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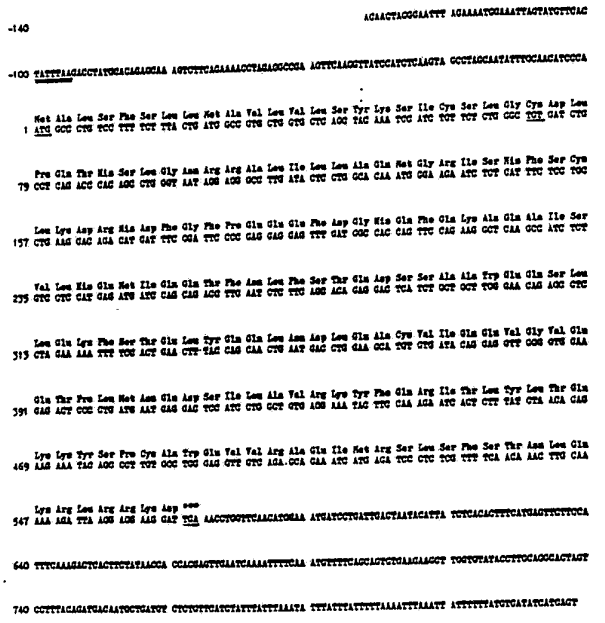
(54) Title: PRODUCTION OF HUMAN INTERFERON-α

(57) Abstract

A DNA molecule which on expression codes for a human interferon-α, designated IFN-αM1, comprises a nucleotide sequence substantially as shown in Figure 2. A recombinant DNA molecule, a cloning vehicle or vector, and a host cell, all containing this nucleotide sequence, are also disclosed. Also disclosed is a polypeptide having human interferon-α activity, comprising an amino acid sequence substantially as shown in Figure 2.



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PRODUCTION OF HUMAN INTERFERON-α

This invention relates to a human interferon-α and to the production thereof. In particular, the invention relates to a complete nucleotide sequence of a human interferon-α gene, and recombinant DNA molecules comprising this nucleotide sequence, as well as processes utilising said recombinant DNA molecules for producing the human interferon-α.

The human interferons are a group of proteins possessing potent antiviral, antiproliferative and immune response modulating activities (1). In view of the potential therapeutic value of the interferons, together with their limited availability from natural sources, considerable effort has been directed towards the cloning and expression of interferon genes. Three distinct types of interferon, α, β and γ, have been described, based on differences in antigenicity and biological characteristics of the molecules (for review, 2).

Sequencing and expression studies of interferon-α (IFN-α) genes have been carried out using both cDNAs derived from induced leukocytes and DNA from human chromosomal libraries. The results obtained indicate that the human genome contains at least thirteen functional, non-allelic IFN-α genes as

well as a number of allelic variants and pseudogenes (3). Complete nucleotide sequences for some IFN-α coding regions have been published, eight derived from cDNA clones and seven from clones of genomic DNA (4,5,6,7,8,9, 9a, 9b), and comparison of the nucleotide sequences of different IFN-α coding regions reveals a high degree of homology (88 to 98%). Differences in the DNA sequence of flanking regions and the location of IFN-α genes in tandem array on a single chromosomal fragment have been the basis for suggesting that the genes are non-allelic (3).

The present invention relates to an IFN-α gene which has been isolated from a human genome library using oligonucleotide probes. The gene, designated IFN-αM1, has been expressed in E. coli using the M13 phage vector, and its nucleotide sequence has been ascertained. This data represents the first complete nucleotide sequence published for this IFN-α genetic locus.

According to a first aspect of the present invention, there is provided a DNA molecule which on expression codes for a human interferon-α, comprising a nucleotide sequence substantially as shown in Figure 2.

It will be appreciated that the nucleotide sequence of this aspect of the invention may be obtained from natural, synthetic or semi-synthetic sources; furthermore, the nucleotide sequence may be a naturally-occurring sequence, or may be related by mutation, including single or multiple base substitutions, deletions, insertions and inversions,

to such a naturally-occurring sequence, provided always that the DNA molecule comprising such a sequence is capable of being expressed as the desired amino acid sequence. The nucleotide sequence may have expression control sequences positioned adjacent to it, such control sequences being derived either from homologous or heterologous sources.

