Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Human Fibroblast and Human Immune Interferon Genes and Their Expression in Homologous and Heterologous Cells [and Discussion]

W. Fiers, E. Remaut, R. Devos, H. Cheroutre, R. Contreras, D. Gheysen, W. Degrave, P. Stanssens, J. Tavernier, Y. Taya and J. Content

Phil. Trans. R. Soc. Lond. B 1982 **299**, 29-38
doi: 10.1098/rstb.1982.0103

Email alerting service Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here**

To subscribe to *Phil. Trans. R. Soc. Lond. B* go to: **http://rstb.royalsocietypublishing.org/subscriptions**

This journal is © 1982 The Royal Society

Downloaded from rstb.royalsocietypublishing.org

Phil. Trans. R. Soc. Lond. B **299**, 29–38 (1982) [29]
Printed in Great Britain

The human fibroblast and human immune interferon genes and their expression in homologous and heterologous cells

By W. Fiers, E. Remaut, R. Devos, H. Cheroutre, R. Contreras, D. Gheysen, W. Degrave, P. Stanssens, J. Tavernier, Y. Taya and J. Content†

Laboratory of Molecular Biology, State University of Ghent, Ledeganckstraat 35, *B*-9000 *Ghent, Belgium*

† *Institut Pasteur du Brabant*, 28 *rue du Remorqueur*, 1040 *Brussels, Belgium*

The genetic information coding for human fibroblast interferon (IFN-β) has been cloned both as a DNA copy (cDNA) and as a genomic clone. Human IFN-β is made as a precursor and consists of a signal sequence 21 amino acid residues long followed by the mature protein 166 amino acids long. A single site for glycosylation is present. The human IFN-β gene does not contain introns.

Transfection of monkey cells with a chimeric SV40 derivative containing the human IFN-β cDNA clone under control of the late SV40 promoter leads to secretion of high levels of IFN-β. When a genomic clone is used in the same vector, IFN-β synthesis can be further enhanced up to 30-fold by treatment with poly(rI)·poly(rC); this shows that a *cis*-active control element is present in the clone. An efficient expression system in *Escherichia coli* was worked out based on a plasmid containing the promoter P_L of bacteriophage λ, which is regulated by a temperature-sensitive repressor. This promoter is followed by a segment derived from bacteriophage MS_2 that contains the ribosome-binding site of the replicase gene. The latter, however, is replaced by the human IFN-β gene. Upon induction, high levels (about 5×10^9 IU l^{-1}) of IFN-β are synthesized by the bacteria; this corresponds to about 2% of the total bacterial protein.

The human immune (type II) interferon (IFN-γ) gene has similarly been cloned. Partly purified mRNA derived from human spleen cells that had been induced with staphylococcal enterotoxin A was used as starting material. A full-length cDNA clone was sequenced. The total cDNA sequence is about 1150 nucleotides long; it contains a single open reading frame coding for 166 amino acids, the first 20 of which constitute the transmembrane signal. There are two sites for glycosylation. The amino acid sequence is quite different from that of IFN-α or IFN-β, although a few similarities can be noted. The untranslated 3′-terminal region is about 550 nucleotides long. The IFN-γ gene was expressed in monkey cells, again by using the SV40-derived vector, and the secreted product was characterized as true human IFN-γ. A genomic clone in the form of a bacteriophage λ derivative was also obtained. The IFN-γ gene extends over at least 5 kilobases and contains at least two introns.

The human fibroblast inferferon gene: IFN-β_1

In the early experiments by Alick Isaacs & Jean Lindenmann (1957), interferon was induced in membrane fragments of chick allantois by infection with influenza virus. It is likely that this treatment produced mainly β or fibroblast-type IFN and some IFN-α. Both IFN-α and IFN-β belong to the acid-stable interferon I group. They have many biological properties in common: both apparently induce the same type of antiviral state in susceptible target cells, they induce the same types of enzymes and polypeptides in their target cells, they share the same receptor, show similar activation of Natural Killer (NK) cells, and so on.

The Royal Society is collaborating with JSTOR to digitize, preserve, and extend access to *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences.* www.jstor.org

Downloaded from rstb.royalsocietypublishing.org

'Fibroblast' interferon, however, is a misnomer because IFN-β can also be induced in many other types of cell; for example leucocytes or lymphoblastoid cell lines can produce some IFN-β as well as producing IFN-α. Not all of the biological properties of IFN-β are identical to those of IFN-α. The spectrum of cell lines that respond in an antiproliferative assay is different, the dose–response curve is different, the species specificity of IFN-β is more restricted than that of IFN-α and so on. Their chemical properties also differ: IFN-β is more labile, is much more hydrophobic and it is a glycoprotein whereas IFN-α is not.

