Molecular cloning of partial cDNA copies of two distinct mouse IFN-β mRNAs

D.Skup[1], J.D.Windass[2], F.Sor[1], H.George[2], B.R.G.Williams[3], H.Fukuhara[1], J.De Maeyer-Guignard[1] and E.De Maeyer[1]

[1]Institut Curie-Biologie, Campus d'Orsay, Bâtiment 110, F-91405 Orsay, France, [2]ICI/University Joint Laboratory, Biochemistry Department, University of Leicester, Leicester LE1 7RH, UK, and [3]Hospital for Sick Children, Toronto, Canada M5G 1X8

Received 10 March 1982; Revised and Accepted 27 April 1982

ABSTRACT

Two cDNA libraries were constructed, using respectively the 12S and the 16S sucrose gradient fractions of polysomal poly $(A)^+$ RNA from mouse C243 cells induced with Newcastle disease virus. Screening of a part of both libraries by mRNA selection hybridization assays revealed the presence of two plasmids hybridizing to an mRNA, whose translation product was characterized as mouse IFN-β. Blot analysis of RNA indicated that mRNA hybridizing to the DNA from both plasmids could be detected in induced but not in uninduced C243 cells. The two cDNA inserts did not cross hybridize and had distinct restriction maps. Sequencing revealed that both inserts represented the end of the coding region and the entire 3' non coding region of two distinct mRNAs. Although different, the putative 39 AA and 65 AA carboxy termini of both Mu IFN-βs display some homology to human IFN-β_1. Thus there are at least two different murine IFN-β genes.

INTRODUCTION

As a result of the molecular cloning of human IFN-α (1,2) and -β cDNAs (3-5) our understanding of the structure of human interferons has increased dramatically over the past two years. It has been unequivocally established that there exist at least 11 distinct IFN-α genes, considered to be non-allelic ; and in addition several allelic variants exist (6). Thus, human IFN-α consists of a large family of molecules, all structurally related to a greater or lesser extent. For human IFN-β, there has sofar only been unequivocal structural evidence for a single molecular species (7). However, studies with IFN-β mRNA suggest the existence of other human IFN-β genes (8,9).

It would be highly desirable to obtain similar information on the structure of the different mouse IFN genes, in view of the importance of the mouse model for the investigation of the many interesting biological effects of IFNs. Mouse IFN, upon electrophoretic analysis under denaturing and reducing conditions, segregates into a broad zone of antiviral activity, with major peaks at 35, 28 and 22 K (10,11). Based on limited N-terminal amino

0305-1048/82/1010-3069$ 2.00/0

acid homology with human IFN-α and β, the 35 and 28 K species have been tentatively identified as murine IFN-β species, and the 22 K species as the murine IFN-α species (12). In this paper we report on cloned recombinant plasmids containing partial complementary sequences to mRNA's coding for mouse interferon. This work demonstrates the existence of at least 2 different IFN-β genes in the mouse.

MATERIALS AND METHODS

Enzymes and radioactive products. Restriction enzymes were purchased from Bethesda Research Laboratories (BRL) and were used as directed by the suppliers. S1 nuclease, terminal deoxynucleotidyl transferase and large fragment E.coli DNA polymerase I (Klenow polymerase), also from BRL, were used as indicated. T4 polynucleotide kinase was purchased from PL Biochemicals Inc., and calf intestinal alkaline phosphatase from Boehringer. AMV reverse transcriptase (batch G380) was a gift of Dr. J. Beard (Life Sciences Inc., Florida, USA). Radioactive nucleotide derivatives and 3'-end labelling kits were obtained from Amersham International Ltd.

Induction of mouse IFN mRNA in C243 cells. Interferon (IFN) mRNA was prepared from a continuous line of mouse C243 cells (14) induced with Newcastle disease virus (NDV) according to a method recently described (11). Cells were harvested 10.5 hours after removal of the inducing virus.

Extraction and purification of polysomal poly(A)$^+$RNA. Polysomes were prepared as described (15) except that 10 mM vanadyl ribonucleoside complex (BRL) was present all the time. Polysomes were dissolved in 0.4 M NaCl, 10 mM Tris-HCl, pH 7.6, 10 mM EDTA, 1 % SDS and digested with 50 µg/ml of proteinase K (Boehringer-Mannheim), for 1 hr, at 37°C. Poly(A)$^+$RNA was purified by two passages through oligo-dT cellulose (PL Biochemicals) followed by sedimentation on a 15-30 % sucrose gradient (centrifugation was carried out for 17 hrs, at 85,000 g, in a SW 27 rotor).

In vitro translation of IFN mRNA. mRNA aliquots derived from each of the sucrose gradient fractions were translated in a micrococcal nuclease-treated rabbit reticulocyte lysate (Amersham International). The translation reaction mixtures were diluted in 100 µl of PBS to be processed for IFN characterization.