The nucleotide sequence of IFN-αM1 according to this invention is further characterised in having a restriction map substantially as shown in Figure 1b.

Of the IFN-α nucleotide sequences previously reported (4,5,6,7,8,9), the IFN-αM1 coding region sequence most closely resembles that of IFN-C (4). IFN-αM1 and IFN-C are 98% homologous at the nucleotide sequence level, however at the level of amino acid homology they differ by seven residues.

Weissmann et al. (3) have published a partial amino acid sequence for an IFN-α denoted α4a (153 of 189 amino acids) and a complete amino acid sequence for another IFN-α denoted α4b. The amino acid sequences were derived from unpublished nucleotide sequences of clones isolated from the same human gene library as that used in the present report (12). IFN-α4a and IFN-α4b are considered to be allelic as they have similar flanking DNA sequences and, on currently published data, have only two amino acid differences (3).

The amino acid sequence predicted for IFN-αM1 is identical to the 153 amino acids of IFN-α4a that have been published (3). Also IFN-αM1 differs from IFN-α4b at the same two amino acid residues as

IFN-α4a. However, the IFN-αM1 coding region contains two restriction enzyme sites (one EcoRII site and one BspI site; indicated by asterisks in Fig.1b) which are not present in either IFN-α4a or IFN-α4b (3). This suggests the existence of the three separate coding regions, IFN-αM1, IFN-α4a and IFN-α4b, in the one individual and hence the presence of at least two genetic loci.

Synthetic oligonucleotides have been used in the screening of cDNA clones (21) but they have not been used extensively in the screening of genome libraries. While the lack of specificity of hybridization presents a problem in the selection of genomic clones with individual oligonucleotides, this can be overcome by using combinations of oligonucleotides. The set of five oligonucleotides used throughout this work (Table 1) was suitable not only for the selection of genomic clones but also for the identification of subclones, for the construction of restriction maps and for priming the chain-termination nucleotide sequencing reactions.

As described in greater detail below, an AluI fragment containing the coding region of the IFN-αM1 gene has been inserted into the HincII site of the phage M13 mp 11, resulting in a fusion of the IFN-αM1 gene and the β-galactosidase gene. E. coli infected with the recombinant M13 phage carrying the fused gene has been cultured and extracts have shown antiviral activity in cytopathic effect inhibition assays. This antiviral activity was completely neutralised by IFN-α antibodies.

In a further aspect of the present invention, there is provided a recombinant DNA molecule which on expression codes for a human interferon-α, comprising a nucleotide sequence substantially as shown in Figure 2, operatively linked to an expression control sequence. The expression control sequence may comprise known initiator and terminator sequences with the interferon nucleotide sequence located between them.

In yet another aspect of this invention, there is provided a recombinant DNA cloning vehicle or vector capable of expressing a human interferon-α, having inserted therein a nucleotide sequence substantially as shown in Figure 2, operatively linked to an expression control sequence. The cloning vehicle or vector may comprise a known bacteriophage or plasmid. This invention further provides a host cell, such as a known E. coli strain, transformed with a recombinant DNA cloning vehicle or a recombinant DNA molecule as described above.

As previously described, the amino acid sequence of IFN-αM1 expressed by the nucleotide sequence of Figure 2 can be predicted on the basis of the known genetic code. Accordingly, in yet another aspect of this invention, there is provided a polypeptide having human interferon-α activity, comprising an amino acid sequence substantially as shown in Figure 2. This polypeptide may comprise either the pre-IFN-α sequence of 189 amino acids and containing a secretion leader of 23 amino acids, as shown in Figure 2, or the mature IFN-α sequence of 166 amino acids as shown.

Finally, this invention provides a method of producing a polypeptide having human interferon-α activity, which comprises the steps of culturing a host cell as described above, and recovering said polypeptide from the culture.