To characterize this system in molecular detail and with the particular intention of making available larger quantities of IFN-β, we decided to clone the human fibroblast IFN gene. The procedure we followed was quite straightforward (Derynck *et al.* 1980). Messenger RNA was isolated from primed and superinduced VGS cells (a human diploid fibroblast cell line). This mRNA was partially purified by centrifugation in a sucrose gradient containing 50% formamide and the resulting fractions assayed for IFN-β mRNA activity by injecting a portion of each fraction into *Xenopus laevis* oocytes. These then translated and secreted IFN, which could be quantitated in a conventional assay. The mRNA present in the active fractions was converted into duplex DNA, tailed and cloned into the *Pst*I site of the plasmid pBR322. By using 0.128 μg insert DNA, a library containing 17000 transformants was obtained. The most tedious part of the project was screening for the colonies that contained genuine IFN-β information. This was done by a procedure called group selection. Plasmid DNA isolated from groups of 50 clones was chemically linked to diazobenzyloxymethyl paper. The paper discs were then used for hybridization with active IFN-β mRNA and both the hybridized (linked to the DNA-paper) fraction and the unhybridized (supernatant) fraction were tested by injection into *Xenopus* oocytes. As many factors exert a negative influence on such experiments, a positive internal control was included each time. This consisted of a small amount of DNA corresponding to Satellite Tobacco Necrosis virus (STNV) added to the plasmid DNA preparation and STNV RNA added to the hybridization mixture (Van Emmelo *et al.* 1980). STNV RNA is an extremely good messenger that can be readily translated *in vitro* in a wheatgerm extract. By using this procedure, several groups of 50 clones were found to retain the human IFN-β mRNA selectively. Such a positive group was then divided into subgroups, analysed as before and, finally, individual positive clones were isolated. The first plasmid obtained was named pHFIF-1 (Derynck *et al.* 1980). Pure IFN-β mRNA can be obtained by hybridization to pHFIF-1 DNA. Upon translation in a reticulocyte system *in vitro*, a polypeptide of 18000 Da was obtained that was immunoprecipitable with antiserum raised against (partly) purified human IFN. This 18000 Da protein corresponds to preinterferon. If dog pancreas microsomes are added to the translation system *in vitro*, processing and glycosylation can also occur. Indeed, instead of the 18000 Da component we then observed a 16500 Da polypeptide corresponding to mature IFN-β and a 20500 Da product corresponding to the glycosylated derivative. The molecular masses given above are based on mobility in sodium dodecyl sulphate – polyacrylamide gels. This mobility is unusually large presumably because of the very hydrophobic nature of IFN-β.

We then proceeded to sequence the DNA of our human IFN-β clones. Here we ran into unexpected difficulties because most of the clones contained bizarre rearrangements. This was possibly due to an unusual primary or secondary structure of the IFN-β mRNA; this problem has been studied and discussed in more detail by Volckaert *et al.* (1981). Nevertheless, it was possible to reconstruct the correct sequence of the IFN-β mRNA and on this basis we were able

