Molecular cloning of human interferon cDNA
(human fibroblast/hybridization-translation assay/DNA sequence analysis)

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**ABSTRACT** A hybrid plasmid, TpIF319, has been shown to contain the sequence for human fibroblast interferon mRNA [Taniguchi, T., Sakai, M., Fuji-Kuriyama, Y., Muramatsu, M., Kobayashi, S. & Sudo, T. (1979) Proc. Jpn. Acad. 55, Ser. B, 464-469]. This conclusion was confirmed by a hybridization-translation assay, using rabbit globin mRNA and its cDNA-containing plasmid as a control. Plasmid TpIF319 was used as a probe and another recombinant plasmid, TpIF319-13, whose cDNA insert consists of about 800 base pairs, was isolated.

Nucleotide sequence analysis of the cDNA revealed that the DNA in fact codes for human fibroblast interferon.

**Interferons** are species-specific glycoproteins with characteristic antiviral activities, produced by various cells upon induction by viruses or double-stranded RNAs as well as other compounds (1, 2). In addition to their antiviral activities, multiple functions of interferons such as antitumor and antifungal activities have also been described (3).

It has been postulated that, within human cells, there may be several interferon structural genes giving rise to distinct interferon molecules (4).

Although various pieces of information are available on the biological activities as well as physicochemical properties of these interferons, no definitive structures of the proteins have emerged, mainly due to the scarcity of interferons produced by human cells (5-7). For the same reason, clinical investigation of human interferons has been limited.

As an approach to solve these problems, we have constructed a hybrid plasmid containing the gene sequence for human fibroblast interferon (8). In this paper, we confirm our previous conclusion and present evidence that we have isolated a cDNA clone that contains the entire coding region of the interferon mRNA.

**MATERIALS AND METHODS**

**mRNA Preparation.** Human fibroblast strain DIP2, a high interferon producer, was generously provided by S. Kobayashi, Toray Industries. Total cytoplasmic RNA was extracted from DIP2 cells after 4 hr of induction by poly(I)-poly(C) (10 μg/ml) in the presence of cycloheximide (1 μg/ml) essentially as described by Sehgal et al. (9). After oligo(dT)-cellulose affinity chromatography, the poly(A)-containing mRNA was further fractionated by 5-25% sucrose gradient centrifugation, and the RNA sedimenting in the 12S region was collected. Starting from 2 x 10^6 cells, about 50 μg of the RNA was obtained. This RNA directed the synthesis of human interferon when injected into Xenopus oocytes and is termed interferon mRNA here. Rabbit globin mRNA was prepared essentially as described by Aviv and Leder (10). About 35% of the RNA was shown to be β-globin mRNA when analyzed by filter hybridization (11) (result not shown).

**Hybrid Plasmid DNA.** Preparation of the hybrid plasmid TpIF319 has been described in the previous paper (8). Plasmid pBR322 containing a rabbit β-globin cDNA insert, termed RβGpBR322 here, was kindly provided by F. Meyer and C. Weissmann, University of Zurich.

**Hybridization-Translation Assay.** The hybridization-translation assay was carried out essentially as described by Taniguchi et al. (8). Human interferon mRNA (2.5 μg) and rabbit globin mRNA were dissolved in 20 μl of a solution containing 20 mM 1,4-piperazinediethanesulfonic acid (Pipes) buffer at pH 6.5, 0.4 M NaCl, and 5 mM EDTA in Eppendorf tubes. It was then brought to dryness by passing nitrogen gas over it. Plasmid DNA was linearized either by HindIII (in the case of TpIF319) or by Pst I (in the case of RβGpBR322) restriction endonuclease digestion and was dissolved in 20 μl of 80% (wt/vol) formamide. DNA was denatured at 85°C for 10 min, then quickly transferred into the mRNA-containing tube and mixed well before hybridization. Thus, the hybridization mixture (20 μl) contained 2.5 μg of interferon mRNA, 1.0 μg of globin mRNA, 20 mM Pipes buffer at pH 6.5, 0.4 M NaCl, 5 mM EDTA, 80% (wt/vol) formamide, and various amounts of the plasmid DNAs (see legend to Fig. 1). The mixture was sealed with paraffin oil and incubated for 4 hr at 53°C. It was then mixed with 0.4 ml of ice-cold 0.45 M NaCl/0.045 M sodium citrate, pH 7.0, solution and passed through a nitrocellulose membrane filter (Sartorius membrane filter, 1 cm diameter, 0.45 μm pore size) at a constant flow rate of 0.5 ml/min. The filter was then washed by 1.5 ml of 0.3 M NaCl/0.03 M sodium citrate solution. When globin mRNA and RβGpBR322 were used as a model system, about 50% of the total mRNA-DNA hybrid could be trapped on the filter, and unspecific binding of the mRNA was negligible under these conditions. The hybridized mRNA was eluted from the filter by three consecutive incubations in 0.6 ml of 90% formamide/20 mM Pipes, pH 6.5/5 mM EDTA/0.5% sodium dodecyl sulfate at 60°C for 2 min (12), then applied on an oligo(dT)-cellulose column. The poly(A)-containing RNA was finally dissolved in 3 μl of 10 mM Tris-HCl, pH 7.5/0.8 mM NaCl and translated in Xenopus laevis oocytes.