The invention will be further described by way of reference to the accompanying drawings, in which:

Figure 1a shows the restriction map of the λM1 PstI fragment containing the IFN-αM1 gene. Restriction sites are indicated by the symbols: ○, PstI; ▲, EcoRI; ●, HindIII. The hatched area indicates the IFN-αM1 coding region. The direction of transcription is from left to right.

Figure 1b shows the restriction map of the λM1 RsaI fragment containing the IFN-αM1 gene and the strategy for sequencing the IFN-αM1 gene. Restriction sites are indicated by the symbols: ◇, RsaI; ▽, BspI; ▼, AluI; □, Sau3AI; △, EcoRII; ●, HindIII. Arrowed segments below the map indicate the extent and direction of nucleotide sequence data obtained from M13 subclones. The asterisks indicate the BspI and EcoRII sites which are present in IFN-αM1 but absent from both IFN-α4a and IFN-α4b (see below).

Figure 2 shows the nucleotide and predicted amino acid sequence of IFN-αM1. The initiation codon for pre-interferon, the codon for the N-terminal amino acid of the mature interferon and the termination codon are underlined. The putative 'TATA' box is underlined twice.

Figure 3 shows the nucleotide sequence of the M13 recombinant phage M13-αM1-B1 in the region of the fusion between the β-galactosidase gene of M13mp11 and the IFN-αM1 gene. The numbers and amino acid sequences refer to segments derived from the β-galactosidase N-terminus, the M13mp11 polylinker, the IFN-αM1 leader and the N-terminus of the mature IFN-αM1 protein.

Materials and Methods

Synthetic oligonucleotides.

Oligonucleotides were synthesized by the solid-phase phosphotriester method (10) and purified by HPLC on a Partisil 10 SAX column operated at ambient temperature and eluted with a gradient of potassium phosphate, pH 6.5, from 1mM in 5% acetonitrile to 0.2M in 30% acetonitrile. The nucleotide sequences of these oligonucleotides and the positions at which they are complementary to IFN-α sequences are presented in Table 1. Oligonucleotide probes were 5'-end labelled using T4 polynucleotide kinase (Boehringer-Mannheim) and [γ-^{32}P]ATP (Amersham) (11). Unincorporated label was separated from the probes by polyacrylamide gel electrophoresis.

Screening of human genome library.

A human genome library in phage γ Charon 4A prepared by Lawn and colleagues (12) was used. Approximately 300,000 plaques were screened by the 'amplified plaque lift' procedure of Woo (13). The hybridization temperatures used are indicated in Table 1.

Analysis of subclones.

Restriction fragments of the γ clones were inserted into plasmid pUC9 (14) and cloned in E. coli ED8654 [SupE, SupF, hsdR$^-$m$^+$S$^+$, met$^-$, trpR]. Colonies were screened by the Grunstein-Hogness colony hybridization method (15). Subsequently, restriction fragments of the selected pUC9 recombinant were inserted into M13mp9 or M13mp11 (16) and used to transform E. coli JM101 [lacpro, thi, supE, F'traD36, proAB, LacI^{q}Z M15]. M13 recombinant plaques were screened by the Benton and Davis procedure (17). Hybridization was carried out using the synthetic oligonucleotide probes (Table 1). Restriction maps were constructed using standard methods, including Southern blotting (18).

DNA sequencing.

Single-stranded DNA was prepared from M13 subclones and sequenced by the dideoxy chain-termination method of Sanger et al. (19). Priming was carried out using synthesized oligonucleotides, either the M13 'universal primer' (5'-GTAAAACGACGGCCAGT-3') or an IFN-α gene-specific oligonucleotide (Table 1).

Expression of cloned IFN-α DNA.

The conditions for infection of E. coli with the recombinant phage and induction with isopropyl β-D-thiogalactopyranoside (IPTG) were as previously reported (20), except that JM101 was used as the host strain.

Interferon assays.

A standard cytopathic effect (CPE) inhibition assay using human HEp-2 cells and Semliki forest virus was used (for review, 1).