Downloaded from rstb.royalsocietypublishing.org


```
                                                                                                                 -20
                                                                                                                 MET-THR-ASN-LYS-CYS-LEU-LEU-GLN-ILE-
mGpppAUU CUAACUGCAA CCUUUCGAAG CCUUUGCUCU GGCACAACAG GUAGUAGGCG ACACUGUUCG UGUUGUUGAC [AUG] ACC.AAC.AAG.UGU.CUC.CUC.CAA.AUU. 100

    -10                                     -1 +1                                               10
ALA-LEU-LEU-LEU-CYS-PHE-SER-THR-THR-ALA-LEU-SER-[MET-SER-TYR-ASN-LEU-LEU-GLY-PHE-LEU-GLN-ARG-SER-SER]-ASN-PHE-GLN-CYS-GLN-
GCU.CUC.CUG.UUG.UGC.UUC.UCC.ACU.ACA.GCU.CUU.UCC.AUG.AGC.UAC.AAC.UUG.CUU.GGA.UUC.CUA.CAA.AGA.AGC.AGC.AAU.UUU.CAG.UGU.CAG. 190

    20                                      30                                      40
LYS-LEU-LEU-TRP-GLN-LEU-ASN-GLY-ARG-LEU-GLU-TYR-CYS-LEU-LYS-ASP-ARG-MET-ASN-PHE-ASP-ILE-PRO-GLU-GLU-ILE-LYS-GLN-LEU-GLN-
AAG.CUC.CUG.UGG.CAA.UUG.AAU.GGG.AGG.CUU.GAA.UAC.UGC.CUC.AAG.GAC.AGG.AUG.AAC.UUU.GAC.AUC.CCU.GAG.GAG.AUU.AAG.CAG.CUG.CAG. 280

    50                                      60                                      70
GLN-PHE-GLN-LYS-GLU-ASP-ALA-ALA-LEU-THR-ILE-TYR-GLU-MET-LEU-GLN-ASN-ILE-PHE-ALA-ILE-PHE-ARG-GLN-ASP-SER-SER-SER-THR-GLY-
CAG.UUC.CAG.AAG.GAG.GAC.GCC.GCA.UUG.ACC.AUC.UAU.GAG.AUG.CUC.CAG.AAC.AUC.UUU.GCU.AUU.UUC.AGA.CAA.GAU.UCA.UCU.AGC.ACU.GGC. 370

    80                                      90                                      100
TRP-ASN-GLU-THR-ILE-VAL-GLU-ASN-LEU-LEU-ALA-ASN-VAL-TYR-HIS-GLN-ILE-ASN-HIS-LEU-LYS-THR-VAL-LEU-GLU-GLU-LYS-LEU-GLU-LYS-
UGG.AAU.GAG.ACU.AUU.GUU.GAG.AAC.CUC.CUG.GCU.AAU.GUC.UAU.CAU.CAG.AUA.AAC.CAU.CUG.AAG.ACA.GUC.CUG.GAA.GAA.AAA.CUG.GAG.AAA. 460

    110                                     120                                     130
GLU-ASP-PHE-THR-ARG-GLY-LYS-LEU-MET-SER-SER-LEU-HIS-LEU-LYS-ARG-TYR-TYR-GLY-ARG-ILE-LEU-HIS-TYR-LEU-LYS-ALA-LYS-GLU-TYR-
GAA.GAU.UUC.ACC.AGG.GGA.AAA.CUC.AUG.AGC.AGU.CUG.CAC.CUG.AAA.AGA.UAU.UAU.GGG.AGG.AUU.CUG.CAU.UAC.CUG.AAG.GCC.AAG.GAG.UAC. 550

    140                                     150                                     160
SER-HIS-CYS-ALA-TRP-THR-ILE-VAL-ARG-VAL-GLU-ILE-LEU-ARG-ASN-PHE-TYR-PHE-ILE-ASN-ARG-LEU-THR-GLY-TYR-LEU-ARG-ASN
AGU.CAC.UGU.GCC.UGG.ACC.AUA.GUC.AGA.GUG.GAA.AUC.CUA.AGG.AAC.UUU.UAC.UUC.AUU.AAC.AGA.CUU.ACA.GGU.UAC.CUC.CGA.AAC [UGA] AGAUC 642

UCCUA GCCUGUGCCU CUGGGACUGG ACAAUUGCUU CAAGCAUUCU UCAACCAGCA GAUGCUGUUU AAGUGACUGA UGGCUAAUGU ACUGCAUAUG AAAGGACACU AGAAG 752

AUUUU GAAAUUUUUA UUAAAUUAUG AGUUAUUUUU AUUUAUUUAA AUUUUAUUUU GGAA[AAUAAA]UUAUUUUUGG UGCAAAAGUC AAAAAAAAA_n ...
```

FIGURE 1. Nucleotide sequence of human IFN-β mRNA and deduced amino acid sequence. The nucleotide sequence is presented as a messenger RNA and was determined on several cDNA clones and also on a genomic clone. The arrow indicates the start of the cDNA clone. The initiation and termination codons are boxed; the AAUAAA sequence commonly present near the poly(A)-addition site in nearly all mRNAs is shown in a dashed box. The coding region is translated into an amino acid sequence consisting of a signal peptide 21 amino acid residues long, followed by the mature IFN-β polypeptide 166 amino acids long. The N-terminal 13 amino acid residues shown in a box were directly determined on authentic IFN-β by Knight *et al.* (1980). The asparagine residue at position 80 is presumably glycosylated.

Downloaded from rstb.royalsocietypublishing.org

to deduce the total amino acid sequence of human (pre)interferon-β (figure 1). Taniguchi *et al.* (1980) independently isolated a human IFN-β gene and their nucleotide sequence differed from ours in only one position. This corresponded to a 'wobble' third letter of a codon, and, as we have observed either T or C in this position in different clones, it may be an example of allelic variation.

The nucleotide sequence of the copy DNA (cDNA) clone revealed a single 'open' reading frame, which can be translated into an amino acid sequence (figure 1). The first 21 residues constitute the signal sequence, which is processed away upon membrane passage. The next 13 amino acid residues were identified as the N-terminal sequence of authentic IFN-β by Knight *et al.* (1980). The mature IFN-β has a length of 166 amino acids. The polypeptide contains three cysteine residues, and by analogy with leucocyte IFN-α, it is likely that Cys 31 and Cys 141 are linked in a disulphide bridge. IFN-β is known to be a glycoprotein and the glycosyl group is undoubtedly linked to the asparagine residue at position 80. It is interesting that leucocyte IFN-α, which is not a glycoprotein, contains an aspartic acid or a glutamic acid residue at the corresponding position (Weissmann 1981).

The clone obtained by reverse transcription of an mRNA is called a cDNA clone. This clone, in a highly radiolabelled form, was used to probe restricted human DNA by 'Southern' analysis. By using human DNA digested with various restriction enzymes, which do not cleave the cDNA information, only one band was revealed after hybridization and autoradiography (Tavernier *et al.* 1981). These results indicate that there is only one human fibroblast IFN gene, as least when judged by the criterion of non-hybridization. In this respect, IFN-β is very different from leucocyte IFN, which exists as a large family of genes.

