Description

The invention concerns novel hybrid interferons, the microbial production thereof via recombinant DNA technology, and their use for the treatment of viral and neoplastic diseases.

The interferons ("IFNs") are a family of polypeptides secreted by a large variety of eukaryotic cells upon exposure to various mitogens and viruses. The interferons have been classified by their chemical and biological characteristics into three groups: IFN-α, IFN-β, and IFN-γ. IFN-α and β genes are expressed predominantly in cells treated with viruses, viral constituents and double stranded RNA. IFN-γ on the other hand, is synthesized in response to mitogens.

Human IFN-α is coded for by a gene family comprising at least 15 non-allelic genes. Human IFN-β and human IFN-γ are encoded by single genes. The primary translation product of IFN-α comprises 189 amino acids (except IFN-α_2: 188 amino acids), from which a signal peptide of 23 amino acids is removed by post-translational modification. Human IFN-β and human IFN-γ consist of 187 and 186 amino acids from which 21 or 20 amino acids constituting the signal peptides are removed. The amino acid sequence homology among IFN-α subtypes is 80-85 % whereas the homology between IFN-β and IFN-α is about 30-40 %. IFN-γ shows only single amino acid homologies with IFN-α, corresponding to 12 % identical residues.

The IFNs exhibit antiviral, immunomodulatory, as well as cytotoxic activities. It has been demonstrated that IFN-α subtypes (e.g. IFN-α_2, IFN-α_1) show different target cell specificities. The antiviral activity of IFN-α_2 and IFN-α_1 is about the same on bovine (MDBK) cells, but IFN-α_2 is seven times more effective than IFN-α_1 on human (WISH) cells, and 30 times less effective than IFN-α_1 in protecting mouse (L 929) cells. [M. Streuli et al., Proc. Natl. Acad. Sci. USA 78, 2848 (1981)]. The evidence that members of the multigene IFN-α family vary in the extent and specificity of their antiviral activity has been confirmed by results obtained from some IFN-α "hybrids" (cf. EP 32134, US 4,414,150). These "hybrids" have been constructed by cleaving two genes coding for interferon-α subtypeswith the restriction enzyme PvuII (or, in two cases, with BglII) and cross-ligating the resulting fragments such that the DNA sequence coding for the amino terminal part of one IFN gene was fused to the DNA sequence coding for the carboxy terminal part of the other IFN gene. Although these hybrid interferons exhibit changes in target cell specificity as compared to the parent interferons it was not demonstrated that there was any attenuation or any restriction of any of the three interferon activities. Such attenuation or restriction may be desirable, for example, in clinical application where it is desirable to focus interferon therapy on a particular problem such as viral infection, without the possibility of complicating factors resulting from other activities of the administered interferon.

It is thus an object of the present invention to provide novel hybrid interferons which show more specific biological activities resulting from the selective or predominant activation of only some of the interferon-induced biochemical pathways, and/or in which some of the undesired side effects of natural interferons which limit their use, such as influenza-like symptoms (e.g. fever, headache and fatigue), gastrointestinal disturbances, decrease of blood pressure and loss of hair, are excluded or reduced.

The hybrid interferons according to the invention are derived from human lymphoblastoid interferon LyIFN-α-2 having the sequence

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN THR PHE ASN LEU PHE
SER THR LYS ASP SER SER ALA ALA LEU ASP GLU THR LEU LEU ASP GLU PHE
TYR ILE GLU LEU ASP GLN GLN LEU ASN ASP LEU GLU SER CYS VAL MET GLN
GLU VAL GLY VAL ILE GLU SER PRO LEU MET TYR GLU ASP SER ILE LEU ALA
VAL ARG LYS TYR PHE GLN ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER SER CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER PHE SER
LEU SER ILE ASN LEU GLN LYS ARG LEU LYS SER LYS GLU

and human lymphoblastoid interferon LyIFN-α-3 having the sequence

CYS ASP LEU PRO GLU THR HIS SER LEU ASP ASN ARG ARG THR LEU MET LEU
LEU ALA GLN MET SER ARG ILE SER PRO SER SER CYS LEU MET ASP ARG HIS
ASP PHE GLY PHE PRO GLN GLU GLU PHE ASP GLY ASN GLN PHE GLN LYS ALA
PRO ALA ILE SER VAL LEU HIS GLU LEU ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU ALA CYS VAL MET GLN
GLU GLU ARG VAL GLY GLU THR PRO LEU MET ASN ALA ASP SER ILE LEU ALA
VAL LYS LYS TYR PHE ARG ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER PRO CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER LEU SER
LEU SER THR ASN LEU GLN GLU ARG LEU ARG ARG LYS GLU

These interferons as well as methods for the preparation thereof have been described in European Patent Application No. 76,489. LyIFN α-2 is related to (but not identical with) human leukocyte interferon LeIF-αB (cf. U.S. Patent 4,414,150) and has a specific activity on primary calf kidney cells and on human embryonic foreskin cells (HEF, each infected with vesicular stomatitis virus) of $1.85 \cdot 10^8$ IU/mg and $2.45 \cdot 10^8$ IU/mg, respectively. LyIFN-α-3 is related to (but not identical with) human leukocyte interferon LeIF-αD (cf. U.S. Patent 4,414,150) and has a specific activity on calf cells and HEF cells (as above) of $1.32 \cdot 10^8$ IU/mg and $3.7 \cdot 10^6$ IU/mg, respectively. Furthermore, LyIFN-α-3 has a higher thermostability and lower antiproliferative activity than LyIFN-α-2.

It is a particular object of the present invention to provide LyIFN-α-2/α-3 hybrid polypeptides which combine the high antiviral titre and/or the higher antiproliferative activity on human cells of LyIFN-α-2 and the high thermostability of LyIFN-α-3.

Especially the invention relates to a hybrid interferon polypeptide having an amino acid sequence composed of two to four sub-sequences corresponding in amino acid identity and number to sub-sequences of human lymphoblastoid interferons LyIFN-α-2 and LyIFN-α-3, said hybrid interferon polypeptide being selected from the group consisting of a polypeptide having an amino acid sequence consisting of amino acids 1 to 150 of LyIFN-α-2 and amino acids 151 to 166 of LyIFN-α-3, a polypeptide having an amino acid sequence consisting of amino acids 1 to 92 of LyIFN-α-2, amino acids 93 to 150 of LyIFN-α-3 and amino acids 151 to 166 of LyIFN-α-2, and a polypeptide having an amino acid sequence consisting of amino acids 1 to 60 of LyIFN-α-2, amino acids 61 to 92 of LyIFN-α-3, amino acids 93 to 150 of LyIFN-α-2 or of LyIFN-α-3 and amino acids 151 to 166 of LyIFN-α-2 or of LyIFN-α-3.

More specifically, the invention relates to the hybrid interferon "$B_1B_2B_3D_4$" having the formula

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN THR PHE ASN LEU PHE
SER THR LYS ASP SER SER ALA ALA LEU ASP GLU THR LEU LEU ASP GLU PHE
TYR ILE GLU LEU ASP GLN GLN LEU ASN ASP LEU GLU SER CYS VAL MET GLN
GLU VAL GLY VAL ILE GLU SER PRO LEU MET TYR GLU ASP SER ILE LEU ALA
VAL ARG LYS TYR PHE GLN ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER SER CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER LEU SER
LEU SER THR ASN LEU GLN GLU ARG LEU ARG ARG LYS GLU

(I),

to the hybrid interferon "$B_1B_2D_3B_4$" having the formula

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN THR PHE ASN LEU PHE
SER THR LYS ASP SER SER ALA ALA LEU ASP GLU THR LEU LEU ASP GLU PHE
TYR ILE GLU LEU ASP GLN GLN LEU ASN ASP LEU GLU ALA CYS VAL MET GLN
GLU GLU ARG VAL GLY GLU THR PRO LEU MET ASN ALA ASP SER ILE LEU ALA
VAL LYS LYS TYR PHE ARG ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER PRO CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER PHE SER
LEU SER ILE ASN LEU GLN LYS ARG LEU LYS SER LYS GLU

(II),

to the hybrid interferon "$B_1D_2B_3D_4$" having the formula

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU SER CYS VAL MET GLN
GLU VAL GLY VAL ILE GLU SER PRO LEU MET TYR GLU ASP SER ILE LEU ALA
VAL ARG LYS TYR PHE GLN ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER SER CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER LEU SER
LEU SER THR ASN LEU GLN GLU ARG LEU ARG ARG LYS GLU

(III),

to the hybrid interferon "$B_1D_2D_3B_4$" having the formula

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU ALA CYS VAL MET GLN
GLU GLU ARG VAL GLY GLU THR PRO LEU MET ASN ALA ASP SER ILE LEU ALA
VAL LYS LYS TYR PHE ARG ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER PRO CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER PHE SER
LEU SER ILE ASN LEU GLN LYS ARG LEU LYS SER LYS GLU

(IV),

to the hybrid interferon "$B_1D_2D_3D_4$" having the formula

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU ALA CYS VAL MET GLN
GLU GLU ARG VAL GLY GLU THR PRO LEU MET ASN ALA ASP SER ILE LEU ALA
VAL LYS LYS TYR PHE ARG ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER PRO CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER LEU SER
LEU SER THR ASN LEU GLN GLU ARG LEU ARG ARG LYS GLU

(V),

and to the hybrid interferon "$B_1D_2B_3B_4$" having the formula

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU SER CYS VAL MET GLN
GLU VAL GLY VAL ILE GLU SER PRO LEU MET TYR GLU ASP SER ILE LEU ALA
VAL ARG LYS TYR PHE GLN ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER SER CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER PHE SER
LEU SER ILE ASN LEU GLN LYS ARG LEU LYS SER LYS GLU

(VI).