RESULTS

A human genome library in phage λ Charon 4A (12) was screened for the presence of IFN-α genes with synthetic oligonucleotides. The sequences of the oligonucleotide probes correspond to a number of different, highly conserved segments within published IFN-α coding regions (Table 1). Using the individual probes approximately 300,000 recombinant phage were screened, resulting in the isolation of 297 putative IFN-α clones. The number of putative positive clones was reduced to twenty-eight by using combinations of the oligonucleotide probes. One clone, designated λM1, which hybridized to all five oligonucleotide probes (Table 1), was selected for detailed analysis.

A PstI fragment of the λM1 DNA to which the oligonucleotide probes hybridized was subcloned into pUC9 by standard methods. Following amplification in E. coli the purified PstI fragment was digested with selected restriction enzymes and the resulting fragments were separated by electrophoresis on an agarose gel. The fragments were transferred to nitrocellulose paper, hybridized to specific oligonucleotide probes and a restriction map of the PstI fragment was derived (Fig.1a).

Digestion of the PstI fragment with Sau3AI resulted in four fragments: a 790 base pair (bp) fragment which hybridized to oligonucleotide probe 5, a 176 bp fragment which hybridized to probe 4, a 269 bp fragment which hybridized to probes 2 and 3, and a 74 bp fragment which hybridized to probe 1. All four

fragments were cloned in both orientations into the BamHI site of the vector M13mp9 (Fig.1b). An RsaI fragment (977 bp), wholly contained within the PstI fragment and hybridizing to all five probes, was similarly cloned in both orientations into the HincII site of the vector M13mp11 (Fig.1b). The identification of recombinant clones of interest and the determination of the orientation of the inserted fragments was achieved by screening the M13 recombinant clones with the appropriate oligonucleotides. Utilizing either the M13 'universal primer' or an IFN-α-specific oligonucleotide as the primer, the nucleotide sequence of these M13 recombinant subclones was obtained by the dideoxy chain-termination method. The sequence determined is shown in Fig.2 and a detailed restriction map derived both from this sequence and restriction enzyme analysis is shown in Fig.1b. Comparison with previously reported IFN-α sequences reveals that the nucleotide sequence of the RsaI fragment contains an entire IFN-α coding region. This coding region specifies a pre-IFN-α of 189 amino acids, consisting of a 23 amino acid leader and a 166 amino acid mature IFN-α protein (Fig.2).

In order to demonstrate that the IFN-αM1 gene codes for a biologically active product, the gene was expressed in E. coli. An AluI fragment of 669 bp (Fig.1b) was cloned into the HincII site of M13mp11 and clones with the correct orientation of the AluI fragment were selected by hybridization with oligonucleotides 4 and 5 (Table 1). One such clone, M13-αM1-B1, was subjected to sequence analysis and shown to have the predicted fused gene. M13-αM1-B1 contained the β-galactosidase promoter and the

N-terminal 15 nucleotides of the β-galactosidase gene coding region, 20 nucleotides of the M13mp11 polylinker sequence, and 25 nucleotides of the IFN-αM1 leader sequence followed by the mature IFN-αM1 coding region (Fig.3). In the fused protein product, it would therefore be predicted that the 23 amino acid interferon leader would be replaced by a 19 amino acid leader (11 residues of which are non-interferon), assuming the N-terminal methionine is removed from the β-galactosidase N-terminus.

Cultures of E. coli (JM101) were infected with the recombinant phage M13-αM1-B1 and induced with the lac operon inducer IPTG. CPE inhibiton assays for antiviral activity detected 6.3×10^5 IU/1 of culture in the spent culture supernatant. Extracts of the pelleted cells contained 1.3×10^6 IU/1 of culture. The interferon activity in both the culture supernatant and in the cell pellet extracts was completely neutralized by both a polyclonal antiserum against human IFN-α (Cantell) and a monoclonal anti-human IFN-α antibody. It may be noted that the level of interferon expression obtained was lower than that previously reported with a M13 vector (20). Factors which may account for this difference include the intrinsic specific activities of the particular interferons, the specific activity of the product of the particular fused gene constructed here (M13-αM1-B1), and the properties of the host E. coli strain.