Characterization as IFN of in vitro translation products.

a) Chromatography on an anti-mouse (α + β) IFN antibody agarose column : 100 µl aliquots of all in vitro translation reactions were individually chromatographed on an immunosorbent column, consisting of anti-mouse (α + β)

goat-immunoglobulins covalently linked to affigel-10 (Biorad) as described (16). After extensive washing of the column with PBS, the proteins retained on the gel were desorbed with 0.1 M citrate buffer (pH 2) and titered for IFN activity.

b) IFN titrations : IFN titrations were performed using a microtiter assay in L cells with vesicular stomatitis virus as challenge. In this system, one unit corresponds to one international unit as measured by reference mouse interferon preparation n° G-002-904-511.

c) Neutralization of the antiviral activity by a specific anti-mouse IFN-serum : Anti-mouse IFN-β serum was a gift of Dr. Lee Kronenberg (Lee Biomolecular, San Diego, Calif., USA). It had been prepared by immunizing rabbits against the 36 K component of poly I.C induced L cells IFN. In preliminary experiments, we confirmed that it neutralized the 28 and 35 K (= IFN-β) but not the 22 K (= IFN-α) component of mouse C243 cell IFN.

d) PAGE electrophoresis and autoradiography : IFN labelled with ^{35}S methionine during *in vitro* translation was analysed by electrophoresis in 15 % polyacrylamide slab gels, according to Laemmli (17) as reported earlier (16). The gel was used for autoradiography on Ultrofilm ^{3}H (LKB).

Preparation and cloning of cDNA. Single stranded cDNA was prepared from both of the size classes of mRNA obtained from the sucrose gradient, which gave IFN on *in vitro* translation. 8-10 µg of mRNA was dissolved in 100 µl of 50 mM Tris-HCl (pH 8.3), 50 mM NaCl, 8 mM $MgCl_2$ and 10 mM DTT. This medium was supplemented with 10 µCi of ^{3}(H)dCTP (25 Ci/mmole) and 1 mM each of dCTP, dATP, dGTP and TTP ; after addition of 10 µg of oligo-dT primer and 50 units of AMV reverse transcriptase, the reaction was incubated for 60 min, at 42°C. At this time, another 50 units of reverse transcriptase in 100 µl of 5 mM DTT and 5 mM Tris-HCl (pH 8.3) were added and the reaction left to continue for 30 min at 47°C. Synthesis of the second DNA strand was by the method of Kay et al. (18). The double-stranded cDNA thus obtained was treated with S1 nuclease (19). Double stranded cDNA was chromatographed on DE-52 cellulose and extended with approximately 25 residues of dCMP/terminus, using terminal deoxynucleotidyl transferase (20). The cDNA elongated with oligo (dC) was annealed with pAT153 DNA (21) which had been tailed with ± 25 residues of dGMP at the PstI site. Annealing mixtures were used to transform *E.coli* strain JA 221 (22), made competent by 0.1 M $CaCl_2$ treatment (23) as described previously (24).

General techniques : Plasmid DNA preparations were performed as reported previously (24). Gel electrophoretic analyses and recovery of DNA fragments

from gels were as previously described (25-27). DNA sequencing was performed by the methods of Maxam and Gilbert (13). Colony hybridizations were according to Grunstein and Hogness (28). Nick translation was according to Rigby et al. (29).

mRNA selection hybridization assay. Denatured plasmid DNA was spotted onto Schleicher and Schüll nitrocellulose filters, which were then baked for 5 hrs at 80°C. The excised spots were prehybridized in 50 % deionized formamide, 0.4 M NaCl, 10 mM Pipes (pH 6.4), 4 mM EDTA and 0.5 mg/ml baker's yeast tRNA. Hybridization was carried out at 41°C, in the same buffer supplemented with poly(A)$^+$RNA from C243 cells ; filters were washed with 1xSSC, 0.5 % SDS, then 0.1xSSC, 0.1 % SDS, at room temperature ; 0.1xSSC 0.5 % SDS then 10 mM Tris-HCl (pH 8.0), 1 mM EDTA at 50°C. RNA was eluted in distilled water at 80°C, precipitated with ethanol and used for *in vitro* translation.

Blot analysis of RNA. Polysomal poly(A)$^+$RNA from mouse C243 cells was fractionated in 1.5 % agarose gels containing 4 mM methylmercury hydroxide for 17 hrs at 25 V (30). RNA was then transferred electrophoretically to Gene-Screen (NEN) at 50 V, for 5.5 hrs in 25 mM Na-phosphate buffer (pH 5.5) at 10°C. The blots were then rinsed with transfer buffer and baked for 2 hrs at 80°C. Blots were prehybridized with 50 % formamide, 0.1 M NaCl, 50 mM Na-phosphate (pH 7.0), 5 mM EDTA, 0.1 % SDS, 0.02 % (w/v) each of bovine serum albumin, Ficoll and polyvinylpyrrolidone (31) and 250 µg/ml of baker's yeast tRNA at 42°C overnight. Following prehybridization, nick-translated plasmids were added to buffer of the same composition but with 10 % w/v Dextran sulfate 500 (Pharmacia) and hybridized overnight at 42°C.

Following hybridization, blots were washed twice with 2xSSC, 0.5 % SDS, 1 mM EDTA at room temperature for 15 min ; twice with 0.1xSSC with 0.1 % SDS and 0.1 M EDTA at 55°C for 30 min, once with 0.1xSSC, 0.1 % SDS, 0.1 M EDTA at 65°C for 30 min. Dried blots were exposed to ^{3}H-Ultrofilm (LKB) with Dupont Lightning Plus intensifying screens.