**Translation of mRNA in X. laevis Oocytes and Analysis of the Products.** The mRNA was injected into X. laevis oocytes as described (13). Fifty nanoliters of a solution of mRNA prepared as described above was injected into each oocyte. Groups of 10 oocytes were incubated for 20 hr at 23°C in 50 μl of incubation medium (14) containing [3H]histidine (1 mCi/ml, specific activity 60 Ci/mmol; 1 Ci = 5.7 x 10^10 becquerels). After incubation, 150 μl of 52 mM Tris/glycine buffer (pH 8.9) was added to the incubation medium and the oocytes were homogenized. The homogenate was centrifuged at 10,000 x g at 4°C for 5 min and the supernatant was carefully removed. Interferon activity was determined according to the procedure of Suzuki et al. (15). In order to check globin synthesis, part of

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the material was electrophoresed on polyacrylamide gels and fluorography was done as described by Bonner and Laskey (16).

**Synthesis of the cDNA, Transformation of Escherichia coli, and Colony Hybridization.** Synthesis of the double-stranded cDNA, construction of the hybrid plasmid by the oligo(A)-oligo(T) method, and transformation of E. coli χ1776 were carried out as described by Taniguchi et al. (8).

Nick translation of the cDNA insert (17) and colony hybridization (18) were carried out according to published procedures. All cloning procedures were conducted in a P3 biocontainment facility at the Cancer Institute as specified by the Guidelines for Research Involving Recombinant DNA Molecules issued in March 1979, by the Ministry of Education, Science and Culture of Japan.

**DNA Sequence Analysis.** The DNA nucleotide sequence was determined by following the method of Maxam and Gilbert (19).

**RESULTS AND DISCUSSION**

**Hybridization-Translation Assay.** Hybrid plasmid TpIF319, in which a cDNA is inserted by the oligo(A)-oligo(T) method at the EcoRI site of the plasmid pBR322, has been shown to hybridize specifically with human interferon mRNA in the preceding paper (8). In order to substantiate this observation, the hybridization-translation assay was carried out by hybridizing a mixture of the interferon mRNA and the rabbit globin mRNA with different kinds of recombinant DNA. As shown in Fig. 1, mRNA that had hybridized with the plasmid TpIF319 DNA gave rise to human interferon synthesis in E. coli, whereas an opposite result was obtained when TpIF319 DNA was replaced with RβGpBR322, a recombinant plasmid containing a rabbit β globin cDNA insert (lane 2). In addition, both human interferon and rabbit β globin were translated from the filter-trapped mRNA when both of the recombinant DNAs were present in the hybridization mixture (lane 3). Approximately 1000 interferon units equivalent was detectable when the same amount of the interferon mRNA as that used for hybridization was incubated under the same conditions without DNA and translated in the frog oocytes. Because about 50% of the mRNA-DNA hybrid can be trapped on the filter under our assay conditions, interferon mRNA must have hybridized with TpIF319 DNA at an efficiency of about 40% of the input or even higher.

The results thus confirm our previous result that the recombinant plasmid TpIF319 DNA contains the cDNA insert specific for the human fibroblast interferon mRNA.

**Isolation of the Recombinant Plasmid TpIF319-13.** Plasmid TpIF319 DNA was digested by several restriction enzymes and a restriction map of the cDNA insert was made as shown in Fig. 2. The length of the cDNA insert was estimated to be about 600 base pairs. This is apparently shorter than the full copy of the interferon mRNA, because the RNA sediments at 12 S, a size corresponding to about 800–900 nucleotides, when analyzed by sucrose gradient centrifugation (8, 9). It was essential to obtain a cDNA clone whose length was close to that of the full copy of interferon mRNA, particularly for the determination of the complete sequence of its coding region as well as for the synthesis of correct interferon molecules in bacteria. We therefore searched for clones containing a longer cDNA copy by the following procedure. Double-stranded cDNA was synthesized on partially purified interferon mRNA, inserted into the EcoRI site of pBR322 by the oligo(A)-oligo(T) method, and transfected into E. coli χ1776 as described (8). TpIF319 DNA was digested by Bgl II and Pst I, and a resulting DNA fragment of about 400 base pairs was isolated (Fig. 2). The DNA was then labeled with 32P by nick-translation (17) and used as probe for in situ colony hybridization (18). About 4000 ampicillin-resistant clones were screened and 15 of them gave a positive response (result not shown). Plasmid DNA was isolated from each of the positive clones and the length of the insert was analyzed by restriction enzyme digestion. Among them, a plasmid termed TpIF319-13 appeared to contain the largest cDNA insert, consisting of about 800 base pairs. A restriction map of TpIF319-13 is presented in Fig. 2.