Table 1. Synthetic oligonucleotides used in the screening of the human genome library and the characterization of IFN-α genes

Oligonucleotide No.	Length in Nucleotides	Nucleotide Sequence	Location of Complementary Sequence[a]	Temp. (°C)[b]
1	19	5'-TCATTCCTTCCTCCTTAAT-3'	Nucleotide 552-570 IFN mRNA-like strand	28
2	27	5'-ATCTCATGATTTCTGCTCTGACAACCT-3'	Nucleotides 494-520 IFN mRNA-like strand	45
3	13	5'-GATCCAGCAGACC-3'	Nucleotides 249-261 IFN non-mRA-like strand	25
4	13	5'-GCTTGAGCCTTCT-3'	Nucleotides 215-227 IFN mRNA-like strand	25
5	12	5'-AGATCACAGCCC-3'	Nucleotides 66-77 IFN mRNA-like strand	20

(a) Nucleotide position 1 of the IFN gene is taken as being the 'A' of the ATG coding for translation initiation in the IFN-α1 gene (9).

(b) The temperature given indicates the empirically determined temperature of hybridization and washing for the oligonucleotide probes.

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It will be appreciated that many modifications and variations may be made to the particular methods described above by way of illustration of the present invention, and that the present invention includes all such modifications which fall within the scope of the invention as broadly described above.

CLAIMS:

1. A DNA molecule which an expression codes for a human interferon-α, designated IFN-αM1, comprising a nucleotide sequence substantially as shown in Figure 2.

2. A DNA molecule according to claim 1, wherein the nucleotide sequence isfurther characterised in having a restriction map substantiallly as shown in Figure 1b.

3. A recombinant DNA molecule which on expression codes for a human interferon-α, designated IFN-αM1, comprising a nucleotide sequence substantially as shown in Figure 2, operatively linked to an expression control sequence.

4. A recombinant DNA molecule according to claim 3, wherein the expression control sequence comprises initiator and terminator sequences with said nucleotide sequence located between said initiator and terminator sequences.

5. A recombinant DNA cloning vehicle or vector capable of expressing a human interferon-α, having inserted therein a nucleotide sequence substantially as shown in Figure 2, operatively linked to an expression control sequence.

6. A cloning vehicle or vector according to claim 5, wherein the vehicle or vector is a bacteriophage.

7. A host cell, transformed with a recombinant DNA cloning vehicle or vector according to claim 5, or a recombinant DNA molecule according to claim 3.

8. A host cell according to claim 7, wherein said host cell is a known strain of E.coli.

9. A polypeptide having human interferon-α activity, comprising an amino acid sequence substantially as shown in Figure 2.

10. A polypeptide according to claim 9, comprising a pre-IFN-α sequence of 189 amino acids, including a secretion leader of 23 amino acids, substantially as shown in Figure 2.

11. A polypeptide according to claim 9, comprising a mature IFN-α sequence of 166 amino acids, substantially as shown in Figure 2.

12. A method of producing a polypeptide having human interferon-α activity which comprises the steps of culturing a host cellaccording to claim 7, and recovering said polypeptide from the culture.