RESULTS

Partial purification of IFN mRNA and cDNA cloning. Polysomal poly (A)$^+$RNA derived from mouse C243 cells induced with Newcastle disease virus was extracted and partially purified successively on oligo-dT-cellulose and on a 15-30 % sucrose gradient, as described in Materials and Methods. Selection of the gradient fractions enriched with IFN mRNA was performed as follows : individual 9S to 18S fractions, a pool of fractions sedimenting

faster than 18S and a pool of fractions sedimenting slower than 9S, were translated in vitro, in rabbit reticulocyte lysates. Each translation mixture was chromatographed on an anti-mouse ($\alpha + \beta$) IFN antibody column and the proteins retained were desorbed at pH 2. This procedure not only resulted in concentration of mouse IFN molecules present in the translation mixture, but also removed the non specific antiviral activity, often present in rabbit reticulocyte lysates. Eluates from the affinity chromatography column were titrated for antiviral activity. For some eluates, the antiviral activity was also tested against encephalomyocarditis virus (EMC) in L cells, and against EMC and VSV in secondary cultures of mouse embryo fibroblasts, to confirm that the acid stable protein(s) retained on the immunosorbent column had a broad antiviral activity in several mouse cell systems, as expected for IFN molecules. The data strongly suggest the existence of a heterogeneity within the mouse IFN mRNA population, since there are two distinct peaks, respectively at 12S and 16S (data not shown). In view of these data, the RNA from each peak was processed separately for cDNA synthesis and cloning.

The double-stranded cDNA derived from each RNA peak was prepared as described in Materials and Methods. Following insertion into plasmids pAT153 and transformation of E.coli JA 221, approximately 3000 colonies from the 12S mRNA peak and 2500 from the 16S mRNA peak were obtained ; in each case 85 % of these colonies were phenotypically tetracycline resistant and ampicillin sensitive, and therefore, probably carry recombinant plasmids.

mRNA hybridization selection of IFN cDNA containing plasmids. Recombinant plasmids harboring mouse IFN cDNA sequences were detected by a variation of the mRNA selection hybridization assay, first described for human leucocyte IFN by Nagata et al. (1). Plasmid DNA was prepared from 13 pools of 20 clones from both the 12S and the 16S derived libraries. These cDNAs were then denatured and spotted onto nitrocellulose filters as described in Materials and Methods and total polysomal poly(A)$^+$RNA derived from NDV induced C243 cells incubated with these filters under stringent hybridization conditions.

RNA hybridizing to the filters was recovered as described in Materials and Methods to be tested for mouse IFN mRNA activity. Two pools of clones, namely pMIF 3 and pMIF 20 were positive, in other words hybridized to an mRNA, whose translation products could be characterized as mouse IFN : they were retained on the antimouse ($\alpha + \beta$) IFN antibody column, were acid stable and displayed broad antiviral activity. Pool MIF 3 was derived from the original 16S poly(A)$^+$ preparation, whereas pool MIF 20 was derived from

the 12S preparation.

Plasmid DNA was then prepared from individual clones of these two pools. Again IFN cDNA was detected by the mRNA selection hybridization assay on nitrocellulose filters. Typical results of this second selection step are given in table 1. Plasmid DNA derived from clone MIF 3/10 (originating from the 16S mRNA peak) and plasmid DNA derived from clone MIF 20/11 (originating from the 12S mRNA peak) hybridized to mRNAs, whose translation products were shown to be mouse IFN. (they displayed all the properties described above for the products of the mRNAs selected by the pools of clones).

When used as probes in colony hybridization (28), inserts from plasmids pMIF 3/10 and pMIF 20/11, both detect further clones containing plasmids, which, in turn, select for IFN mRNAs (data not shown).

Further characterization of the mouse IFNs translated from mRNAs selected by plasmids pMIF 3/10 and pMIF 20/11. As shown above, the translational products of the two mRNAs hybridizing, on nitrocellulose filters, to pMIF 3/10 and pMIF 20/11, display three characteristic properties of mouse α and β IFN molecules, namely broad antiviral activity, resistance to pH 2, and affinity for anti-mouse IFN (α + β) antiserum. To determine whether the mouse IFN translated by each of the mRNAs selected by hybridization were of

TABLE 1

IFN titrations of translation products of mRNA selected by individual plasmids

Origin of mRNA		IFN units**
Polysomal poly (A)+		36
Selected by pMIF 3/3	(16S)*	< 6
Selected by pMIF 3/5	(16S)	< 6
Selected by pMIF 3/6	(16S)	< 6
Selected by pMIF 3/10	(16S)	48
Selected by pMIF 20/1	(12S)	< 6
Selected by pMIF 20/6	(12S)	< 6
Selected by pMIF 20/8	(12S)	< 6
Selected by pMIF 20/11	(12S)	96
Selected by pAT153		< 6

*12S and 16S indicates the sucrose gradient mRNA fraction from which the cDNA inserted into a given plasmid pool (pMIF) was derived.

**First dilution was 1/6.

the α or of the β type, neutralization tests were carried out, with an anti-mouse IFN-β serum. Results given in table 2, show that the antiviral activity present in the translational products derived from the mRNA selected by pMIF 3/10 and from the mRNA selected by pMIF 20/11 were completely neutralized by the antimouse IFN-β antiserum.