The most preferred hybrid interferons according to the invention are "$B_1D_2D_3D_4$" (V) and "$B_1B_2D_3B_4$"-(II).

A hybrid interferon according to the invention can be prepared by recombinant DNA technique comprising the steps, for example, of

a) preparing a DNA comprising the structural gene coding for said hybrid interferon by in vitro recombination at the sites corresponding to amino acids 60, 92 and 150, respectively, of interferons LyIFN-α-2 and LyIFN-α-3 or by chemical DNA synthesis and incorporating the obtained DNA into an appropriate expression vector,

b) transferring the obtained hybrid vector carrying the hybrid IFN structural gene into a recipient host,

c) selecting transformed hosts from untransformed hosts, usually by culturing under such conditions where only the transformed hosts survive,

d) culturing the transformed hosts under conditions which allow expression of the hybrid IFN structural gene, and

e) isolating the expressed hybrid IFN.

The methods applied will become more apparent from the detailed description which follows and from the accompanying drawings in which:

Fig. 1 compares the amino acid sequences of mature LyIFN-α-2 and LyIFN-α-3. Only those amino acids of LyIFN-α-3 which differ from corresponding amino acids of LyIFN-α-2 are depicted. The amino acid sequence of LyIFN-α-3 is otherwise identical to that of LyIFN-α-2.

Fig. 2 depicts the nucleotide sequences of the coding regions and parts of the 3'extracistronic regions of LyIFN-α-2 and LyIFN-α-3. The ATG translation initiation codons, the termination triplets and convenient restriction sites are underlined. The coding triplets are numbered.

Fig. 3 shows the restriction maps of the HindIII-PstI small segments of plasmids pBR(AP)/LyIFN-α-2 and pBR(AP)/LyIFN-α-3. The white bar (LyIFN-α-3) and black bar (LyIFN-α-2) delimit the coding regions of the mature IFNs. At the bottom the subdivision of the primary structure of IFN polypeptide (166 amino acids) into four sections is shown.

1. Preparation of hybrid vectors containing a hybrid IFN structural gene

The cDNAs of human interferon-α subtypes α-2 and α-3 have been cloned in E. coli. Thus, plasmid pBR(AP)/LyIFN-α-2 and pBR(AP)/LyIFN-α-3 include HindIII-PstI (or EcoRI-PstI) DNA inserts containing the coding regions of LyIFN-α-2 and LyIFN-α-3 under the control of the β-lactamase promoter (cf. European Patent Application No. 76,489). The restriction maps of said inserts are depicted in Fig. 3. It can easily be seen that the genes share some common restriction sites (located at positions 60, 92 and 150 of the IFN amino acid sequences) which can be used advantageously to construct novel genes coding for hybrid interferons having favourable biological and/or physical properties.

Thus, recombinants (single or multiple exchanges) between LyIFN-α-2 and LyIFN-α-3 genes can be generated by in vitro recombination at the PvuII site (position 92) and/or at the Sau3A sites (positions 60

and 150). Since the primary structure of the interferons can thus be divided into 4 sections (first section amino acids 1 to 60, second section amino acids 61 to 92, third section amino acids 93 to 150 and fourth section amino acids 151 to 166, see Fig. 3), the hybrid interferons of the present invention can be designated by four letters each letter being indicative of the section present. Thus, for example, hybrid interferon (I) being composed of sections 1 to 3 of interferon LyIFN-α-2 ("B", LyIFN-α-2 is related to IFN-αB, supra) and section 4 of LyIFN-α-3 ("D", LyIFN-α-3 is related to IFN-αD, supra) is designated IFN "$B_1B_2B_3D_4$".

In order to facilitate the production of the desired hybrid interferon the DNA inserts containing the LyIFN-α-2 and LyIFN-α-3 coding regions, respectively, have to be modified in such a manner that for religation purposes the restriction sites HindIII (or EcoRI), PvuII and PstI are unique to them. It is evident from Fig. 3 that there is an additional PvuII site in the 3' extracistronic region of the gene coding for LyIFN-α-2. This site can easily be removed, for example by partial cleavage of a hybrid vector containing the LyIFN-α-2 coding region with PvuII, ligating the resulting linearized DNA to a linker containing a DNA sequence which is recognized by the restriction endonuclease PstI, cleaving with PstI, religating the digests at low DNA concentration and selecting for plasmids (e.g. by restriction analysis) having the desired modification, viz. a shortened 3'extracistronic region with the PvuII site eliminated. Analogously, vectors used to clone IFN genes are advantageously modified such that the restriction sites HindIII, PvuII and PstI are unique to them.

The invention relates to hybrid vectors comprising a DNA sequence which codes for any of the hybrid interferons of the formulae I-VI and is operatively linked to an expression control sequence, and to processes for the preparation thereof.

The vector is selected depending on the host cells envisaged for transformation. Examples of suitable hosts are microorganisms, which are devoid of or poor in restriction enzymes or modification enzymes, such as yeasts, for example Saccharomyces cerevisiae, and strains of bacteria, in particular strains of Escherichia coli, for example E. coli X1776, E. coli HB 101, E. coli W3110, E. coli HB101/LM1035, E. coli JA221 or E. coli K12 strain 294, Bacillus subtilis, Bacillus stearothermophilus, Pseudomonas, Haemophilus, Streptococcus and others, and furthermore cells of higher organisms, in particular established human or animal cell lines. The above strains of E. coli, for example E. coli HB101 and E. coli JA221, and furthermore Saccharomyces cerevisiae are preferred as the host microorganism.

In principle, all vectors which replicate and express the hybrid IFN genes according to the invention in the chosen host are suitable. Examples of vectors which are suitable for the expression of the hybrid interferon genes in an E. coli strain are bacteriophages, for example derivatives of λ bacteriophages, or plasmids, such as, in particular, the plasmid ColE1 and its derivatives, for example pMB9, pSF2124, pBR317 or pBR322. The preferred vectors of the present invention are derived from plasmid pBR322. Suitable vectors contain a complete replicon and a marker gene, which allows to select and identify the hosts transformed with the expression plasmids on the basis of a phenotypical trait. Suitable marker genes impart to the host, for example, resistance towards heavy metals, antibiotics and the like, Furthermore, preferred vectors of the present invention contain, outside the replicon and marker gene regions, recognition sequences for restriction endonucleases, so that the hybrid IFN gene and, if appropriate, the expression control sequence can be inserted at these sites. The preferred vector, the plasmid pBR322, contains an intact replicon, marker genes which confer resistance towards tetracycline and ampicillin (tet^R and amp^R) and a number of unique recognition sites for restriction endonucleases, for example PstI (cleaves in the amp^R gene, the tet^R gene remains intact), BamHI, HindIII and SalI (all cleave in the tet^R gene, the amp^R gene remains intact), NruI and EcoRI.

Several expression control sequences can be used for regulation of the gene expression. In particular, expression control sequences of highly expressed genes of the host to be transformed are used. In the case of pBR322 as the hybrid vector and E. coli as the host microorganism, for example, the expression control sequences (which contain, inter alia, the promoter and the ribosomal binding site) of the lactose operon, tryptophan operon, arabinose operon and the like, the β-lactamase gene, the corresponding sequences of the phage λ N gene or the phage fd-coat protein gene and others, are suitable. Whilst the plasmid pBR322 already contains the promoter of the β-lactamase gene (β-lac gene), the other expression control sequences must be introduced into the plasmid.

Vectors which are suitable for replication and expression in yeast contain a yeast replication start and a selective genetic marker for yeast. Hybrid vectors which contain a yeast replication start, for example chromosomal autonomously replicating segment (ars), are retained extrachromosomally within the yeast cell after the transformation and are replicated autonomously. Furthermore, hybrid vectors which contain sequences homologous to the yeast 2μ plasmid DNA can be used. Such hybrid vectors will get integrated by recombination into 2μ plasmids already existing within the cell, or replicate autonomously. 2μ sequences

are particularly suitable for plasmids with a high transformation frequency and permit high copy numbers. The preferred yeast vector of the present invention is the plasmid pJDB207.

Suitable marker genes for yeasts are, in particular, those which impart antibiotic resistance to the host or, in the case of auxotrophic yeast mutants, genes which complement host lesions. Corresponding genes impart, for example, resistance towards the antibiotic cycloheximide or provide for protrophy in an auxotrophic yeast mutant, for example the URA3, LEU2, HIS3 or, in particular, TRP1 gene. Yeast hybrid vectors furthermore preferably contain a replication start and a marker gene for a bacterial host, in particular E. coli, so that the construction and cloning of the hybrid vectors and their intermediates can take place in a bacterial host.

Expression control sequences which are suitable for expression in yeast are, for example, those of highly expressed yeast genes. Thus, the promoters of the TRP1 gene, the ADHI or ADHII gene, acid phosphatase (PH03 or PH05) gene, isocytochrome gene or a promoter involved with the glycolytic pathway, such as the promoter of the enolase, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 3-phosphoglycerate kinase (PGK), hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase and glucokinase genes, can be used. Preferred vectors of the present invention contain promoters with transcriptional control, e.g. the promoters of the PH05, ADH II and GAPDH genes, which can be turned on or off by variation of the growth conditions. For example, the PH05 promoter can be repressed or derepressed solely by increasing or decreasing the concentration of inorganic phosphate in the medium.

Promoters for use in mammalian cells are for example viral promoters, such as HTLV, SV40, vaccinia promoter and the like.

