of the total poly(A)$^+$ RNA of induced FS11 cells. There is only 1/1000th to 1/500th of this RNA in uninduced FS11 cells. The mRNA sequences hybridizing with A341 DNA also appeared in FS11 cells exposed for 1 hr to poly(rI·rC) at 100 μg/ml without cycloheximide, but interferon (200 units/ml) did not induce this RNA in the cells (not shown).

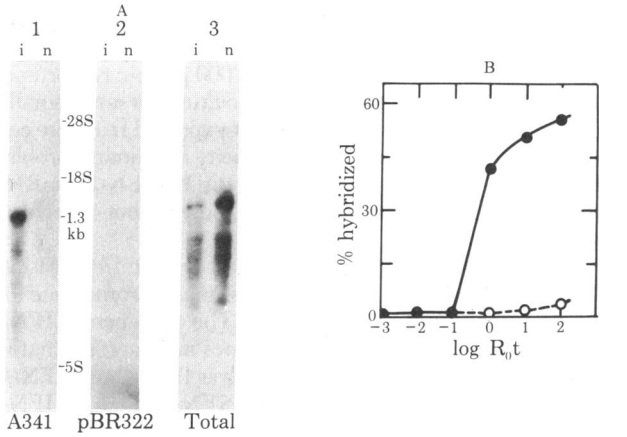

FIG. 5. Hybridization of Hu IFN-β2 cDNA to mRNA from induced and noninduced cells. (*A*) Agarose gel blot on DBM-paper: Poly(A)$^+$ RNA (4 μg) from induced (i) or noninduced (n) FS11 cells was heated 30 min at 50°C in 15 μl of 50% (vol/vol) dimethyl sulfoxide/15% (vol/vol) glyoxal/10 mM potassium phosphate buffer, pH 7.0. After electrophoresis in a 1.5% agarose gel, RNAs were transferred to DBM-paper (17) and hybridized with the following [^{32}P]DNAs: Lanes 1, *Eco*RI-digested and nick-translated (16) plasmid A341 DNA (6×10^6 cpm; 10^8 cpm/μg); lanes 2, same from pBR322 DNA; lanes 3, total cDNA of induced FS11 mRNA (0.6×10^6 cpm; 10^9 cpm/μg). RNA migration positions are indicated; kb, kilobase. (*B*) R_0t analysis of FS11 RNA hybridization to A341 cDNA. R_0 is RNA concentration (mol of nucleotide/liter); t is incubation time (hr). Nick-translated insert DNA of plasmid A341, excised with *Pst* I (2×10^3 cpm; 10^6 cpm/μg) was denatured at 100°C and hybridized for 20 hr at 62°C in 25 μl of 0.6 M NaCl/1 mM EDTA/25 mM Tris·HCl, pH 7.5, with 0.08–8000 ng of poly(A)$^+$ RNA from induced (●—●) and noninduced (o---o) FS11 cells. Hybrids resistant to S1 nuclease were measured (18, 19).

DISCUSSION

The finding that two interferon mRNAs are induced in human fibroblasts by poly(rI·rC) is surprising because the major part of interferon activity from such cells behaves as a single species of 20,000 M_r, which was purified to homogeneity and its amino acid sequence was partially determined (1). This interferon appears to be coded for by the smaller of the two interferon mRNAs (20,000 mRNA or Hu IFN-β1 mRNA) as shown by the *in vitro* translation–immunoprecipitation experiments and by the fact that cDNA with the nucleotide sequence corresponding to the amino acid sequence determined for this major interferon hybridizes only to the small interferon mRNA. This was also directly demonstrated by Sehgal and Sagar (4), using the clone of Taniguchi *et al.* (2). We calculate that the 11–12S Hu IFN-β1 mRNA is about 850–900 nucleotides long, which is in good agreement with the cDNA sequencing data (2, 3). On the other hand, the 14S interferon mRNA (23,000 mRNA or Hu IFN-β2 mRNA), which is about 1300 nucleotides long, appears to code for a larger protein of 23,000–26,000 M_r, but not for the 20,000 M_r protein, as clearly seen from the sucrose gradient translation pattern. Evidence for interferon biological activity associated with the 23,000 protein is based on different sucrose gradient mRNA fractionations as well as on the hybridization–translation experiments using a cloned cDNA that hybridizes to the 1300-nucleotide-long mRNA. Both inhibition of VSV growth and induction of the (2′-5′)oligo(A) synthetase could be demonstrated with the product of the 23,000 mRNA and with that of the 20,000 mRNA. We have also observed that the 23,000 protein produced by translation *in vitro* binds strongly to Cibacron blue-Sepharose and elutes with 60% (vol/vol) ethylene glycol/0.5 M NaCl, as does the 20,000 protein and fibroblast interferon produced *in vivo* (1). Nucleotide sequence analysis (unpublished) of the Hu IFN-β2 cDNA clones (corresponding to the 23,000 mRNA) shows that the 23,000 protein does not contain the same amino terminal sequence as that found by Knight *et al.* (1) for the major fibroblast interferon. There is, however, a marked homology between the Hu IFN-β2 (23,000) and β1 (20,000) interferons as determined by DNA sequencing (2, 3), especially in the region of codons 45–52, (which contains also the most conserved sequences of the α and β interferons). The β2 interferon sequence differs strikingly from that in IFN-α and IFN-β1, because it is much longer at the NH_2-terminal and shorter at the COOH-terminal. Although the 23,000 protein is prominent in the translation products of mRNA from 4-hr-induced fibroblasts, it was not yet detected in the immu-

noprecipitated [^{35}S]methionine-labeled proteins from the culture fluid of fibroblasts collected 24 hours after induction (unpublished). It is possible that the 23,000 protein is shortened by processing *in vivo*, is present in cell cultures in much smaller amounts, is rapidly degraded, or is not exported from the cell. Some authors have reported heterogeneity in human fibroblast interferon preparations (20, 21), and the Hu IFN-β2-mRNA product may correspond to one of these minor interferon fractions.

In somatic cell hybrids, production of human fibroblast interferon can be observed either with human chromosome 9 or with chromosomes 2 and 5 (22–24). The presence of IFN-β genes on different human chromosomes may suggest that independent genes (or gene clusters) such as IFN-β1 and IFN-β2 have evolved in human cells. The Hu IFN-β1 and Hu IFN-α genes, on the other hand, appear to be derived from a common gene (25). The finding that cycloheximide-treated cells have only IFN-β2 mRNA suggests that this mRNA could be induced in cells without virus infection. We recently observed that the (2′-5′)oligo(A) synthetase increases when Friend cells reach confluency and that this increase is blocked by anti-interferon antibodies (unpublished). Very small amounts of interferon may be produced spontaneously by cells to control their own growth. Like IFN-β1 mRNA, the IFN-β2 mRNA is, however, much more induced when poly(rI·rC) is added, and is also induced with poly(rI·rC) without cycloheximide. Use of the cloned IFN-β1 and IFN-β2 DNAs will allow comparison, in more detail, of the induction of these two mRNAs and determination of whether their protein products have different functions in the multiple facets of interferon's antiviral and anticellular action.

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