Almost the whole of the human genome has been cloned in a bacteriophage λ derivative by Maniatis and his colleagues (Lawn *et al.* 1978). From this library, a genomic IFN-β clone was isolated (Degrave *et al.* 1981). Detailed restriction mapping and DNA sequencing revealed that the cDNA was completely collinear with the genomic clone. This means that the IFN-β gene does not contain introns. Similar findings have also been made by Streuli *et al.* (1980) for the IFN-α genes. The region preceding the start of the mRNA contains the putative promoter, where the eukaryotic RNA polymerase II binds and initiates transcription. The nucleotide sequence of this region reveals a number of structural elements that are thought to be characteristic for eukaryotic promoter sequences. Not unexpectedly, in view of the similarity of the control mechanism of gene induction and switch-off, the 5′-flanking region and the 3′-flanking region are quite homologous to the corresponding regions of the IFN-α gene.

Expression of the human IFN-β gene in monkey cells and in *Escherichia coli*

We wished to express the cloned IFN-β gene in conditions approaching those occurring in the natural situation, so that we could start a study of the control mechanisms that normally govern IFN-β expression (including cell specificity, induction, priming, turn-off and superinduction). For this reason, we transferred the IFN-β cDNA insert into the vector pSV529 (Gheysen & Fiers 1982). This vector contains information from the bacterial plasmid pBR322 that allows it to be selected and propagated in *Escherichia coli*, and it contains 1.4 genome equivalents of SV40. The SV40 information (T-antigen and origin of DNA replication) allows the plasmid to be replicated to a high copy number upon transfection of kidney cells of the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

African Green Monkey (the AP-8 cell line was used). Moreover, in the eukaryotic cell, owing to the partial duplication, the SV40 information can be recombined out, thus removing the superfluous bacterial plasmid DNA. The SV40 DNA segment, however, has been modified by removal of the gene coding for the main structural protein (VP1). This was replaced by a single restriction site into which the IFN-β cDNA gene was cloned. Three to four days after infection, large quantities of human IFN-β were released into the medium (titres of more than 3000 IU ml^{-1}† were obtained). Considering that only about 5% of the monkey cells in the monolayer effectively took up DNA (as revealed by T-antigen staining), such results revealed a very active synthesis of IFN-β indeed. Various controls proved that the product was genuine human IFN-β, produced under transcriptional control of the SV40 late promoter. Undoubtedly, under these conditions, the product is correctly processed and is presumably glycosylated.

In the above construction, one would not expect any effect of addition of the inducer poly(rI)·poly(rC), and indeed no effect was found. But we also used an SV40 derivative into which the genomic human IFN-β clone had been inserted. In this construction, about 280 nucleotides upstream from the natural starting site of IFN-β mRNA synthesis were derived from the IFN-β genomic clone. Now a very clear induction effect was observed upon addition of poly(rI)·poly(rC): the increase in IFN-β production after 72 h was 30-fold. These results indicate that the 5′-flanking region together with a putative polymerase II promoter contains a *cis*-acting control element that responds directly or indirectly to the poly(rI)·poly(rC) treatment. In principle, this could involve negative control, i.e. a repressor being inactivated by the induction procedure; or it could be positive control, an essential activator element being formed upon induction, similar to the cyclic AMP plus its corresponding binding protein in *E. coli*. Although no unequivocal data are available as yet, we favour the latter explanation at present, because, unlike many other eukaryotic promoters, transcription of the human IFN-β gene is apparently not correctly initiated in nuclei of *Xenopus laevis* oocytes, and it is unlikely that this is due to repression.

Another important reason for cloning the human IFN-β gene is to work out a procedure for efficient expression in microorganisms such that abundant quantities of IFN-β can be produced economically. Here I must digress somewhat, to discuss briefly an efficient expression system in *E. coli* that we have developed (Remaut *et al.* 1981). This system involves the leftward promoter P_L of bacteriophage λ cloned on the multicopy plasmid pBR322 or a suitable derivative. This promoter is controlled by the repressor protein C_I and by *cro*. We use a C_I repressor in a temperature-sensitive form (the C_I857 mutant), and the corresponding gene is present either on a compatible plasmid, or else on the bacterial chromosome, while the *cro* gene is absent. This expression system offers many advantages: P_L is a strong promoter giving rise to very efficient transcription at 42 °C, complete shut-off is possible at 28 °C and this allows the bacteria to grow despite a harmful or even lethal gene being cloned in the transcription unit (Kastelein *et al.* 1982), and the C_I repressor is not titrated out by an excess of operators because the C_I gene is autoregulatory (Ptashne *et al.* 1976). To illustrate the use of this system, we placed the tryptophan synthetase gene A under this P_L expression control, and obtained synthesis of the polypeptide A in amounts corresponding to almost 40% of the total newly made protein (Remaut *et al.* 1981). Likewise, when the bacteriophage MS_2 polymerase (or replicase) gene was placed under P_L control, synthesis of this polypeptide corresponded to 35% of total protein synthesis, although after a while this product reached concentrations that were toxic to

† Units with reference to NIH human fibroblast interferon standard (GO23-9OC-527).