**DNA Sequence Analysis.** We have determined a part of the nucleotide sequence corresponding to the terminal regions of the mRNA by the chemical cleavage method of Maxam and Gilbert (19). Although the complete amino acid sequence of the human fibroblast interferon is not yet available, Knight et al. have sequenced part of the amino acid sequence of the human interferon in the previous paper (8). The sequence data were analyzed using a computer program to determine the complete sequence of the cDNA insert. The sequence analysis was done on an IBM 3081 computer equipped with an IBM 3081 computer.
has also been in amino sequence DNA interferon with the region of 100 phoblastoid to sponding (20). The region determined fragment corresponded (20), region according determined fragment. The nucleotide sequence of fragment A was determined according to Maxam and Gilbert (19).

al. (20) have presented a 13-amino-acid sequence at the NH₂-terminal region of the protein. The sequence corresponding to 20 NH₂-terminal amino acids of the human lymphoblastoid interferon has also been reported by Zoon et al. (21). At the left end of the cDNA insert (see Fig. 2), the sequence of about 100 nucleotides was determined; it is presented in Figs. 3 and 4. It is evident that this region corresponds to the 5' coding region of the interferon mRNA, because the sequence predicts synthesis of a polypeptide whose amino acid sequence is identical with the reported NH₂-terminal region of the purified human fibroblast interferon (Fig. 4).

The nucleotide sequence data thus corroborate our conclusion that the cDNA we have cloned is a copy of the human fibroblast interferon mRNA. We consider that the cDNA insert of TpIF319-13 contains the entire coding region of the mRNA, because the interferon seems to consist of about 170 amino acids [molecular weight approximately 20,000 (3)] and the cDNA insert, which consists of about 800 base pairs, is long enough to code for such a protein.

From the nucleotide sequence analysis, in addition, we found an additional sequence that could code for a polypeptide proximal to the NH₂-terminal region of interferon. This may correspond to the signal peptides, which are found in almost all the secretory proteins (22). The polypeptide deduced from the DNA sequence is in fact very rich in hydrophobic amino acids, typical for the signal peptide (22). If translation begins with the methionine codon that is present in phase 21 codons proximal to the first amino acid of the interferon, then the primary translation product of human fibroblast interferon mRNA would contain 21 additional amino acids, which would eventually be cleaved off during secretion (22).

One interesting observation regarding interferons is that the molecules produced in human fibroblasts and in cultures of human buffy coat cells exhibit a number of distinct properties (23-26), suggesting that fibroblast interferon and leukocyte interferon are different proteins (4). Such proteins may be coded for by distinct structural genes. Alternatively, one gene could give rise to two different mRNAs, by means of a post-transcriptional modification system such as splicing (27). We are so far unable to find a sequence that could code for the NH₂-terminal sequence of the human lymphoblastoid interferon, leukocyte type interferon (7), either in or out of the reading frame of fibroblast interferon mRNA. It will be of great interest to compare the structures of fibroblast and leukocyte interferon mRNAs when the leukocyte interferon gene is cloned and analyzed.

One important objective of the interferon cDNA cloning is its expression in bacteria. Because we now know where the interferon protein synthesis starts on mRNA, we should be able to insert the cDNA in a correct position under a suitable bacterial promoter. It will be quite important to let the gene express correctly, because fused or incomplete polypeptides synthesized in bacteria may not be useful in clinical applications, even if such proteins exhibit antiviral properties.

Note Added in Proof. After this paper was submitted, the complete nucleotide sequence of the cDNA from TpIF319-13 was published (28). We also learned that human leukocyte interferon cDNA was cloned and its nucleotide sequence was determined (29).

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\begin{align*}
\text{Met} & \text{ Thr Asn Lys Cys Leu Leu Gln Ile Ala Leu Leu Cys Phe Ser Thr} \\
& \ldots \text{AAAAA GTC AAC ATG ACC AAC AAG TGT CTC CTC CAA ATT GCT CTC CTG TTG TGC TTC TCC ACT} \\
& \ldots \text{TTTTT CAG TTG TAC TGG TTG TTC ACA GAG GAG GTT TAA C6A GAG GAG AAC AGC AAG AGG T6A}
\end{align*}
\]

\[
\begin{align*}
\text{Met} & \text{ Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser} \\
& \text{Thr Ala Leu Ser Met Ser Tyr Asn Leu Leu Gly Phe Gln Arg Ser Ser} \\
& \text{ACA GCT CTT TCC ATG AGC TAC AAC TTG CTT GGA TTC CTA CAA AGA AGC AGC} \\
& \text{TGT C6A GAA AGG TAC TCG ATG TTG AAC GAA CCT ACT GAT GTT TCT TCG TCG}
\end{align*}
\]

FIG. 4. DNA sequence of the insertion in TpIF319-13, corresponding to the 5' coding region of human fibroblast interferon mRNA. The DNA sequence at the 5' coding region of the cDNA was determined using fragments A, B, C, and D (see Fig. 2). The sequence of the first 13 amino acids of the NH₂-terminal region of the human fibroblast interferon reported by Knight et al. (20) is underlined; it agrees with the sequence presented in the lower part in this figure, which has been deduced from the DNA sequence. The amino acid sequence of the putative signal peptide has also been deduced from the DNA sequence and is shown in the upstream part of the figure.
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