The IFN-β like nature of the translated mouse IFNs was further indicated by electrophoretic analysis. Translation of the mRNAs hybridizing to pMIF 3/10 and pMIF 20/11 cDNA was performed in vitro in the presence of ^{35}S-methionine. The translation mixtures were chromatographed on an antimouse IFN (α + β) antibody column, eluted with SDS and analysed by SDS polyacrylamide slab gel electrophoresis followed by autoradiography. Translational products selected by plasmids not hybridizing with IFN mRNA, also processed similarly, served as controls. The results of the analysis are shown in figure 1. There are no detectable radioactive products on the two gel tracks loaded with material translated from mRNA selected by plasmids that do not select IFN mRNA (tracks C and D), as is the case where no mRNA was added to the translation reaction (track B). However, the translational products derived from the mRNA selected by pMIF 3/10 (track F) or from the mRNA selected by pMIF 20/11 (track G) show proteins migrating with an apparent size of about 34 K. This MW is consistent with the 28-35 K range reported for mouse IFN-β molecules (12) and is obviously too large for mouse IFN-α, which has a MW of 22 K (12).

TABLE 2

Neutralization by anti-mouse IFN-β serum of the translation products of the mRNAs selected respectively by pMIF 3/10 and pMIF 20/11

Type of mouse IFN	Titer	Titer after contact with antiserum
Mu IFN-α-C243	9600	9600
Mu IFN-β-C243	38400	< 6000
Translation product of mRNA selected by pMIF 3/10	96	< 6
Translation product of mRNA selected by pMIF 20/11	96	< 6

Mouse C243 cell IFN was purified by two step affinity chromatography in poly (U) and antibody column as reported earlier (16). The α and β types populations were separated by SDS-PAGE to be eluted. Mu IFN-α represents the eluate of the IFN migrating with an apparent MW of 22 K. Mu IFN-β is the eluate of the IFN migrating at 28-35 K (16).

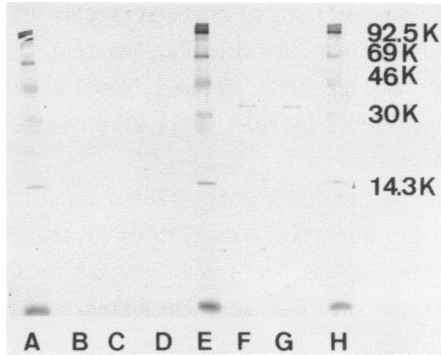

Fig. 1 : SDS-PAGE of translation products of the mRNAs selected by pMIF 3/10 and pMIF 20/11 (autoradiography).
Tracks A, E and H : ^{14}C labelled protein markers.
Track B : no mRNA added to the translation system.
Track C : product of translation of mRNA selected by plasmid pMIF 3/6.
Track D : product of translation of mRNA selected by plasmid pMIF 20/8.
Track F : product of translation of mRNA selected by pMIF 3/10.
Track G : product of translation of mRNA selected by pMIF 20/11.

Expression in induced and uninduced C243 cells of IFN mRNAs related to plasmids pMIF 3/10 and pMIF 20/11. Significant levels of IFN are not produced by uninduced C243 cells, and IFN mRNA has not been detected in such cells (D. Skup, unpublished data). It was therefore expected that plasmids pMIF 3/10 and pMIF 20/11 would hybridize strongly to mRNA species derived from induced cells, but hardly, or not at all, to mRNA derived from uninduced cells. To test this hypothesis, a blot hybridization assay was carried out using polysomal poly(A)$^+$RNA derived from NDV induced and uninduced C243 cells. Following extraction, poly(A)$^+$RNA was fractionated on a 1.5 % agarose gel containing 4 mM methylmercury hydroxide. The RNA from the gel was transferred to Gene Screen and the resulting blot was hybridized to nick translated pMIF 3/10 and pMIF 20/11V The result is shown in figure 2. Neither pMIF 3/10 nor pMIF 20/11 hybridize to the RNA from uninduced C243 cells (Tracks A and C). However, both pMIF 3/10 and pMIF 20/11 hybridize strongly to two bands of mRNA from induced cells (tracks B and D) ; these bands correspond to the positions at which 16 and 12S RNA would migrate.

Physical analyses on plasmids pMIF 3/10 and pMIF 20/11. In the preceding sections strong biological evidence is presented that plasmids pMIF 3/10 and pMIF 20/11 contain cDNA copies of mouse IFN mRNA molecules. Further-

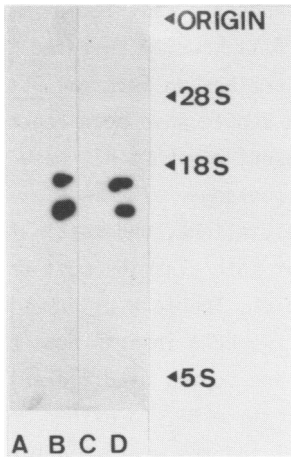

Fig. 2 : Blot hybridization assay of poly(A)$^+$RNA derived from uninduced versus NDV-induced C243 cells.
Tracks A and B : hybridization to ∿ 3 x 10^6 dpm of nick translated plasmid DNA from MIF 3/10 (specific activity ∿ 4 x 10^7 cpm/µg).
A : RNA was from uninduced cells (4 µg).
B : RNA was from NDV-induced cells (4 µg).
Tracks C and D : hybridization to ∿ 3 x 10^6 dpm of nick translated plasmid DNA from MIF 20/11 (specific activity ∿ 4.2 x 10^7 cpm/µg) .
C : RNA from uninduced cells (4 µg).
D : RNA from NDV-induced cells (4 µg).
Positions of 28, 18 and 5S markers, as visualized with ethidium bromide, are given on the right hand side of the figure.

more, evidence is given that the translation products of the mRNA species complementary to the cDNA inserts of both plasmids are of the β type. Nevertheless, we were unable to detect cross hybridization of the cDNA inserts in Grunstein-Hogness (28) colony hybridization experiments. The two inserts must therefore either be copies of two rather distinct mRNA species or alternatively they may be incomplete copies of different regions of a single IFN mRNA molecule.