The promoter is operatively linked to the coding region so as to ensure effective expression of the hybrid interferon. In one embodiment of the present invention, the promoter is directly linked to the coding region of mature hybrid IFN with a translation start signal (ATG) inserted at the junction. A preferred region for joining the promoter to the hybrid IFN coding region is between the major mRNA start and the ATG of the gene naturally attached to said promoter.

In another embodiment of the present invention, especially if yeast is used as the host microorganism, a signal sequence is included in the construction. Suitable signal sequences are those naturally linked to the promoter used. For example the PH05 signal sequence, furthermore the signal sequences of the yeast invertase or α-factor genes may be used. Those combinations are favoured which allow a precise cleavage between the signal sequence and the mature hybrid IFN amino acid sequence. Additional sequences, such as pro- or spacer-sequences which may or may not carry specific processing signals can also be included in the constructions to facilitate accurate processing of precursor molecules. Alternatively fused proteins can be generated containing internal processing signals which allow proper maturation in vivo or in vitro.

Preferably, the hybrid vectors according to the present invention comprise also the 3'-flanking sequence of a gene which contains the proper signals for transcription termination and polyadenylation. Suitable 3'-flanking sequences are for example those of the gene naturally linked to the promoter used. In the case yeast is used as the host microorganism and the PH05 promoter is used as expression control sequence, the 3'-flanking sequence is preferably that of the PH05 gene.

In a preferred embodiment, the present invention relates to hybrid vectors capable of replication and phenotypical selection in a host strain comprising a promoter and a DNA sequence encoding any of the novel hybrid interferons, said DNA sequence being positioned together with transcription start and termination signals as well as translation start and stop signals in said hybrid vectors under the control of said promoter such that in a transformed host it is expressed to produce a hybrid interferon according to the invention.

The hybrid vectors according to the present invention can be produced by in vitro ligation of appropriate segments of the LyIFN-α-2 and LyIFN-α-3 coding regions and inserting the resulting hybrid IFN gene into a suitable vector or by ligating a particular IFN DNA segment to a linearized vector DNA already containing the remaining sections in such a way that the gene coding for the desired hybrid interferon is formed. It is preferred that the DNA segment donating the amino terminal end sequence is already fused to the expression control sequence.

Appropriate segments of the LyIFN-α-2 and LyIFN-α-3 coding regions are especially taken from the preferred starting vectors of the present invention, viz. the pBR322 derived plasmids pAM2 and pAM21. The plasmid pAM2 contains a HindIII-PstI insert with the LyIFN-α-3 coding region under the control of the β-lactamase promoter. The vector part of plasmid pAM2 is resistant to PvuII. The plasmid pAM21 contains a HindIII-PstI insert with the LyIFN-α-2 coding region having a unique PvuII site at position 92 and being under the control of the β-lactamase promoter. The vector part of plasmid p21 is likewise resistant to PvuII.

Especially, the gene coding for hybrid interferon "$B_1B_2B_3D_4$" is prepared by ligating the large PstI-PvuII fragment of plasmid pAM21, the small Sau3A-PvuII fragment of plasmid pAM21 and the small Sau3A-PstI fragment of plasmid pAM2. This construction results in a plasmid (pJC344) containing the hybrid interferon "$B_1B_2B_3D_4$" coding region under the control of the β-lactamase promoter. In a similar manner, the gene coding for hybrid interferon "$B_1B_2D_3B_4$" is prepared by ligating the large PvuII-PstI fragment of plasmid pAM21, the small Sau3A-PvuII fragment of plasmid pAM2 and the small Sau3A-PstI fragment of plasmid pAM21. This construction results in a plasmid (pJC342) containing the hybrid interferon "$B_1B_2D_3B_4$"coding region under the control of the β-lactamase promoter. The gene coding for hybrid interferon "$B_1D_2D_3D_4$" can be prepared by ligating the small HindIII-Sau3A fragment of plasmid pAM21, the small Sau3A-PvuII fragment of plasmid pAM2 and the large PvuII-HindIII fragment of plasmid pAM2. This construction results in a plasmid (pAM94) containing the hybrid interferon "$B_1D_2D_3D_4$" coding region under the control of the β-lactamase promoter. Analogously, the gene coding for hybrid interferon "$B_1D_2B_3B_4$" is prepared by ligating the small HindIII-Sau3A fragment of plasmid pAM21, the small Sau3A-PvuII fragment of plasmid pAM2 and the large PvuII-HindIII fragment of plasmid pAM21. This construction results in a plasmid (pAM90) containing the hybrid interferon "$B_1D_2B_3B_4$" coding region under the control of the β-lactamase promoter. Plasmids containing the hybrid interferon "$B_1D_2B_3D_4$" coding rgion (e.g. plasmid pDM1) or the hybrid interferon "$B_1D_2D_3B_4$" coding region (e.g. plasmid pDM2) may be prepared in a similar manner. These plasmids are suitable for replicating and expressing the hybrid IFN genes in E. coli. If desired, the DNA insert coding for any of said hybrid interferons may be excised from the corresponding plasmid and introduced into a different vector DNA. In this manner, plasmids may be obtained which contain the hybrid interferon coding region under the control of a promoter other than the β-lactamase promoter and/or allow the gene to be expressed in a host different from E. coli.

The preparation of DNA comprising the structural gene for any of said hybrid interferons may also be performed by means of chemical synthesis. Suitable methods for the synthesis of DNA have been presented in summary form by S.A. Narang, I Tetrahedron 39, 3 (1983) J. The known synthesis techniques allow the preparation of polynucleotides towards 20 bases in length, in good yield, high purity and in a relatively short time. Suitably protected nucleotides are linked with one another by the phosphodiester method I K.L. Agarwal et al., Angew. Chem. 84, 489 (1972) J, or the even more efficient phosphotriester method I C.B. Reese, Tetrahedron 34, 3143 (1972) J or phosphite triester method I R.L. Letsinger et al., J. Am. Chem. Soc. 98, 3655 (1976) I. Simplification of the synthesis of the oligonucleotides and polynucleotides is made possible by the solid phase method, in which the nucleotide chains are bound to a suitable polymer. Itakura et al. I J. Am. Chem. Soc. 103, 706 (1981) J use trinucleotides linked by the phosphotriester method in the solid phase synthesis, instead of individual nucleotides, and these can thus be condensed, in a short time and with good yields, for example, to give a polynucleotide with 31 bases. The actual double-stranded DNA can be built up enzymatically from chemically prepared short segments. For this, Khorana et al.
I J. Biol. Chem. 251, 565 (1976) J use overlapping polynucleotide sequences from both DNA strands, which are held together in the correct arrangement by base-pairing and are then chemically linked by the enzyme DNA ligase. Another possibility comprises incubating in each case one polynucleotide sequence from the two DNA strands with a short overlapping segment in the presence of the four required deoxynucleoside triphosphates with a DNA-polymerase, for example DNA-polymerase I, a Klenow fragment of polymerase I or T_4 DNA polymerase, or with AMV (avian myeloblastosis virus) reverse transcriptase. The two polynucleotide sequences are thereby held together in the correct arrangement by base-pairing and are supplemented with the required nucleotides by the enzyme to give a complete double-stranded DNA I S.A. Narang et al., Anal. Biochem. 121, 356 (1982) J. Itakura et al. I J. Biol. Chem. 257, 9226 (1982) J describe how, on the basis of this principle, a segment 132 base pairs long of the human leukocyte interferon α_2-gene can be built in the presence of DNA-polymerase I (Klenow fragment) from 4 chemically synthesised fragments 39 to 42 bases in length, a 40 % saving in chemical synthesis in comparison with the method which uses only ligase being achieved. A suitable procedure for preparing the DNAs according to the present inventin has been described, for example, in European Patent Application No. 146,785 the teaching of which is herein incorporated by reference.

The chemically synthesized DNA comprising the structural gene for any of the hybrid interferons according to the invention may be inserted into a vector DNA containing an expression control sequence in a manner such that the expression control sequence regulates the expression of said gene.

2. Transformation of the host cells

The invention also relates to a process for the preparation of a transformed host, which comprises

transforming a host with an expression vector containing a DNA sequence which codes for any of the hybrid interferons according to the invention and is regulated by an expression control sequence.

Examples of suitable hosts are the above-mentioned microorganisms, such as strains of Saccharomyces cerevisiae, Bacillus subtilis and Escherichia coli. The transformation with the expression plasmids according to the invention is carried out, for example, as described in the literature, thus for S. cerevisiae (A. Hinnen et al., Proc. Natl. Acad. Sci. USA 75, 1929 (1978)], B. subtilis (Anagnostopoulos et al., J. Bacteriol. 81, 741 (1961)] and E. coli (M. Mandel et al., J. Mol. Biol. 53, 159 (1970)].

Accordingly, the transformation procedure of E. coli cells includes Ca^{2+} -pretreatment of the cells so as to allow DNA uptake, and incubation with the hybrid vector. The cells are transferred to a selective growth medium which allows separation of the transformed cells from the parent cells. Cells which do not contain the vector will not survive in such a medium. The transformation of yeast comprises, for example, the steps of (1) enzymatic removal of the yeast cell wall by means of glucosidases, (2) treatment of the obtained spheroplasts with the vector in the presence of polyethyleneglycol and Ca^{2+} -ions and (3) regeneration of the cell wall by embedding the spheroplasts into agar. Preferably, the regeneration agar is prepared in a way to allow regeneration and selection of the transformed cells at the same time.

The invention relates also to the transformed hosts obtainable by the route described.

3. Cultivation of the transformed host cells and isolation of the expressed hybrid interferon

The invention concerns further a method for the preparation of the hybrid interferons of the formulae I-VI, characterized in that host cells transformed with a hybrid vector which contains a DNA sequence coding for any of said hybrid interferons are cultured, and the hybrid interferon is isolated.