13. A polypeptide having human interferon-α activity, produced by the method according to claim 12.

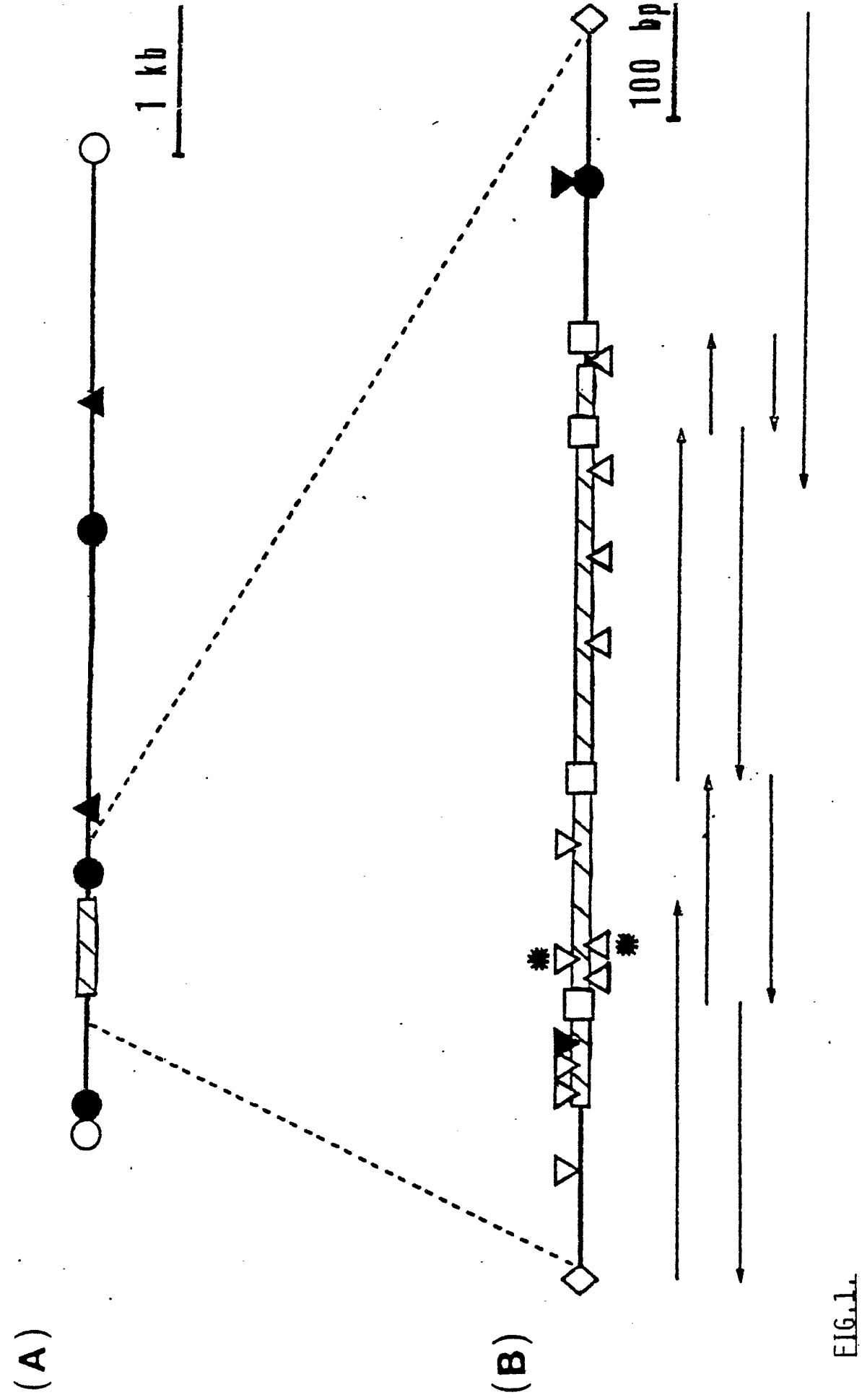

FIG.1.

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-140                                                                                                ACAACTAGGGAATTT AGAAAATGGAAATTAGTATGTTCAC

-100 TATTTAAGACCTATGCACAGAGCAA AGTCTTCAGAAAACCTAGAGGCCGA AGTTCAAGGTTATCCATCTCAAGTA GCCTAGCAATATTTGCAACATCCCA

       Met Ala Leu Ser Phe Ser Leu Leu Met Ala Val Leu Val Leu Ser Tyr Lys Ser Ile Cys Ser Leu Gly Cys Asp Leu
   1 ATG GCC CTG TCC TTT TCT TTA CTG ATG GCC GTG CTG GTG CTC AGC TAC AAA TCC ATC TGT TCT CTG GGC TGT GAT CTG

       Pro Gln Thr His Ser Leu Gly Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln Met Gly Arg Ile Ser His Phe Ser Cys
  79 CCT CAG ACC CAC AGC CTG GGT AAT AGG AGG GCC TTG ATA CTC CTG GCA CAA ATG GGA AGA ATC TCT CAT TTC TCC TGC