To obtain an estimate of the size of the cDNA inserts of plasmids pMIF 3/10 and pMIF 20/11, they were digested with PstI and subjected to polyacrylamide gel electrophoretic analysis. As the poly dG-poly dC tailing method of cloning into a PstI site normally regenerates PstI sites at either side of the insert (32), this analysis suggested that the cDNA insert in pMIF 3/10 was about 290 base pairs long and that in pMIF 20/11 was about 350 base pairs long. Further restriction enzyme analysis of pMIF 3/10 confirmed its

size and enabled us to construct the restriction map shown in figure 3 (top). A similar analysis of pMIF 20/11 however revealed that the apparent insert size was incorrect. This insert has an internal PstI site and lacks one of the two flanking sites which should have been regenerated in cloning. pMIF 20/11 actually has a cDNA insert of about 470 base pairs and its restriction map is as shown in figure 3 (bottom).

Despite the initial, slight, underestimate in size of plasmid pMIF 20/11, it is still clear that both plasmids must only be partial copies of either a 12 or 16S mRNA species. The lack of any similarity in the restriction maps (figure 3) of the two cDNA inserts does not therefore resolve the question as to whether they are copies of different mRNA species or copies of different regions of the same mRNA. To extend the comparison between plasmids pMIF 3/10 and pMIF 20/11 and the mRNA molecules from which they were derived the nucleotide sequence of both cDNA inserts was determined, according to the strategy shown in figure 3. The sequences of the inserts are shown in figure 4. These sequences demonstrate conclusively that the two cDNA inserts must have been derived from two different mRNA species. Both inserts contain poly dA.poly dT tracts at one end, preceded 18 or 21 base pairs away by the AATAAA polyadenylation signal (33). These must represent copies of the extreme 3' ends of the corresponding mRNA molecules. The lack of other obvious

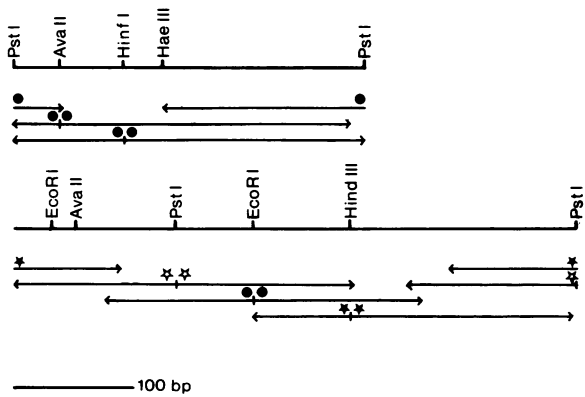

Fig. 3 : Restriction maps and DNA sequence determination strategies for the cDNA inserts of plasmids pMIF 3/10 (top) and pMIF 20/11 (bottom). Arrows indicate the direction and extent of the sequence determined from each labelling position. Those arrows without bars indicate fragments whose sequences were determined from flanking pAT 153 MspI (HpaII) sites. Fragment labelling methods are indicated : 5' labelling ● , 3' fill in labelling ★ and 3' cordycepin labelling ✡

sequence homology between the inserts proves therefore that they are copies of the 3' ends of distinct mRNA species.

The sequences of the regions of both cDNA inserts before the AATAAA sequences have the appearance of typical 3' non-coding regions of mRNA, in that there are extensive oligo dA and oligo dT tracts present (33). However,

A

HisCysLeuTrpThrAspGlnValLeuValGlySerGluAspTyrGlnSerArgHisPheAlaCysLeu
GTCACTGTTTGTGGACGGATCAGGTCCTCGTGGGCTCTGAGGACTACCAGAGCCGTCACTTTGCTTGCCTG

ProArgAsnLeuGlyLeuCysThrTrpArgSerLeuGlyAlaArgTer
CCACGGAATCTAGGCTTGTGCACTTGGAGATCCCTTGGGGCCCGATGACCTGAAGCCTTCCCCTAGGAAAA

ACTGAAGCCTGAACACTGTCTACTTTTCCTCCATCTTTCTTTCTCTTAGATGGTGAAATAAAGAACTATCA

GACAGCAGCAAAAAAAAAAAAAAAAAAAAAA

B

HisLeuGlnThrAsnGluPheLeuArgAsnValPheGluLeuGlyProProValMetLeuAspAlaAla
ACCACTTGCAGACAAATGAATTCCTTCGAAATGTATTTGAGGTTGGACCCCCTGTGATGCTTGATGCTGCA