The transformed host cells are cultured by methods known in the art in a liquid medium containing assimilable sources of carbon, nitrogen and inorganic salts.

Various sources of carbon can be used for culture of the transformed hosts according to the invention. Examples of preferred sources of carbon are assimilatable carbohydrates, such as glucose, maltose, mannitol or lactose, or an acetate, which can be used either by itself or in suitable mixtures. Examples of suitable sources of nitrogen are amino acids, such as casaminoacids, peptides and proteins and their degradation products, such as tryptone, peptone or meat extracts; and furthermore yeast extracts, malt extract and also ammonium salts, for example ammonium chloride, sulfate or nitrate, which can be used either by themselves or in suitable mixtures. Inorganic salts which can also be used are, for example, sulfates, chlorides, phosphates and carbonates of sodium, potassium, magnesium and calcium.

The medium furthermore contains, for example, growth-promoting substances, such as trace elements, for example iron, zinc, manganese and the like, and preferably substances which exert a selection pressure and prevent the growth of cells which have lost the expression plasmid. Thus, for example, ampicillin is added to the medium if the expression plasmid contains an amp^R gene. Such an addition of antibiotic substances also has the effect that contaminating antibiotic-sensitive microorganisms are destroyed. If a yeast strain which is auxotrophic in, for example, an essential amino acid, is used as the host microorganism the plasmid preferably contain a gene coding for an enzyme which complement the host defect. Cultivation of the yeast strain is performed in a minimal medium deficient in said amino acid.

Culture is effected by processes which are known in the art. The culture conditions, such as temperature, pH value of the medium and fermentation time, are chosen so that a maximum titre of hybrid interferon is obtained. Thus, an E. coli or yeast strain is preferably cultured under aerobic conditions by submerged culture with shaking or stirring at a temperature of about 20 to 40 °C, preferably about 30 °C, and a pH value of 4 to 8, preferably at about pH 7, for about 4 to 30 hours, preferably until maximum yields of the hybrid interferon are reached.

It is surprisingly found that the maximum titres of the hybrid interferons according to the invention, for example of hybrid interferons "$B_1D_2D_3B_4$", "$B_1D_2B_3D_4$", "$B_1D_2D_3D_4$" and "$B_1D_2B_3B_4$", attainable in crude extracts of, for example, transformed yeast cultured under standard conditions are distinctly higher than those of interferon-α-2 and hybrid interferons "$B_1B_2D_3D_4$" and "$D_1D_2B_3B_4$" which are closely related to hybrid interferons "BD" and "DB" described in U.S. Patent 4,414,150.

When the cell density has reached a sufficient value, the culture is interrupted and the hybrid interferon is released from the cells of the host. For this purpose, the cells are destroyed, for example by treatment with a detergent, such as SDS or triton, or lysed with lysozyme or a similarly acting enzyme. If yeast is used as host microorganism the cell wall may be removed by enzymatic digestion with a glucosidase. Alternatively or additionally, mechanical forces, such as shearing forces (for example X-press, French press, Dyno mill) or shaking with glass beads or aluminium oxide, or alternating freezing, for example in liquid nitrogen, and thawing, for example to 30 ° to 40 °C, as well as ultra-sound can be used to break the cells.

The resulting mixture, which contains proteins, nucleic acids and other cell constituents, is enriched in proteins, including hybrid interferon, in a manner which is known per se, after centrifugation. Thus, for example, most of the non-protein constituents are removed by polyethyleneimine treatment and the proteins, including hybrid interferon, are precipitated, for example, by saturation of the solution with ammonium sulfate or with other salts. Bacterial proteins can also be precipitated by acidification with acetic acid (for example 0.1 %, pH 4-5). Further purification steps include, for example, ultrafiltration, diafiltration, gel electrophoresis, chromatographic processes, such as ion exchange chromatography, size exclusion chromatography, HPLC, reverse phase HPLC and the like, separation of the constituents of the mixture according to molecular size by means of a suitable Sephadex column, dialysis, affinity chromatography, for example antibody, especially monoclonal antibody, affinity chromatography, and other known processes, especially those known in the art.

Surprisingly, the hybrid interferons according to the invention have higher thermstabilities than interferon LyIFN-α-2 and hybrid interferon "$B_1B_2D_3D_4$". Thus, the temperature at which 50 % of the antiviral activity is lost (temperature increase of IFN solutions at 1 °C/min) is 62.8 °C in the case of LyIFN-α-2 and 63 °C in the case of hybrid interferon "$B_1B_2D_3D_4$" whereas the corresponding values of, for example, hybrid interferons "$B_1D_2D_3D_4$", "$B_1D_2B_3B_4$", "$B_1D_2B_3D_4$" and "$B_1D_2D_3B_4$" are 65 °C, 64.7 °C, 65.3 °C and 64.2 °C, respectively.

The invention concerns furthermore the hybrid interferons of the formulae I-VI whenever prepared according to the methods of the present invention.

The invention concerns also the hybrid interferons obtainable according to the inventive process.

The invention concerns especially the hybrid vectors, the transformed host cells, the hybrid interferon polypeptides and the processes for the preparation thereof as described in the Examples.

Biological properties of the hybrid interferons and pharmaceutical formulations

The hybrid interferons according to the present invention exhibit interesting biological properties which qualify them as valuable pharmaceuticals.

The hybrid interferons of the invention are distinguished by a very potent antiviral activity on mammalian cells, such as bovine and especially human cells. Thus, the antiviral titres determined as the reduction of cytopathic effect according to the method of S. Rubinstein et al. ǀ J. Virol. 37, 755 (1981) using vesicular stomatitis virus (VSV) as the challenge virus on bovine (MDBK) and human (WISH) cells ǀ are as follows:

Table 1

Antiviral titres of hybrid interferons using VSV as challenge virus		
interferon	specific activity/mg protein	
	bovine	human
"$B_1D_2D_3D_4$"	$1.25 \cdot 10^8$	$1.3 \cdot 10^8$
"$B_1D_2B_3B_4$"	$1.25 \cdot 10^8$	$1.3 \cdot 10^8$
"$B_1B_2D_3B_4$"	$9.5 \cdot 10^7$	$>2 \cdot 10^8$
"$B_1D_2B_3D_4$"	$1.9 \cdot 10^8$	$7.7 \cdot 10^7$
"$B_1D_2D_3B_4$"	$7.2 \cdot 10^7$	$3.8 \cdot 10^7$
"$B_1B_2B_3D_4$"	$1.05 \cdot 10^8$	$8 \cdot 10^7$
"$D_1D_2B_3B_4$" (DB)	$8.5 \cdot 10^7$	$3.3 \cdot 10^5$
"$B_1B_2D_3D_4$" (BD)	$1.15 \cdot 10^8$	$2.33 \cdot 10^8$
α-2 ("B")	$1.05 \cdot 10^8$	$2.13 \cdot 10^8$
α-3 ("D")	$5.5 \cdot 10^7$	$6.7 \cdot 10^6$

Accordingly, the antiviral action of the hybrid interferons according to the invention is not confined to human cells but is invariably interspecific. Thus, the antiviral activities of hybrid interferons "$B_1D_2D_3D_4$", "$B_1D_2B_3B_4$", "$B_1B_2D_3B_4$", "$B_1D_2B_3D_4$" and "$B_1B_2B_3D_4$" exhibited on human cells are comparable in degree to those of parent interferon α-2 and hybrid interferon "$B_1B_2D_3D_4$" which is closely related to hybrid interferon "BD" disclosed in U.S. patent No. 4,414,150, but are distinctly superior to parent interferon α-3 (by factor 10-30) and to reference hybrid interferon "$D_1D_2B_3B_4$" (by factor 230-600).

Apart from the general antiviral activity on human cells, the hybrid interferons according to the invention

give rise to a more specific response of target cells. Thus, various intracellular enzymes are induced on interferon action. Among these enzymes is (2'-5') oligoisoadenylate synthetase ("2-5 A synthetase") whichs generates (2'5') linked oligonucleotides, and these in turn activate a latent endoribonuclease which cleaves viral mRNA. It is surprisingly found that the hybrid interferons according to the invention, such as "$B_1D_2D_3D_4$", "$B_1D_2B_3B_4$", "$B_1B_2B_3D_4$" and "$B_1B_2D_3B_4$", are particularly active inducers of 2-5 A synthetase activity in human cells. Thus, at 10^3 units/ml hybrid interferons "$B_1D_2D_3D_4$", "$B_1D_2B_3B_4$", "$B_1B_2B_3D_4$" and "$B_1B_2D_3B_4$ give a 17.5 fold, 11.4 fold, 8.5 fold and 9.6 fold increase, respectively, in 2-5 A synthetase activity in Daudi cells compared to a 5.6, 3.9, 4.0 or 4.3 fold increase on action of parent interferons α-2 and α-3 and reference hybrid interferons "$B_1B_2D_3D_4$" and "$D_1D_2B_3B_4$", respectively.

Furthermore, the hybrid interferons according to the present invention show antiproliferative activities. The antiproliferative activity can be assayed as follows: interferon containing solutions are incubated with $5*10^4$ Daudi cells per ml in 2 ml RPMI medium containing 10 % foetal calf serum. Cells multiplication is determined after 3 days incubation by counting viable cells in an haemocytometer using the Trypan blue exclusion test. Thus, the concentration of hybrid interferon "$B_1D_2D_3D_4$", "$B_1D_2B_3B_4$", "$B_1B_2B_3D_4$", "$B_1B_2D_3B_4$", "$B_1D_2B_3D_4$" and "$B_1D_2D_3B_4$" required to induce a 50 % inhibition of Daudi cell multiplicaltion is about 4, 4, 1.3, 1.3, 4 and 4 units/ml, respectively, hence approximately equivalent to parent interferon α-2 and reference hybrid interferon "$B_1B_2D_3D_4$" (about 1.3 units/ml) and superior to parent interferon α-3 (about 20 units/ml) and reference hybrid interferon "$D_1D_2B_3B_4$" (about 400 units/ml).