Downloaded from rstb.royalsocietypublishing.org

the cells (Remaut *et al.* 1982*a*). Starting from this construction, we were able to produce a new chimeric plasmid that again contained the P_L promoter followed by the same region of bacteriophage MS_2 except that it extended exactly to the position of the initiation codon of the polymerase gene and hence included the same ribosome-binding site. But the gene itself was now that of mature IFN-β, which fortuitously also commences with an AUG codon. Upon induction, this system produces human interferon in amounts equivalent to about 2% of the total protein synthesis (Remaut *et al.* 1982*b*). Shifting a culture at a bacterial density of only 4×10^8 cells ml^{-1} still produces 5×10^9 IU l^{-1}, i.e. more than 10 mg l^{-1} interferon, but improved fermentation conditions will undoubtedly enhance the yield still further. It is remarkable that after shift-up to 42 °C, the culture ceases growth almost completely. This is a rather specific effect, not seen with many other similar plasmid constructions containing other genes. Possibly the human IFN-β, by its very hydrophobic nature, interacts with some essential bacterial component, like the inner side of the cell membrane.

Clearly the major goal of high level production of IFN-β by *E. coli* has now been achieved. The next challenge will be to determine whether, and in what conditions, human IFN-β will be useful as an antiviral or as an antitumour agent.

Cloning of the human immune interferon (IFN-γ) gene

IFN-γ is a type II interferon, it is acid-labile, and is induced in (certain) T-lymphocytes by mitogens, by alloantigens or some other treatments. There are several good reasons for exploring the possible use of IFN-γ for cancer treatment and perhaps for the treatment of some immune diseases. It has a more pronounced antiproliferative effect on tumour cells than has IFN-β (Crane *et al.* 1978; Blalock *et al.* 1980), and it can activate NK cells more than IFN-β. IFN-γ retains its activity on cells that are resistant to IFN-α or IFN-β; when tested together with IFN-α or IFN-β, it acts synergistically (Fleischmann *et al.* 1979, 1980), and IFN-γ is not inhibited by gangliosides (Ankel *et al.* 1980). Very recently, Gray *et al.* (1982) reported the first successful cloning of human IFN-γ and its expression. We have done similar work, which is summarized briefly below (see also Devos *et al.* 1982*a*). Cultures of lymphocytes obtained from human spleens were used as starting material. After induction with staphylococcal enterotoxin A, IFN-γ was produced in the culture medium and reached titres of about 10000 laboratory units ml^{-1} (this is equivalent to about 200 leucocyte IU ml^{-1}). This was genuine immune-type IFN, not only because of the nature of the induction procedure used, but also as judged by the criterion of insensitivity towards anti-α or anti-β antisera. Messenger RNA was prepared from such induced splenocyte cultures and the $poly(A)^+$ RNA was fractionated on a sucrose gradient. As with IFN-β, portions of the fractions were injected into *Xenopus* oocytes and after three days, the IFN secreted was assayed by a conventional assay for protection against cytopathogenic effect. IFN-γ mRNA moved at about 13 *S*, i.e. slightly faster than IFN-β mRNA (Devos *et al.* 1982*a*). Other portions of the same gradient fractions were tested by translation *in vitro* in a wheatgerm extract, and this showed that the fractions active in the oocyte assay system coded for polypeptides ranging in size from 15000 to 35000 Da. The active fractions were pooled and recentrifuged through a sucrose gradient in the presence of 50% formamide. Usually, two partly resolved active peaks were observed, and it is still not certain whether this is due to real heterogeneity or to discrete steps in the unfolding of the IFN-γ mRNA. The mRNA in the active fractions was pooled, converted to double-stranded DNA, fractionated by size, tailed and

Downloaded from rstb.royalsocietypublishing.org

cloned. In one experiment more than 8000 clones were obtained. A clone containing genetic information corresponding to IFN-γ was then sought by the hybridization–elution–oocyte translation method as described above for IFN-β. Plasmid DNA was purified by sucrose gradient centrifugation and was immobilized on nitrocellulose filters. After hybridization in the presence of excess mRNA from induced splenocytes, followed by washing and elution, the resulting mRNA was injected into *Xenopus laevis* oocytes. By this procedure, several positive groups were identified that could then be broken down into subgroups; finally a single positive clone, pHIIF-SV-γ0, was identified. By using the insert DNA of this first clone as a radioactive probe, at least three clones of almost full length were isolated; these were designated pHIIF-SV-γ1, γ5 and γ10. The nucleotide sequence was determined by the Maxam & Gilbert technique (1977). There is only one long open reading frame that codes for 166 amino acids and starts near the 5′-end, while at the 3′-end of the same strand, the AAUAAA polyadenylation signal is present. It is remarkable that the untranslated 3′-terminal region is nearly 600 nucleotides long and accounts for almost half of the messenger length.