ThrLeuLysThrMetLysIleSerArgPheGluArgHisLeuTyrAsnSerAlaAlaPheLysAlaArgThr
ACACTTAAAACCATGAAGATTTCTCGTTTTGAAAGGCATTTATATAACTCTGCAGCTTTCAAAGCTCGAACA

LysAlaArgSerLysCysArgAspLysArgAlaAspValGlyGluPheLeuTer
AAAGCTCGAAGCAAATGCCGAGATAAGAGAGCAGATGTTGGAGAATTCTTGTAGATGTCTGAATTTGATGGC

TGTTTTCTAATCTCTTCCTTTATTATTATTTTTGCTACTTCTAATGTATATAAGCTTTTAGAGACAGTTTTT

TATCTTGGTCAACTTAAATAATTTTTGATGTAGGGGTGGGTTGTATTTTAATTTAATGTACAGTGTTACAAA

TTAATGAGTTCTTTATTCTCTGTAAAAATAACTGGTAACCACAAATAAAGTGTTTGTGATGTTTGGTCGTAA

AAAAAAAAAAAAA

Fig. 4 : DNA sequence of the cDNA inserts of plasmids pMIF 3/10 and pMIF 20/11
The sequence of the non-coding strand only, together with the corresponding amino acids, are given for the most probable open reading frame. G-C tails are not shown. The AATAAA polyadenylation signal is boxed.
A : sequence of the cDNA insert from pMIF 3/10
B : sequence of the cDNA insert from pMIF 20/11.

analysis of the sequence of the inserts for open translation reading frames shows that both have relatively extensive ones entering the opposite end of the insert to the poly dA.poly dT tract. The amino acid sequence of these open reading frames is 39 amino acids long for pMIF 3/10 and 65 amino acids long for pMIF 20/11 (figure 4). If these amino acid sequences correspond to the authentic carboxy terminal sequences of the polypeptide products of the mRNA species from which pMIF 3/10 and pMIF 20/11 were derived, then the pMIF 3/10 mRNA must have a 3' non-coding region of 102 nucleotides and the pMIF 20/11 mRNA one of 231 nucleotides.

DISCUSSION

In this present publication we report the molecular cloning of cDNA sequences which correspond to partial copies of two distinct mouse IFN-β mRNAs. The identification of these cDNA clones and additional proof of their identity rests on compelling biological evidence on the characterization of the complementary mRNA species and their translation products.

Firstly, our screen for IFN cDNA clones was based not only on the ability of the recombinant plasmids to hybridize specifically to mRNA species which can be translated *in vitro* to give antiviral products, but the *in vitro* translation products were first selected by binding to a specific anti-mouse IFN antibody column. Elution of the *in vitro* translation products from the antibody column was at pH 2, and a well documented characteristic of interferon is the resistance of its activity to such low pHs (34). The antiviral activity of the *in vitro* translation products of the mRNA species hybridizing to both cDNA clones is neutralized specifically by an anti-mouse IFN-β serum.

The natural 35 K mouse IFN-β species being glycosylated (16), one would expect its *in vitro* translation product to migrate faster on PAGE gel. The size of the ^{35}S-methionine labelled *in vitro* translation products of the specifically hybridizing mRNA species, (± 34 K), is quite close to that of *in vivo* produced mouse IFN-β (12). Since the size of the sugar moiety is unknown, it is impossible to predict the size of the unglycosylated product ; moreover, the presence of a leader sequence on the *in vitro* translation product may compensate, to some extent for the absence of carbohydrates.

Both plasmids pMIF 3/10 and pMIF 20/11 hybridize to mRNA species present in NDV-induced C243 cells but which are absent in mRNA from uninduced cells, as would be expected for IFN cDNA clones. We have found that other clones from our cDNA library which contain cDNA sequences which cross-hybridize to the first two plasmids, pMIF 3/10 and pMIF 20/11 also hybridize spe-

cifically to C243 cell mRNA species which give in vitro translation products with antiviral activity. In addition, it has recently been demonstrated that there are nucleotide sequences present in the genomes of mice which are complementary to the cDNA inserts of these plasmids (unpublished results).

Despite the evident similarities in the behaviour of the IFN mRNA molecules of which these plasmids contain copies, they are obviously distinct. NDV-induced C243 cell IFN mRNA populations were found to be heterogeneous, as judged by their sedimentation profiles on sucrose gradients. Two distinct peaks of activity, migrating at approximately 12 and 16S were found. Plasmid pMIF 3/10 originated from the 16S IFN mRNA, plasmid pMIF 20/11 from 12S mRNA. The two plasmid inserts fail to cross-hybridize detectably and nucleotide sequence analysis demonstrates that there is no significant homology between the regions of the two mRNA species represented in these cDNA inserts. Thus there must be at least two forms of IFN-β mRNA expressed in NDV induced mouse C243 cells. Furthermore, these two forms must be much more distantly related than the genes of the human IFN-α family, judging from the nucleotide sequences we have determined. Additional evidence for heterogeneity within the mRNA populations containing sequences complementary to the cDNA inserts in both plasmids pMIF 3/10 and pMIF 20/11 is provided by the RNA blot analysis of NDV induced C243 cells mRNA. Each plasmid hybridizes to two different sizes of mRNA, corresponding approximately to the size classes of C243 cell IFN mRNA found on sucrose density gradients. This may seem surprising, but a possible explanation could be the existence of further heterogeneity of families of related mRNAs.