The antiproliferative activity against human cancers can also be demonstrated in vivo. Thus, various cancers of human origin, such as cancers of the breast, colon and lung, ovarian cancer and melanoma, have been propagated in nude mice. Small fragments of the propagated tumors are isolated and implanted by trochar under the venal capsule of (Balb/c x DBA/2)F_1 (CDF_1) mice. IFN is administered intramuscularly at two daily doses of from $5*10^5$ to $5*10^7$ IU/kg/inj. at days 1 to 5 (day of administration: day 0). At day 6 the animals are sacrificed, the final tumor sizes are measured and compared to the initial tumor sizes (before IFN treatment) and to the tumor sizes in untreated control mice. Upon treatment with the hybrid interferons according to the invention, for example "$B_1D_2D_3D_4$" and "$B_1B_2D_3B_4$", a considerable and significant inhibition of tumor growth and, partially, regression of tumors is observed. Surprisingly, the antiproliferative activity of the hybrid interferons is superior to that of parental interferons α-2 and α-3 as well as to that of hybrid interferons "$B_1B_2D_3D_4$" and "$D_1D_2B_3B_4$".

The antiviral and antiproliferative properties of the hybrid interferons according to the invention make these polypeptides useful for treating viral infections, such as influenza and other respiratory tract virus infections, herpes virus infections, rabies and hepatitis infection, and neoplastic diseases, such as melanoma, renal cancer and hairy cell leukemia, of the human or animal body, optionally in combination with other antiviral or antitumor agents, preferably in the form of pharmaceutical preparations that contain a therapeutically effective amount of the active ingredient optionally together or in admixture with inorganic or organic, solid or liquid, pharmaceutically acceptable carriers which are suitable preferably for parenteral administration.

Parenteral formulations are especially injectable fluids that are effective in various manners, such as intravenously, intramuscularly, intraperitoneally, intranasally, intradermally or subcutaneously. Such fluids are preferably isotonic aqueous solutions or suspensions which can be prepared before use, for example from lyophilised preparations which contain the active ingredient alone or together with a pharmaceutically acceptable carrier. The pharmaceutical preparations may be sterilised and/or contain adjuncts, for example preservatives, stabilisers, wetting agents and/or emulsifiers, solubilisers, salts for regulating the osmotic pressure and/or buffers. The present pharmaceutical preparations, which may, if desired, contain further pharmacologically valuable substances, are produced in a manner known per se, for example by means of conventional dissolving of lyophilising processes, and contain from approximately 0.1 % to 100 %, especially from approximately 1 % to approximately 50 %, and in the case of lyophilisates up to 100 %, of the active ingredient. It is worthy of note that the hybrid interferons according to the invention, such as "$B_1D_2D_3D_4$", can be lyophilised and redissolved without any loss of activity. In contrast thereto, lyophilised preparations of parental interferons α-2 and α-3 give turbid solutions with a remarkable decrease in activity. The latter interferons can therefore only be stored in buffered solutions.

The invention also concerns a method for producing a pharmaceutical composition characterized in that a pharmacologically active compound of the present invention is admixed with a pharmaceutically acceptable carrier.

The particular mode of administration and the dosage will be selected by the attending physician taking into account the particulars of the patient, the disease and the disease state involved. For instance, viral infections are usually treated by daily or twice daily doses over a few days to a few weeks whereas treatment of neoplasms typically involves daily or multidaily doses over weeks or months. The same daily

dose levels, viz. about 10^6 to 10^7 units, as are used in conventional interferon therapy may be applied.

Purification of hybrid interferon by monoclonal antibody

There has been developed a hybridoma cell line with the designation 144 PS 22-6-19, which has been deposited on March 14, 1985 at the "Collection Nationale de Cultures de Microorganismes", Institut Pasteur, Paris, under the number I-424. This hybridoma cell line is a hybrid of the mouse myeloma cell line Sp2/O-Ag14 and of a B lymphocyte of the spleen of a Balb/c mouse immunized with natural human IFNα. It is a stable cell line, which secretes the monoclonal antibody with the designation 144 BS. The cell line may be kept in culture or deep-frozen in liquid nitrogen and reactivated by thawing.

The monoclonal antibody 144 BS is prepared applying processes known per se, characterised in that the hybridoma cell line 144 BS 22-6-19

a) is cultivated in vitro and the monoclonal antibody isolated from the culture supernatant, or

b) is propagated in vivo in a suitable mammal and the monoclonal antibody recovered from body fluids of said mammal.

The hybrid interferons according to the invention can be purified by immunoaffinity chromatography with the aid of the monoclonal antibody 144 BS. The monoclonal antibody is bound to a suitable carrier of organic or inorganic origin, such as cross-linked agarose, dextran or polyacrylamide in suitably functionalized form, optionally after activation, applying general methods known in the art. For instance, a carrier bearing activated ester functions is suspended in an aqueous buffer solution, mixed with a solution of the monoclonal antibody, filtered, resuspended and washed to remove excess monoclonal antibody, and treated with a solution of irrelevant proteins to block free reactive positions of the carrier. This antibody-coated carrier is used to selectively bind the hybrid interferons according to the invention in glycosylated or unglycosylated form. To that end carrier material is suspended in a suitable aqueous solvent, for example a salt solution, such as NaCl solution, or a buffer solution, such as phosphate-buffered NaCl solution, $NaHCO_3$ solution or 3-(N-morpholino)propanesulphonic acid solution, and brought into contact with the solution containing the hybrid interferon, for example is poured into a chromatography column and the solution containing the hybrid interferon is introduced and pumped through the carrier material, if desired under pressure. Unbound proteins and other impurities are washed away with aqueous solutions, for example buffer solutions in a pH range of from approximately pH 5 to approximately pH 9 and/or salt solutions, for example NaCl solution. The hybrid interferon bound to the antibody on the carrier material is eluted with suitable aqueous solutions, for example buffer solutions in a pH range of from approximately pH 2 to approximately pH 5, such as glycine buffer or citric acid, or pH gradients of differing composition or salt solutions, for example concentrated NH_4SCN solution. The eluate containing the hybrid interferon is optionally neutralized, and the purified hybrid interferon is isolated therefrom according to known procedures.

The following Examples serve to illustrate the present invention but should not be construed as a limitation thereof.

Experimental Part

Abbreviations used in the Examples:

DTT	1,4-dithiothreitol
TNE	solution containing 50 mM Tris•HCl pH 7,5, 100 mM NaCl, 2 mM EDTA
Tris	tris-(hydroxymethyl)-aminomethane
EDTA	ethylenediamine tetraacetic acid
TE	solution containing 10 mM Tris, 50 µM EDTA
N-medium	contains Bacto-Tryptone (10 g, Difco), yeast extract (1 g, Difco), glucose (1 g), NaCl (8 g), $CaCl_2 \cdot 2H_2O$ (249 mg) per 1 l solution
TBE	solution containing 89 mM Tris, 89 mM boric acid, 2 mM EDTA
BSA	bovine serum albumin
HAT	hypoxanthine/aminopterin/thymidin
Ig	immunoglobulin
IU	international units (of interferon activity)
MAb	monoclonal antibody
PBS	phosphate buffered saline
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis

Part I: Construction and cloning of interferon α-2/α-3 hybrids in Escherichia coli

All hybrid interferon protein coding sequences described below have been operatively linked to the β-lactamase promoter, cloned into a pBR322 derived expression plasmid and transformed into E. coli HB101.

Example 1: Construction of PvuII resistant vectors carrying DNA sequences of the lymphoblastoid IFNs α-2 and α-3

pBR322 is cut with PvuII (Biolabs), and the linear DNA is ligated to a [^{32}P]-BclI linker [d-(ATGTGTGATCACACAT) synthesized by the solid phase phosphotriester method, cf. H. Rink et al., Nucleic Acids Research 12, 6369 (1984)] as follows:
The restricted plasmid DNA (1 µg, about 0.7 pmole ends) is ligated to ^{32}P-labelled BclI linker DNA (about 40 pmole ends) in 50 µl reaction volume containing 20 mM Tris-HCl pH 7.8, 10 mM $MgCl_2$, 10 mM DTT, 0.5 mM rATP and 40 U/µl T_4 DNA Ligase (Biolabs). After 2-3 h incubation at 15°C, the aqueous phase is extracted with an equal volume of phenol/chloroform, and the DNA is precipitated by the addition of 1/9 volume 10x TNE, 2.5 volumes of ethanol and keeping at -20°C over night. The DNA pellet is resuspended in 50 µl TE pH 8.0 and centrifuged through a 5-23 % sucrose gradient (50 mM Tris-HCl pH 8.0/1 mM EDTA) in a Kontron TST 60 rotor for 4 h at 58000 rpm and 15°C. The gradient is fractioned from top to bottom, and the radioactivity in each fraction is monitored by Cerenkov counting. Fractions of interest are pooled and the DNA precipitated with TNE and ethanol. The DNA pellet is resuspended in restriction buffer and digested with BclI and again precipitated after extraction of the aqueous phase with phenol/chloroform. This linear DNA is then recirculated at a concentration of 5 µg/ml under conditions identical to those described for the linker ligation.