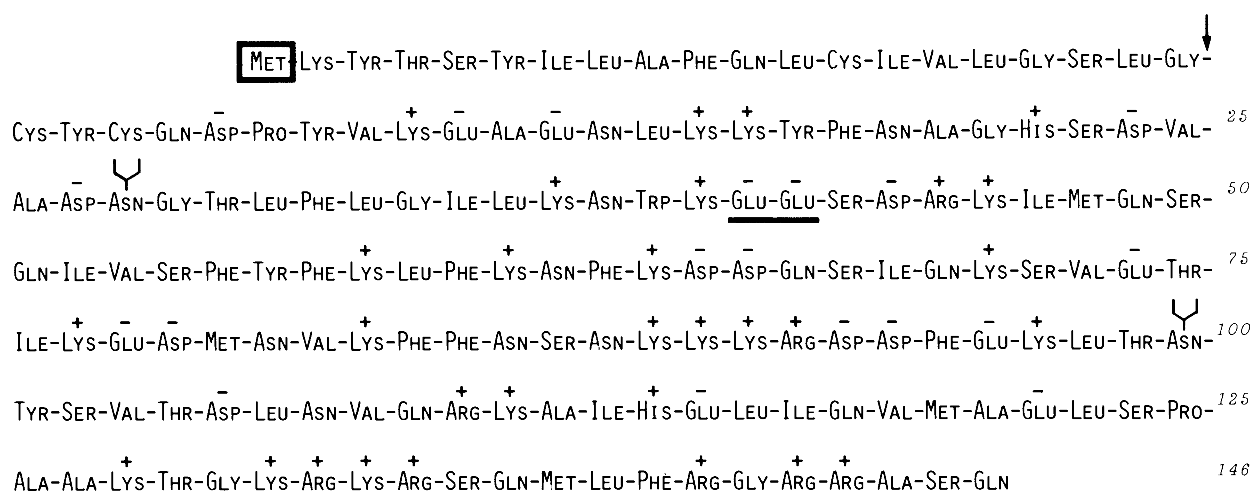

Figure 2. Amino acid sequence of human IFN-γ. The first 20 amino acids constitute the putative signal sequence (the arrow indicates the position of cleavage). Glycosylation presumably occurs on the asparagine residues at positions 28 and 100. The charged residues are as marked and the glutamic acid – glutamic acid doublet, which occurs in all human interferons at about the same position, is also indicated.

The open reading frame can be translated into an amino acid sequence (shown in figure 2). As IFN-γ is a secreted protein one might expect that it would start with a signal sequence. This sequence is presumed to be 20 amino acid residues in length, as residues 18–21, Ser-Leu-Gly-Cys, are also found in IFN-α1 and several other leucocyte IFNs and in these the signal cleavage occurs between the glycine and the cysteine residues (Weissmann 1981). The mature IFN-γ protein would then have a length of 146 amino acids. This is rather surprising as several groups have suggested a molecular mass of 45000 Da or more for authentic IFN-γ (Langford *et al.* 1979; De Ley *et al.* 1980; Fiers *et al.*, unpublished). However, Yip *et al.* (1982) observed that under denaturing conditions, IFN-γ migrated as two peaks at 20000 and 25000 Da. Considering that authentic IFN-γ is glycosylated, these results are compatible with our nucleotide sequence data; the two peaks possibly differ only in the extent of glycosylation. The molecular mass of the native species might thus be due to the formation of a complex, for example a dimer.

There are two likely sites for glycosylation, namely position 28 and position 100. The molecule is not as hydrophilic as IFN-α (and if it is a dimer one would not expect it to be), but neither

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

is it as hydrophobic as IFN-β. There are a large number of charged residues and, more particularly, there are two clusters of four consecutive basic residues, Lys-Lys-Lys-Arg at positions 89–92, and Lys-Arg-Lys-Arg at positions 131–134. In this respect, it may be recalled that IFN-γ is known to be a very basic molecule (Yip *et al.* 1982). Although a superficial study reveals no striking similarity between the amino acid sequence of IFN-γ and IFN-α or IFN-β, Epstein (1982) has nevertheless pointed out a number of identical residues revealed when IFN-γ and IFN-α are aligned. The significance of this homology has yet to be assessed, but particularly noticeable is the glutamic acid – glutamic acid doublet present at positions 41 and 42 in IFN-γ and IFN-α. This doublet is conserved not only in all IFN-α's so far sequenced, but it also occurs in IFN-β (positions 42 and 43). This conservation is intriguing as IFN is supposed to act without internalization and the receptors of IFN-γ and IFN-α or IFN-β are different (Branca & Baglioni 1981). The sequence shown in figure 2 differs in one residue from that published by Gray *et al.* (1982); we found an arginine at position 140 whereas Gray *et al* (1982) reported a glutamine.