Although neither the nucleotide sequences of the pMIF 3/10 and pMIF 20/11 cDNA inserts nor the amino acid sequences of the carboxy terminal regions of the probable translation products of the corresponding mRNA species display extensive homology, both amino acid sequences do contain regions which appear to show some homology to regions of human IFNs. This point is illustrated in figure 5. In figure 5A, a region of the major open reading frame of the pMIF 3/10 insert is shown, compared by amino acid and corresponding coding sequences to the regions of human IFN-β_1 from amino acids 140-148 and to human IFN-α_1 from amino acids 138-146 (35). Allowing for the deletion of one amino acid and its codon from the human sequences, 6 out of 10 amino acids coincide between the pMIF 3/10 derived sequence and that of IFN-β_1. Four of these amino acids are also conserved in the human IFN-α_1 sequence as well. One of the amino acid differences between the pMIF 3/10 sequence and the human ones is also a very conservative one : leu ——

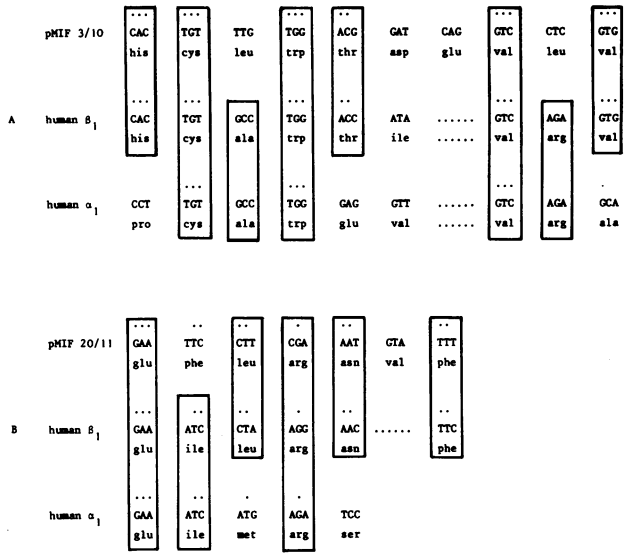

Fig. 5 : Comparison of regions of homology of sequences of the cDNA insert of plasmids pMIF 3/10 and pMIF 20/11 to regions of the sequences of human IFN-β_1 and IFN-α_1 and their mRNAs.
A : pMIF 3/10 insert from nucleotide 3 to 33 with AA sequence of most probable reading frame. Human IFN-β_1 mRNA sequence from AA 140-148 (assume deletion between AA 145 and 146). Human IFN-α_1 mRNA sequence from AA 138-146 (assume deletion between AA 143 and 144).
B : pMIF 20/11 insert from nucleotides 18 to 38 with 11 sequence of most probable reading frame. Human IFN-β_1 mRNA sequence from AA 149-154 (assume deletion between AA 153 and 154). Human IFN-α_1 mRNA sequence from AA 147-151.
Boxed AA show homology. Dotted nucleotides are homologous to the corresponding pMIF sequence. Sequences of IFN-β_1 and -α_1 are from (35).

ala. This is particularly striking as the leucine residue lies in the center of a run of five conserved amino acids in a region previously identified as being highly conserved between human α and β IFNs (35). Just as there is a striking similarity between the amino acids in this region of the probable protein derived from the mRNA from which pMIF 3/10 was derived and the human IFNs there also appears to be a surprisingly high conservation of the codons used to code for the identical amino acids. Part B of figure 5 shows a comparison of a region of the insert of pMIF 20/11 and the polypeptide probably coded for by the corresponding mRNA with the region from amino acids 149-154 of human IFN-β_1 (35), and 147-151 of human IFN-α_1. Again, allowing for one amino acid deletion in the human IFN-β_1

sequence, 5 out of 7 amino acids are identical between the pMIF 20/11 coded polypeptide and the human IFN-β_1 segment. Two of these amino acids are also conserved in the human IFN-α_1. We feel that such a degree of homology between the probable translation products of the mRNA species for which plasmids pMIF 3/10 and pMIF 20/11 were derived and human interferons is too high to be due to chance. Thus some evolutionary relationship between these proteins seems probable. Whether the mouse IFN-β genes of which pMIF 3/10 and pMIF 20/11 contain partial copies might correspond more closely with the other human IFN-β genes described (8, 9) is at present unclear.

Thus our work has demonstrated that there are at least 2 forms of IFN-β in the mouse, both of which appear to show some homology with human IFN-β_1. Future work should allow the isolation of plasmids containing more complete copies of the mRNA species from which the pMIF 3/10 and pMIF 20/11 inserts were derived and of corresponding regions of genomic DNA. These will be most useful in both extending the comparisons between the sequence and organisation of these mouse IFN-β genes with human genes and in producing individual species of murine interferons for studying the various biological effects of different interferon species. In addition, the availability of mouse interferon cDNA probes will greatly facilitate the study of the regulation and control of mouse IFN synthesis *in vivo* and *in vitro*.

ACKNOWLEDGEMENTS

We are indebted to A. Cachard, L. Eusebe and J. Hurst for excellent technical assistance, and to K.S.N. Prasad for help in mRNA extraction. We also thank Drs. W. Brammar and P. Meacock for valuable discussion of the work.