The ligated DNA is cut with the enzymes HindIII, PvuII (to elimate residual intact pBR322) and PstI, and the HindIII-PstI large fragment (3600 bp + linker) containing the origin of replication and the amp^R gene is isolated by sucrose gradient centrifugation.

Hind III-PstI inserts from CG-pBR(AP)/LyIFN-α-2 (1160 bp) and from CG-pBR(AP)/LyIFN-α-3 (1020 bp) (European Patent Application No. 76,489) obtained by sucrose gradient centrifugation are each ligated into the DNA prepared above, resulting in plasmids pAM4 and pAM2, containing the LyIFN-α-2 and LyIFN-α-3 coding regions, respectively, under the control of the β-lactamase promoter.

Ligated DNA is directly used to transform competent E. coli as follows: The DNA ligation mixture (or fractions of it) is transferred into 150 µl 15 mM Tris-HCl pH 7.5, 10 mM $CaCl_2$, 10 mM $MgCl_2$, 10 mM NaCl, 0.5 mM EDTA, and 50 µl of the $CaCl_2$-treated recipient E. coli HB 101 are added. The mixture is kept at 0°C for 20 min., then transferred to 42°C for 2 min., and after cooling to room temperature 1 ml of N-medium is added. The incubation is continued at 37°C for 1 h (shaker platform, 250 rpm) after which 600 µl are plated on McConkey-Agar (Difco) containing tetracycline (10 µg/ml). The petri dishes are incubated at 37°C for 16-18 h, when bacterial colonies can be observed.

Plasmid DNA is obtained from the transformants by the following procedure. 10 ml N-medium containing tetracycline (10 µg/ml) is inoculated with one transformant colony each and the cultures are

agitated at 37°C (shaking platform, 150-300 rpm) until they had reached an optical density of 0,9-1,1 (650 nm). The cells are harvested and resuspended in an equal volume of N-medium containing tetracycline (10 µg/ml) and chloramphenicol (80 µg/ml). Incubation is continued at 37°C for another 18 h to amplify the number of plasmid copies per cell. The bacteria are harvested and the bacterial pellet is resuspended in 50 mM Tris HCl pH 8 (10 ml per gram of wet cell weight) and lysozyme (Sigma) is added to a final concentration of 2 mg/ml. After 10 min at 0°C the solution is adjusted to 50 mM EDTA, after 10 min at 0°C Triton X 100 is added to a final concentration of 0.8 % and the preparation is kept at 0°C for 1 h followed by centrifugation in a Sorvall SS-34 rotor for 30 min at 18000 rpm. After extraction of the clear supernatant with phenol followed by two extractions with chloroform RNase A (Sigma) is added to a final concentration of 25 µg/ml and the solution is incubated at 37°C for 1 h. For removal of RNA, NaCl (1 M final concentration) and polyethylene glycol 6000 (7.5 % final concentration) are added and the preparation is kept at -10°C for 2 h, followed by centrifugation for 10 min at 10.000 rpm and 0°C in a Sorvall SS-34 rotor. The pellets are resuspended in 200 µl TNE and again extracted with phenol/chloroform (1:1) before reprecipitation (by addition of 500 µl ethanol, 5 min at -80°C, 10 min Eppendorf centrifuge). The DNA pellets are resuspended in 20-40 µl of TE. Typical yields resulting from this procedure are about 20 µg of plasmid DNA.

The prepared plasmid DNAs (pAM4 and pAM2) are subjected to restriction enzyme analysis. The structure of pAM4 and pAM2 is confirmed.

Example 2: Elimination of the 3' extracistronic PvuII site from pAM 4

Unlike pAM2 (α-3), pAM4 (α-2) still contains two PvuII sites (cf. Fig. 3). In order to facilitate the construction of hybrid IFN genes at the PvuII site within the IFN coding region, the second PvuII site within the extracistronic region is eliminated.

This modification of plasmid pAM4 is essentially a deletion of the 3' extracistronic IFN α-2 cDNA sequence between PstI and the 3' extracistronic PvuII site (see Fig. 3). pAM4 is partially digested with PvuII l 5 µg pAM4 in 50 mM NaCl, 6 mM $MgCl_2$, 6 mM 2-mercaptoethanol, 10 mM Tris•HCl pH 7.5, 1 unit/µg PvuII (Biolabs) added, digest for 5 min. at 37°C, stop by phenol extraction J and is ligated to l ^{32}P J -PstI linker (Collaborative Research, procedure as described in Example 1). The DNA mixture is transformed into E. coli HB 101 as above and the plasmids of 16 colonies are screened by restriction enzyme analysis using PstI, PvuII and HindIII for the presence of a deletion having the correct size (246 bp). Out of the 16 colonies screened three are unmodified pAM4, one is a multimer, five have a large deletion (620 bp) and seven carries the desired small deletion (246 bp).One clone with a shortened IFN α-2 insert (Hind III-PstI small fragment now 907 bp) is selected and is designated pAM21.

Example 3: Construction of Pvu hybrids with crossover sites at amino acid 92

Plasmids pAM2 (α-3) and pAm21 (α-2) are each cut with HindIII and PvuII which results in the excision of DNA fragments (521 bp) containing the β-lactamase promoter sequences and the N-terminal halves of the IFN genes (coding for amino acids 1 to 92). The large and small fragments (about 4Kb and 521 bp, respectively) are separated by sucrose gradient centrifugation, and the large fragments are dephosphorylated by treatment of 1 µg DNA fragments in 50 µl 50 mM Tris pH 8 with 1 unit of calf intestine alkaline phosphatase per 10 pmole 5'ends for 30 min at 37°C. Hybrid IFN "$B_1B_2D_3D_4$" and "$D_1D_2B_3B_4$" genes are constructed by ligation of the appropriate large and small DNA fragments described above l large HindIII-PvuII fragment (~ 4Kb) from pAM2 ligated to small HindIII-PvuII fragment (521 bp) from pAM21 resulted in type $B_1B_2D_3D_4$ IFN, etc. J. Ligation is carried out in 10 µl volumes containing 20 mM Tris•HCl pH 7.8, 10 mM $MgCl_2$, 10 mM DTT, 0.5 mM rATP, 40 units/µl T_4DNA ligase (Biolabs) and approximately 500 ng of large DNA fragments, and 20-40 ng of small DNA fragments. After 5 h at 15°C, 5 µl of the ligation mixtures are used to transform E. coli HB101 as above, resulting in about 1000 transformed colonies each. Three colonies are picked each, their plasmid DNA is isolated and is analyzed with the enzymes HindIII, PvuII, EcoRI, TaqI and PstI. Two colonies harbouring plasmid DNAs of the predicted structure l pAM 27 ("$B_1B_2D_3D_4$") and pAM 33 ("$D_1D_2B_3B_4$") J are selected.

Example 4: Construction of hybrid IFN genes crossed over at amino acid 150

Plasmids pAM 2 (α-3) and pAM 21 (α-2) are each digested with PvuII and PstI. The DNA fragments are separated by sucrose gradient centrifugation into PvuII-PstI large fragments (about 4Kb, encoding the N-terminal 92 amino acids of IFNs α-2 and α-3), and PvuII-PstI small fragments (386 and 495 bp in length,

respectively, encoding the C-termini of IFNs α-2 and α-3). In a second step, the purified PvuII-PstI small fragments from pAM2 and pAM21 are each ^{32}P-labelled as follows:
The DNA fragments (~ 1 µg) are each dissolved in 50 µl 50 mM Tris-HCl pH 8.0 and dephosphorylated by treatment with 1 U/10 pmole 5' ends alkaline phosphatase for 30 min. at 37°C. The enzyme is inactivated by incubation for 1 h at 65°C, and the DNA is purified by adsorption and elution from DEAE-Cellulose and precipitated in ethanol. The DNA is phosphorylated in a 20 µl reaction volume containing 50 mM Tris-HCl pH 9.5/10 mM $MgCl_2$/(5 mM DTT, 30-40 µl of lyophilized γ-[^{32}P] -ATP (Amersham, 6000 Ci/mmol, 1 mCi/ml) and 0.5 U/µl T_4 Kinase (P.L. Biochemicals). After 20 min. at 37°C, the reaction is stopped by adding EDTA to 10 mM. An excess (~ 4µg) of corresponding untreated DNA fragment is then added, and the solution is extracted with phenol and chloroform. DNA is separated from residual γ-[^{32}P] -ATP by chromatography through Sephadex G-50 in TNE. The excluded peak fractions as monitored by Cerenkov counting are pooled and precipitated with ethanol.

The ^{32}P labelled PvuII-PstI small fragments are each digested completely with Sau3A, and the restricted DNA is separated by electrophoresis in a 6 % polyacrylamide gel using TBE as running buffer. 4 DNA fragments corresponding to PvuII-Sau3A (172 bp each, encoding amino acids 93 to 150 of IFNs α-2 and α-3), and to Sau3A-PstI (214 bp and 323 bp, respectively encoding amino acids 151 to 166 of IFNs α-2 and α-3) are isolated from the gel.

By religation of 3 appropriate fragments (see Table 2), 4 hybrid IFN types crossed over at amino acid 150 are generated. The corresponding plasmids are reconstructed in a series of steps; in a first ligation reaction equimolar amounts of PvuII-Sau3A and Sau3A-PstI DNA fragments are ligated in 10 µl of ligation buffer containing 20 mM Tris·HCl pH 7.8, 10 mM $MgCl_2$, 10 mM DTT, 0.5 mM rATP and 40 units/µl T4 DNA ligase for 3-6 hours at 15°C (resulting in concatemeric DNA). After digestion with PvuII-PstI and extraction with phenol/chloreform (1:1), an excess of the appropriate PvuII-PstI large fragment I dephosphorylated: typically 1 µg of DNA in 50 µl 50 mM Tris·HCl pH 8 (about 0.6 pmole 5'ends) treated with 0.06 units of calf intestine alkaline phosphatase for 30 min at 37°C] is added and the DNA is ligated again. E. coli HB 101 is transformed and 4 clones carrying DNA of the predicted structure as determined by restriction analysis (PvuII, Sau3A, TaqI and EcoRI) are picked. The clones are designated E. coli HB 101 pJC334, pJC37, pJC342 and pJC344 (cf. Table 2).