       Leu Lys Asp Arg His Asp Phe Gly Phe Pro Glu Glu Glu Phe Asp Gly His Gln Phe Gln Lys Ala Gln Ala Ile Ser
 157 CTG AAG GAC AGA CAT GAT TTC GGA TTC CCC GAG GAG GAG TTT GAT GGC CAC CAG TTC CAG AAG GCT CAA GCC ATC TCT

       Val Leu His Glu Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala Trp Glu Gln Ser Leu
 235 GTC CTC CAT GAG ATG ATC CAG CAG ACC TTC AAT CTC TTC AGC ACA GAG GAC TCA TCT GCT GCT TGG GAA CAG AGC CTC

       Leu Glu Lys Phe Ser Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu
 313 CTA GAA AAA TTT TCC ACT GAA CTT TAC CAG CAA CTG AAT GAC CTG GAA GCA TGT GTG ATA CAG GAG GTT GGG GTG GAA

       Glu Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu
 391 GAG ACT CCC CTG ATG AAT GAG GAC TCC ATC CTG GCT GTG AGG AAA TAC TTC CAA AGA ATC ACT CTT TAT CTA ACA GAG

       Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr Asn Leu Gln
 469 AAG AAA TAC AGC CCT TGT GCC TGG GAG GTT GTC AGA GCA GAA ATC ATG AGA TCC CTC TCG TTT TCA ACA AAC TTG CAA

       Lys Arg Leu Arg Arg Lys Asp ***
 547 AAA AGA TTA AGG AGG AAG GAT TGA AACCTGGTTCAACATGGAA ATGATCCTGATTGACTAATACATTA TCTCACACTTTCATGAGTTCTTCCA

 640 TTTCAAAGACTCACTTCTATAACCA CCACGAGTTGAATCAAAATTTTCAA ATGTTTTCAGCAGTGTGAAGAAGCT TGGTGTATACCTTGCAGGCACTAGT

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FIGURE 2

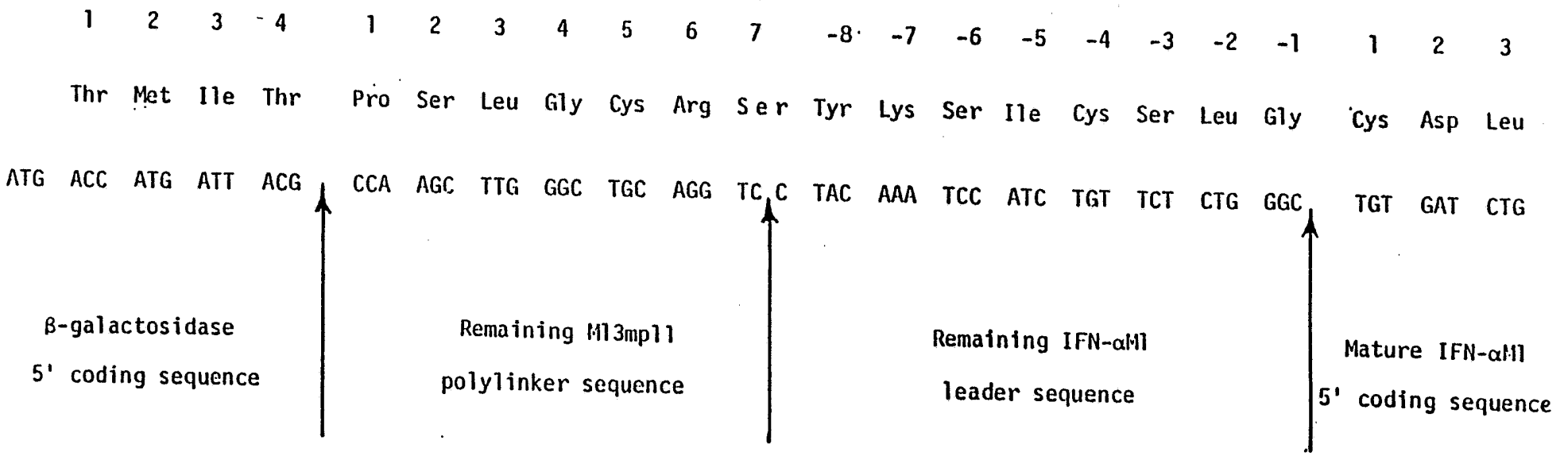
1 2 3 4 1 2 3 4 5 6 7 -8 -7 -6 -5 -4 -3 -2 -1 1 2 3
Thr Met Ile Thr Pro Ser Leu Gly Cys Arg Ser Tyr Lys Ser Ile Cys Ser Leu Gly Cys Asp Leu
ATG ACC ATG ATT ACG CCA AGC TTG GGC TGC AGG TCC TAC AAA TCC ATC TGT TCT CTG GGC TGT GAT CTG
β-galactosidase
5' coding sequence
Remaining M13mp11
polylinker sequence
Remaining IFN-αM1
leader sequence
Mature IFN-αM1
5' coding sequence