This work was supported by the Ligue Nationale Française contre le Cancer, the Fondation pour la Recherche Médicale Française, ICI-Pharma and the C.N.R.S. (AI 03 18 58). B.W. was supported by the Canadian MRC.

REFERENCES

1. Nagata, S., Taira, H., Hall, A., Johnsrud, L., Streuli, M., Ecsödi, J., Boll, W., Cantell, K., and Weissmann, C. (1980) Nature 284, 316-320.
2. Goeddel, D.V., Yelverton, E., Ullrich, A., Heyneker, H.L., Miozzari, G., Holmes, W., Seeburg, P.H., Dull, T., May, L., Stebbing, N., Crea, R., Maeda, S., McCandliss, R., Sloma, A., Tabor, J.M., Gross, M., Familletti, P.C., and Pestka, S. (1980) Nature 287, 411-416.
3. Taniguchi, T., Ohno, S., Fujii-Kuriyama, Y., and Muramatsu, M. (1980) Gene 10, 11-15.
4. Derynck, R., Content, J., De Clercq, E., Volckaert, G., Tavernier, J., Devos, R., and Fiers, W. (1980) Nature 285, 542-547.
5. Goeddel, D.V., Shepard, H.M., Yelverton, E., Leung, D., Crea, R.,

Sloma, A., and Pestka, S. (1980) Nucleic Acids Res. 8, 4057-4074.
6. Brack, C., Nagata, S., Mantei, N., and Weissmann, C. (1981) Gene 15, 379-394.
7. Tavernier, J., Derynck, R., and Fiers, W. (1981) Nucleic Acids Res. 9, 461-471.
8. Weissenbach, J., Chernajovsky, Y., Zeevi, M., Shulman, L., Soreq, H., Nir, U., Wallach, D., Perricaudet, M., Tiollais, P., and Revel, M. (1980) Proc. Natl. Acad. Sci. USA 77, 7152-7156.
9. Sehgal, P.B., and Sagar, A.D. (1980) Nature 287, 95-97.
10. Cabrer, B., Taira, H., Broeze, R.J., Kempe, T.D., Williams, K., Slattery, E., Konigsberg, W.H., and Lengyel, P. (1979) J. Biol. Chem. 254, 3681-3684.
11. Cachard, A., and De Maeyer-Guignard, J. (1981) Ann. Virol. 132E, 307-312.
12. Taira, H., Broeze, R.J., Jayaram, B.M., Lengyel, P., Hunkapiller, M.W., and Hood, L.E. (1980) Science 207, 528-530.
13. Maxam, A.M., and Gilbert, W. (1977) Proc. Natl. Acad. Sci. USA 74, 560-564.
14. Oie, H.K., Gazdar, A.F., Buckler, C.E., and Baron, S. (1972) J. gen. Virol. 17, 107-109.
15. Skup, D., Zarbl, H., and Millward, S. (1981) J. Mol. Biol. 151, 35-55.
16. De Maeyer-Guignard, J., Tovey, M.G., Gresser, I., and De Maeyer, E. (1978) Nature 271, 622-625.
17. Laemmli, U.K. (1970) Nature 227, 680-685.
18. Kay, R.M., Harris, R., Patient, R.K., and Williams, J.G. (1980) Nucleic Acids Res. 8, 2691-2707.
19. Shenk, T.E., Rhodes, C., Rigby, P.W.J., and Berg, P. (1975) Proc. Natl. Acad. Sci. USA 72, 989-993.
20. Roychoudhury, R., Jay, E., and Wu, R. (1976) Nucleic Acids Res. 3, 101-116.
21. Twigg, A.J., and Sherratt, D. (1980) Nature 283, 216-218.
22. Clarke, L., and Carbon, J. (1978) J. Mol. Biol. 120, 517-532.
23. Mandel, M., and Higa, A. (1970) J. Mol. Biol. 53, 159-162.
24. Windass, J.D., and Brammar, W.J. (1979) Molec. Gen. Genet. 172, 329-337.
25. Sharp, P.A., Sugden, B., and Sambrook, J. (1973) Biochemistry 12, 3055-3063.
26. Maniatis, T., Jeffrey, A., and van DeSande, H. (1975) Biochemistry 14, 3787-3794.
27. Smith, H.O. (1980) Methods in Enzymology 65, 371-380.
28. Grunstein, M., and Hogness, D.S. (1975) Proc. Natl. Acad. Sci. USA 72, 3961-3965.
29. Rigby, P.W.J., Dieckmann, M., Rhodes, C., and Berg, P. (1977) J. Mol. Biol. 113, 237-251.
30. Bailey, J.M., and Davidson, N. (1976) Anal. Biochem. 70, 75-85.
31. Denhardt, D.T. (1966) Biochem. Biophys. Res. Commun. 23, 641-646.
32. Williams, J.G. (1981) In : "Genetic Engineering", 1, 1-59, Academic Press.
33. Proudfoot, N.J., and Brownlee, G.G. (1976) Nature 263, 211-214.
34. Interferon Nomenclature (1980) Nature 286, 110.
35. Taniguchi, T., Mantei, N., Schwarzstein, M., Nagata, S., Muramatsu, M., and Weissmann, C. (1980) Nature 285, 547-549.