Table 2

Origin of DNA fragments used for construction of the hybrid IFNs crossed over at amino acid 150. In brackets the IFN type and the section of αIFN encoded on the fragments are indicated.				
resultant hybrid IFN type	clone and plasmid designation	ligated DNA fragments (length)		
		PvuII-PstI (about 4Kb)	PvuII-Sau3A (170 bp)	Sau3A-PstI (210 bp, α-2) (320 bp, α-3)
"$D_1D_2D_3B_4$"	pJC334	AM2(α-3_{1-92})	Am2(α-3_{93-150})	Am21(α-$2_{151-166}$)
"$D_1D_2B_3D_4$"	pJC337	AM2(α-3_{1-92})	Am21(α-2_{93-150})	Am2(α-$3_{151-166}$)
"$B_1B_2D_3B_4$"	pJC342	AM21(α-2_{1-92})	Am2(α-3_{93-150})	Am21(α-$2_{151-166}$)
"$B_1B_2B_3D_4$"	pJC344	AM21(α-2_{1-92})	Am21(α-2_{93-150})	Am2(α-$3_{151-166}$)

Example 5: Construction of hybrid IFN genes crossed over at amino acid 60

The construction of these hybrid IFN genes is accomplished by essentially the same procedure as described in Example 4.

Plasmids pAM2 (α-3) and pAM21 (α-2) are each cut with HindIII and PvuII, and the fragments are separated by sucrose gradient centrifugation. The HindII-PvuII small fragments (521 bp, containing the β-lac promoter region and encoding the N-terminal 92 amino acids of IFNs α-2 and α-3) are ^{32}P-labelled and partially digested with Sau3A (0,5 units of enzyme per µg of DNA, 5 minutes at 37°C, stop reaction by phenol extraction). The resulting mixture of fragments is resolved by electrophoresis in 6 % polyacrylamide gels, and 4 fragments (two 424 bp HindIII-Sau3A fragments and two 97 bp Sau3A-PvuII fragments) are extracted from the gel. Religation of 3 appropriate fragments by the stepwise procedure as described in Example 4 (see Table 3), and transformation into E. coli HB 101 results in transformants of only 2 of the expected 4 constructions: pAM65 ("$D_1B_2D_3D_4$") and pAM90 ("$B_1D_2B_3B_4$").

Table 3

Origin of DNA fragments used for the construction of two hybrid IFN genes crossed over at amino acid 61. In brackets the IFN type and the section of αIFN encoded on the fragments are indicated.				
resultant hybrid IFN type	clone and plasmid designation	DNA fragment type (length)		
		HindIII-Sau3A (424 bp)	Sau3A-PvuII (97 bp)	HindIII-PvuII (about 4Kb)
$D_1B_2D_3D_4$	pAM65	Am2(α-3_{1-61})	Am21(α-2_{62-92})	AM2(α-3_{93-166})
$B_1D_2B_3B_4$	pAM90	Am21(α-2_{1-61})	Am2(α-3_{62-92})	AM21(α-2_{93-166})

For the construction of the other two hybrid IFN genes crossed over at amino acid 61, the plasmids pAM65 and pAM90 are each cut with HindIII and PvuII, and the small HindIII-PvuII DNA fragments (521 bp) are isolated after electrophoresis in agarose gels. Ligation of the HindIII-PvuII small fragment of pAM90 with the HindIII-PvuII large fragment of pAM2 results in pAM94 ($B_1D_2D_3D_4$), and ligation of the small HindIII-PvuII fragment of pAM65 with the HindIII-PvuII large fragment of pAM21 yields plasmid pAM76 ($D_1B_2B_3B_4$). The structure of the 4 above plasmid DNAs is confirmed by restriction enzyme analysis.

The respective clones are referred to as E. coli HB101 pAM65, pAM90, pAM94 and pAM76.

Part II: Construction and clonsing of interferon α-2/α-3 hybrids in Saccharomyces cerevisiae

All hybrid interferon protein coding sequences described below have been linked to the PH05 promoter and the PH05 transcription termination signals, cloned into the yeast 2 µ vector pJDB207, and transformed into yeast strain S. cerevisiae GRF18.

Example 6: Construction of expression plasmid p31RT (Δ72)

Plasmid pJDB207/IF2(5_1)Δ72 (European Patent Application No. 100,561) is digested with HindIII and EcoRI and a 470 bp fragment comprising the carboxyl-terminal end of interferon α-2 and PH05 transcription termination signals is isolated by agarose gel electrophoresis and electroelution l R. Yang et al. Methods Enzym. 68, 176 (1979) J using Micro-Collodion Bags (Sartorius, Göttingen, FRG). Plasmid p31R (cf. EP 100,561) is digested with HindIII and EcoRI and the 4.1 kb vector part is isolated using the same technique. After ligation of 200 ng of vector and insert DNA using E. coli T4 DNA ligase and transformation of E. coli HB 101 to ampicillin resistance a plasmid called p31RT(Δ72) containing the PH05 promoter, the C-terminal end of interferon α-2 and PH05 transcription termination signals is isolated and its structure is confirmed by restriction analysis.

PH05 expression plasmid p31RT(Δ72) (see example 6) is digested with EcoRI and XhoI and the recessed 3'ends are filled in with dCTP, dGTP, dATP and dTTP using the Klenow fragment of DNA polymerase I of E. coli. The blunt ends are subsequently dephosphorylated with bacterial alkaline phosphatase. Plasmids pJC334, pAM90 and pJC337 (Examples 4 and 5) are digested with TaqI (pJC334

and pAM90), or with TaqI and EcoRI (pJC337), and the 3' recessed ends are filled with the corresponding deoxynucleotides as described above. DNA fragments of approximately 560 base pairs are isolated by agarose gel electrophoresis and electroelution using Micro-Collodion Bags as above. Ligation of 200 ng prepared vector part of plasmid p31RT(Δ72) and 200 ng eluted DNA fragments using T4 DNA ligase and transformation of E. coli HB 101 to ampicillin resistance gives rise to plasmids p31R/IFN AM104, (containing the hybrid interferon "$D_1D_2D_3B_4$" coding sequence), p31R IFN AM119 (containing the hybrid interferon "$B_1D_2B_3B_4$" coding sequence) and p31R IFN AM114 (containing the hybrid interferon "$D_1D_2B_3D_4$" coding sequence), respectively. By cutting these plasmids with BamHI and HindIII a DNA fragment of approximately 1300 bp of the configuration PH05 promoter - IFN protein coding sequence - PH05 transcription termination signal is isolated by agarose gel electrophoresis and electroelution. This fragment is cloned between the HindIII and BamHI sites of plasmid pJDB207. The plasmids are transformed into yeast strain GRF18 to Leu$^+$ (cf. EP 100,561, cf. also Example 14). The resulting clones are referred to as S. cerevisiae GRF 18/pJDB207 PH05/IFN AM104, GRF18/pJDB207 PH05/IFN AM114 and GRF18/pJDB207 PH05/IFN AM119.

Example 8: Construction of plasmid pJDB207 PH05/IFN AM129

Plasmid pAM94 (Example 5) is completely cut with TaqI and partially (in the presence of 10 ng/ml ethidium bromide) with EcoRI. A DNA fragment of about 580 bp is isolated by agarose gel electrophoresis and electroelution and ligated to the EcoRI and XhoI cut, gel purified, vector part of plasmid p31RT(Δ72) (cf. Example 6). After transformation of E. coli HB101 to ampicillin resistance the plasmid p31R PH05/IFN AM129 is isolated. This plasmid containing the interferon "$B_1D_2D_3D_4$" coding region is digested with HindIII and BamHI, and the 1300 bp DNA fragment is ligated into pJDB207 as described in Example 7. The plasmid is transformed into yeast strain GRF 18 as above. The resulting clone is designated S. cerevisiae GRF18/pJDB 207 PH05/IFN AM129.

Example 9: Construction of plasmids pJDB207 PH05/IFN AM 106, AM110 and AM112

Plasmids pJDB207 PH05 IFN/AM 104, AM114 and AM129 (see Examples 7 and 8) are digested with BamHI and PvuII and the 6.8 Kb vector parts containing the respective C-terminal ends of the interferon coding regions are isolated by agarose gel electrophoresis and electroelution. Likewise, plasmid pJDB207R/(α-2)Δ72 (European Patent Application No. 100,561) is digested with BamHI and PvuII and a DNA fragment of 800 bp is isolated. Ligation of the corresponding fragments and transformation of E. coli HB101 gives rise to plasmids pJDB207 PH05/IFN AM106 (containing the hybrid interferon "$B_1B_2B_3D_4$" coding region), AM112 (containing the hybrid interferon "$B_1B_2D_3B_4$" coding region) and AM110 (containing the hybrid interferon "$B_1B_2D_3D_4$" coding region). Yeast strain GRF18 is transformed as usual. The resulting clones are referred to as S. cerevisiae GRF18 pJDB207 PH05/IFN AM106, pJDB207 PH05/IFN AM112 and pJDB207 PH05/IFN AM110.