In order to prove that one is dealing with the correct clone, one has to be able to express the genetic information in a biologically active form. For this purpose, the IFN-γ cDNA was used in the SV40-derived vector SV529 described above. Upon transfection of AP-8 monkey cells, IFN activity was detected in the supernatant after 2 or 3 days of incubation (Devos *et al.* 1982*b*). This IFN was indeed of the immune type, as it was not inactivated by anti-IFN-α serum or by anti-IFN-β serum, but it was neutralized by anti-IFN-γ serum. Two anti-IFN-γ sera were used, one generously provided by M. P. Langford and G. J. Stanton (Houston), had been raised in rabbits whereas the other was prepared in mice in our own laboratory. Furthermore, the IFN-γ produced by the transfected monkey cells was active on human cells, but not on cells of bovine, murine or feline origin, as is true of authentic IFN-γ.

Using the cDNA clone as a radioactive probe, we again screened the Maniatis human DNA library and by this process we isolated two phages that showed positive hybridization. They turned out to be closely related and presumably they represent the single human IFN-γ gene. Upon injection of this chimeric phage DNA into the nucleus of *Xenopus laevis* oocytes, active synthesis of IFN-γ was again detected, showing that we had isolated a complete and functional gene (at least as far as coding information is concerned). The human cDNA probe hybridized to a region with a length of more than 5 kilobases. This indicates that unlike the genes for IFN-α and IFN-β, the IFN-γ gene does contain introns. By hybridization with separate fragments of the cDNA, a preliminary map of the genomic clone was drawn up and it was deduced that at least two introns must be present.

Obviously, now that a full-length cDNA clone is available, many avenues for further research are open: the gene structure, the induction mechanism, the structure and mechanism of action of IFN-γ, and so on. Moreover, in view of its intriguing and unique biological properties, it is of the utmost importance to obtain human IFN-γ in quantities large enough to enable a proper evaluation of its potential clinical usefulness.

These research projects were supported by grants from Biogen NV, the Fonds voor Geneeskundig Wetenschappelijk Onderzoek and the Geconcerteerde Onderzoekakties of the Belgian Ministry of Science. We thank S. Kaplan for taking care of the manuscript.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

References

Ankel, H., Krishnamurti, C., Besancon, F., Stefanos, S. & Falcoff, E. 1980 Mouse fibroblast (type I) and immune (type II) interferons: pronounced differences in affinity for gangliosides and in antiviral and antigrowth effects on mouse leukemia L-1210R cells. *Proc. natn. Acad. Sci. U.S.A.* **78**, 3469–3472.

Branca, A. A. & Baglioni, C. 1981 Evidence that types I and II interferons have different receptors. *Nature, Lond.* **294**, 768–770.

Blalock, J. E., Georgiades, J. A., Langford, M. P. & Johnson, H. M. 1980 Purified human immune interferon has more potent anticellular activity than fibroblast or leucocyte interferon. *Cell. Immunol.* **49**, 390–394.

Crane, J. L., Glasgow, L. A., Kern, E. R. & Younger, J. S. 1978 Inhibition of murine osteogenic sarcomas by treatment with type I or type II interferon. *J. natn. Cancer Inst.* **61**, 871–874.

Degrave, W., Derynck, R., Tavernier, J., Haegeman, G. & Fiers, W. 1981 Nucleotide sequence of the chromosomal gene for human fibroblast (β) interferon and of the flanking regions. *Gene* **14**, 137–143.

De Ley, M., Van Damme, J., Claeys, H., Weening, H., Heine, J. W., Billiau, A., Vermylen, C. & De Somer, P. 1980 Interferon induced in human leucocytes by mitogens: production, partial purification and characterization. *Eur. J. Immunol.* **10**, 877–883.

Derynck, R., Content, J., De Clercq, E., Volckaert, G., Tavernier, J., Devos, R. & Fiers, W. 1980 Isolation and structure of a human fibroblast interferon gene. *Nature, Lond.* **285**, 542–547.

Devos, R., Cheroutre, H., Taya, Y. & Fiers, W. 1982*a* Isolation and characterization of IFN-γ mRNA derived from mitogen-induced human splenocytes. *J. Interferon Res.* **2**. (In the press.)

Devos, R., Cheroutre, H., Taya, Y., Degrave, W., Van Heuverswyn, H. & Fiers, W. 1982*b* Molecular cloning of human immune interferon cDNA and its expression in eukaryotic cells. *Nucl. Acids Res* **10**. (In the press.)

Epstein, L. B. 1982 Interferon-gamma: success, structure and speculation. *Nature, Lond.* **295**, 453–454.

Fleischmann, W. R. Jr, Georgiades, J. A., Osborne, L. C. & Johnson, H. M. 1979 Potentiation of interferon activity by mixed preparations of fibroblast and immune interferon. *Infect. Immun.* **26**, 248–253.

Fleischmann, W. R. Jr, Kleyn, K. M. & Baron, S. 1980 Potentiation of antitumor effect of virus-induced interferon by mouse immune interferon preparations. *J. natn. Cancer Inst.* **65**, 963–969.

Gheysen, D. & Fiers, W. 1982 Expression and excretion of human fibroblast β_1 interferon in monkey cells following transfection with a recombinant SV40 plasmid vector. *J. molec. appl. Genet.* (In the press.)