FIG.3.

INTERNATIONAL SEARCH REPORT

International Application No PCT/AU 84/00263

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.[3] C12N 15/00, 1/20, C07H 21/04, C07C 103/52, C07G 17/00
C12P 19/34 // C12N 5/00, C07G 7/00

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
IPC	C12N, C07G

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X,P	WO, A, 84/03300 (INSTITUT ORGANICHESKOGO SINTEZA AKADEMII NAUK LATVIISKOI SSR, RIGA et al) 30 August 1984 (30.08.84)	(1,10)
X	AU, A, 72462/81 (HOFFMANN-LA ROCHE F. & CO. A.G. and GENETECH INC.) 7 January 1982 (07.01.82), see figures 3-4, 8-9.	(1,20)
X	WO, A, 83/02460 (CETUS CORPORATION) 21 July 1983 (21.07.83), see figures 3,5 and 16.	(1,10)
X,P	WO, A, 84/00776 (CETUS CORPORATION) 1 March 1984 (01.03.84), see figures 3,5 and 6.	(1,5)
X	WO, A, 83/02457 (CETUS CORPORATION) 21 July 1983 (21.07.83), see figures 3,5 and 6	(1,10)
X	Archives of Biochemistry and Biophysics, Volume 221, No. 1, issued 1983, February 15 (New York, U.S.A.), S. Pestka, "Human Interferons - from Protein Purification and Sequence to Cloning and Expression in Bacteria: Before, Between, and Beyond", see page 16 figure 17, page 18 figure 18, and page 26 figure 26. CONT.	(1,10)

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
27 March 1985 (27.03.85)	4 April 1985 (04.04.85)
International Searching Authority [1]	**Signature of Authorized Officer [20]**
AUSTRALIAN PATENT OFFICE	J.H. CHAN

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X	Science, Volume 212, issued 1981, June 5 (Washington, D.C., U.S.A.), R.M. Lawn et al, "DNA Sequences of Two Closely Linked Human Leukocyte Interferon Genes", see pages 1159-1162.	(1,10)
X	Nature, Volume 290, issued 1981, March 5 (London, U.K.) D.V. Goeddel, "The structure of eight distinct cloned human leukocyte interferon cDNAs", see pages 20-26.	(1,10)
X	Philosophical Transactions of the Royal Society London, Series B, Volume 299, No. 1094, issued 1982, September 24, (London, U.K.), C. Weissmann et al, "Structure and expression of human IFN-α genes", see pages 7-28, particularly page 12.	(1,10)

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ....., because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers ....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers ....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON
INTERNATIONAL APPLICATION NO. PCT/AU **84/00263**

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Members		
AU 72462/81	BR 8104189	DD 202307	DD 210304
	DD 210305	DE 3125706	DK 2910/81
	EP 43980	ES 503528	FI 812067
	FR 2486098	GB 2079291	IL 63197
	JP 57079897	LU 83461	MC 1396
	MW 25/81	NL 8103151	NO 812247
	PL 231940	PT 73289	SE 8104093
	YU 1622/81	ZA 814375	ZW 14781
WO 8403300	FR 2541579	GB 8426019	SE 8404803
WO 8302460	AU 11529/83	EP 98864	
WO 8400776	AU 19462/83	EP 116090	
WO 8302457	AU 11527/83	EP 98862	

END OF ANNEX