Gray, P. W., Leung, D. W., Pennica, D., Yelverton, E., Najarian, R., Simonsen, C. C., Derynck, R., Sherwood, P. J., Wallace, D. M., Berger, S. L., Levinson, A. D. & Goeddel, D. V. 1982 Expression of human immune interferon cDNA in *E. coli* and monkey cells. *Nature, Lond.* **295**, 503–508.

Isaacs, A. & Lindenmann, J. 1957 Virus interference. I. The interferon. *Proc. R. Soc. Lond.* B **147**, 258–267.

Kastelein, R., Remaut, E., Fiers, W. & Van Duin, J. 1981 Lysis gene expression of RNA phage MS2 depends on a frameshift during translation of the overlapping coat protein gene. *Nature, Lond.* **295**, 35–41.

Knight, E. Jr, Hunkapiller, M. W., Karant, B. D., Hardy, R. W. F. & Hood, L. E. 1980 Human fibroblast interferon: amino acid analysis and amino terminal amino acid sequence. *Science, Wash.* **207**, 525–526.

Langford, M. P., Georgiades, J. A., Stanton, G. J., Dianzani, F. & Johnson, H. M. 1979 Large-scale production and physicochemical characterization of human immune interferon. *Infect. Immun.* **26**, 36–41.

Lawn, R. M., Fritsch, E. F., Parker, R. C., Blake, G. & Maniatis, T. 1978 The isolation and characterization of linked δ- and β-globin genes from a cloned library of human DNA. *Cell* **15**, 1157–1174.

Maxam, A. M. & Gilbert, W. 1977 A new method for sequencing DNA. *Proc. natn. Acad. Sci. U.S.A.* **75**, 560–564.

Ptashne, M., Backman, K., Humayun, M. Z., Jeffrey, A., Maurer, R., Meyer, B. & Sauer, R. T. 1976 Autoregulation and function of repressor in bacteriophage lambda. Interaction of a regulatory protein with sequences in DNA mediate intricate patterns of gene regulation. *Science, Wash.* **194**, 156–161.

Remaut, E., Stanssens, P. & Fiers, W. 1981 Plasmid vectors for high-efficiency expression controlled by the P_L promoter of coliphage lambda. *Gene* **15**, 81–93.

Remaut, E., De Waele, P., Marmenout, A., Stanssens, P. & Fiers, W. 1982*a* Functional expression of individual plasmid-coded RNA bacteriophage MS_2 genes. *J. EMBO* **1**, 205–209.

Remaut, E. *et al.* 1982*b* (In preparation.)

Streuli, M., Nagata, S. & Weissmann, C. 1980 At least three human type α-interferons: structure of α_2. *Science, Wash.* **209**, 1348–1347.

Taniguchi, T., Ohno, S., Fujii-Kuriyama, Y. & Muramatsu, M. 1980 The nucleotide sequence of human fibroblast interferon cDNA. *Gene* **10**, 11–15.

Tavernier, J., Derynck, R. & Fiers, W. 1981 Evidence for a unique human fibroblast interferon (IFN-β_1) chromosomal gene, devoid of intervening sequences. *Nucl. Acids Res.* **9**, 461–471.

Van Emmelo, J., Devos, R., Ysebaert, M. & Fiers, W. 1980 Construction and characterization of a plasmid containing a nearly full-size DNA copy of Satellite Tobacco Necrosis Virus RNA. *J. molec. Biol.* **143**, 259–271.

Volckaert, G., Tavernier, J., Derynck, R., Devos, R. & Fiers, W. 1981 Molecular mechanisms of nucleotide-sequence rearrangements in cDNA clones of human fibroblast interferon mRNA. *Gene* **15**, 215–222.

Weissmann, C. 1981 The cloning of interferon and other mistakes. In *Interferon 3* (ed. I. Gresser), pp. 101–139. New York and London: Academic Press.

Yip, Y. K., Roy, H. L., Urban, C. & Vilček, J. 1981 Partial purification and characterization of human γ (immune) interferon. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1601–1605.

Yip, Y. K., Barrowclough, B. S., Urban, C. & Vilček, J. 1982 Molecular weight of human gamma interferon is similar to that of other human interferons. *Science, Wash.* **215**, 411–413.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

Discussion

In discussion Professor Fiers said that although there was evidence for several species of IFN-β, their cloned gene was present only once in the genome. Sehgal's results could be due to there being other IFNs in his system, the genes for which do not hybridize with the clone obtained by Fiers's group. Glycosylation did not seem to affect the biological activity of the interferon, though it may alter other properties.

Now that the gene for interferon had been cloned it would be possible to use this to study the basis of the disease in patients showing deficiency of IFN. Dr P. G. Boseley said that he had found cross-hybridization between human IFN-α and IFN-β genes and DNA of birds and reptiles and between IFN-α and bony fish DNA.

BIOLOGICAL SCIENCES THE ROYAL SOCIETY B PHILOSOPHICAL TRANSACTIONS OF

BIOLOGICAL SCIENCES THE ROYAL SOCIETY B PHILOSOPHICAL TRANSACTIONS OF