Example 10: Construction of plasmids pJDB207 PH05/IFN DM 1 and DM 2

Plasmid pJDB207 PH05/IFN AM119 (Example 7) is digested with HindIII and BglII and the 7.5 Kb vector part containing the N-terminal end of the interferon coding region is isolated by agarose gel electrophoresis and electroelution. Likewise, plasmids pJDB207 PH05/IFN AM114 and pJDB207 PH05/IFN AM104 (see Example 7) are each digested with the same enzymes and DNA fragments of about 600 bp containing the C-terminal end of the interferon coding region are isolated. The corresponding fragments are ligated with T4 DNA ligase. After transformation of E. coli HB101 plasmids pJDB207 PH05/IFN DM1 containing the hybrid interferon "$B_1D_2B_3D_4$" coding region and pJDB207 PH05/IFN DM2 containing the hybrid interferon "$B_1D_2D_3B_4$" coding region are obtained. Yeast strain GRF 18 is transformed as described above. The resulting clones are referred to as S. cerevisiae GRF18 pJDB207 PH05/IFN DM1 and S. cerevisiae GRF18 pJDB207 PH05/IFN DM2.

Example 11: Construction of plasmid pJDB207 PH05/IFN HRI43

Plasmid pJDB207R/IF(α-2)Δ72 (European Patent Application No. 100,561) is digested with BamHI and PvuII and a DNA fragment of 6.8 kb is isolated. Likewise plasmid pJDB207R/IF (α-3) (EP 100,561) is digested with the same enzymes and a DNA fragment of 800 bp is isolated. Ligation of the two DNA fragments and transformation of E. coli HB101 to ampicillin resistance gives plasmid pJDB207 PH05/IFN

HRi43 containing the hybrid interferon "$D_1D_2B_3B_4$" coding region. Yeast strain GRF18 is transformed as above. The resulting clones are referred to as S. cerevisiae GRF18 pJDB207 PH05/IFN HRi43..

Example 12: Transformation of Saccharomyces cerevisiae GRF18 and induction of interferon production

Plasmid pJDB207 PH05/IFN AM129 ("$B_1D_2D_3D_4$") is introduced into Saccharomyces cerevisiae strain GRF18 (α, his 3-11, his 3-15, leu 2-3, leu 2-112, can^R) in analogy as described (Hinnen et al., Proc. Natl. Acad. Sci., USA 75, 1929 (1978)) One µg of plasmid DNA is added to 100 µl of a spheroplast suspension and the mixture is treated with polyethylene glycol. The spheroplasts are mixed with 10 ml regeneration agar and plated onto yeast minimal medium plates without leucine. After incubation for 3 days at 30°C, about 1000 transformed cells are obtained.

One single yeast colony from the yeast transformation plates [named Saccharomyces cerevisiae GRF 18/pJDB207 PH05/IFN AM129] is picked into 10 ml of yeast minimal medium in a 100 ml Erlenmeyer flask, and grown at 30°C at 200 rpm for 24 hrs to a density of about 2-3x10^7 cells/ml. The cells are washed once with 20 ml of low-P_i minimal medium [as "Difco yeast minimal medium without amino acids" supplemented with 20 g/l glucose, but prepared from the components according to the recipe of Difco (Difco Manuals Difco Laboratories, Detroit, USA) except that 0.03 g/l KH_2PO_4 plus 1 g/l KCl is used instead of 1 g/l KH_2PO_4]. Three ml of the resuspended cells are used to inoculate 300 ml low-P_i minimal medium and 300 ml normal minimal medium, respectively, in 1000 ml Erlenmeyer flasks. Incubation is at 30°C at 160 rpm. Induction of the PH05 promoter is followed by measuring the appearance of acid phosphatase activity in whole cells as described (Toh-e et al., J. Bacteriol. 113, 727 (1973). The cells are grown to about 1-2x10^7 cells/ml (26-30 hrs of incubation).

Example 13: Preparation of yeast cell extracts and determination of the interferon titre

Cells from the 300 ml culture medium (see Example 12) at a density of 1-2x10^7/ml are collected by centrifugation in a Sorvall GSA rotor for 5 min at 8000 rpm at 4°C. The cells are washed once with 100 ml H_2O, resuspended in 6 ml ice cold lysis mix [0.1 M potassium phosphate buffer pH 7.4, 1 % (v/v) Triton X-100,0.0001M PMSF (Merck)] and transferred to a 30 ml corex tube. The suspension is centrifuged again for 5 min in a Sorvall SS-34 rotor at 8000 rpm at 4°C and resuspended in 3 ml lysis mix at 0°C. Four grams of glass beads (0.4 mm in diameter) are added to the cells and the suspension is shaken on a Vortex Mixer (Scientific Instruments Inc., USA) at full speed for 30 sec and then cooled for 1 min in an ice bath. This shaking procedure is repeated 5 to 10 times until more than 90 % of cells are broken (check under light microscope). Cell debris and glass beads are removed from the solution by centrifugation for 10 min at 8000 rpm at 4°C in a Sorvall HB-4 rotor. The supernatant is transferred to Eppendorf tubes, frozen in liquid nitrogen and stored at -60°C. Interferon activity is determined according to S. Rubinstein et al. [J. Virol 37, 755 (1981)] to be 7*10^9 units/ml cell extract.

In an analogous manner as described in Examples 12 and 13, S. cerevisiae strain GRF18 is transformed with plasmids pJDB207 PH05/IFN AM119 ("$B_1D_2B_3B_4$"), AM106 ("$B_1B_2B_3D_4$"), AM112 ("$B_1B_2D_3B_4$"), DM1 ("$B_1D_2B_3D_4$"), DM2 ("$B_1D_2D_3B_4$"), AM110 ("$B_1B_2D_3D_4$") and HRi43 ("$D_1D_2B_3B_4$"). The resulting strains are cultured. The cells are harvested and the interferon titres are determined as above. The IFN titres are as follows:

S. cerevisiae GRF18/pJDB207 PH05/IFN AM119: 5*10^9 units/ml
S. cerevisiae GRF18/pJDB207 PH05/IFN AM106: 7*10^7 units/ml
S. cerevisiae GRF18/pJDB207 PH05/IFN AM112: 2*10^8 units/ml
S. cerevisiae GRF18/pJDB207 PH05/IFN DM1 : 3*10^9 units/ml
S. cerevisiae GRF18/pJDB207 PH05/IFN DM2 : 5*10^9 units/ml
S. cerevisiae GRF18/pJDB207 PH05/IFN AM110: 3*10^8 units/ml
S. cerevisiae GRF18/pJDB207 PH05/IFN HRi43: 1*10^9 units/ml

Under the same conditions, S. cerevisiae strains GRF18/pJDB207R/IF (α-2) and /IF (α-3) (EP 100,561) give titres of 1*10^8 and 3*10^9 units/ml, respectively.

Example 14: Production of hybrid interferon "$B_1D_2D_3D_4$" by transformed yeast cells on a 30 l scale

Yeast strain GRF 18 bearing the plasmid pJDB207 PH05/IFN AM129 encoding the gene for hybrid interferon "$B_1D_2D_3D_4$" downstream from the acid phosphatase promoter PH05 is streaked onto the surface of an agar culture of YNB medium. The plate is incubated at 30°C until confluent growth is observed. A sterile loop is used to transfer a portion of the surface culture to a shake flask containing the pre-culture

medium, IFN/21 containing

Yeast extract (Difco)	10.0 g
L-asparagine*H_2O	6.6 g
KH_2PO_4	1.0 g
$MgSO_4*7H_2O$	1.9 g
L-histidine	0.02 g
D-glucose (monohydrate)	33.0 g per 1 l deionised water.

The 500 ml flask has a single baffle and contains 100 ml of medium. The medium is prepared using deionised water end has a pH value of approximately 6.0. The glucose is sterilised separately. After inoculation the first pre-culture is incubated for 24 h at 30 °C on an orbital shaker with 5 cm throw at a speed of 250 rev/min. The first pre-culture flask provides the inoculum for the second pre-culture flasks. These flasks receive an inoculum of 1 % v/v. The medium and incubation conditions are identical with those for the first pre-culture. Sufficient flasks from the second pre-culture stage are combined to provide 1 % v/v inoculum for the 30 l fermenter (3 flasks/fermenter). The production fermenter has an operating volume of 30 l, contains four baffles and a single six-bladed disc turbine agitator with a diameter of 115 mm. The agitation rate is 600 rev/min, air is sparged at 1 vol/vol/min and the overpressure is maintained at 0.3 bar. The fermentation temperature is 30 °C. The fermenter is sterilised with medium IFN/23 containing

Yeast extract (Difco)	2.0 g
L-aspargine*H_2O	6.6 g
$MgSO_4*7H_2O$	1.0 g
L-histidine	0.02 g
D-glucose (monohydrate)	33.0 g per 1 l deionised water.

glucose being sterilised separately and added to give a final volume of 30 l.
After inoculation the fermentation pH value is controlled so that it does not fall below 6.0 by addition of sodium hydroxide. The fermentation has a duration of about 18 h or until the maximum yield of interferon is reached.
The optical density which is a convenient measure of the growth of the yeast reaches a value between 6-7 OD units. The glucose is largely but not completely consumed and there is an induction of acid phosphatase activity concomitant with the production of interferon.
The interferon titre can be measured in crude extracts prepared by mechanical cell discription in the laboratory and is $7*10^8$ units/l cell extract. The protein content of the crude extract is approximately 1 mg/ml.
At the end of the fermentation the culture broth may be cooled to 10 °C if necessary prior to harvesting and recovery of the hybrid interferon.
30 l culture broth of pH 6.0 is cooled to 10 °C and the cells are separated with a Sharples® centrifuge. The clear supernatant contain no IFN activity. The obtained cell mass is adjusted with Buffer X (2 mM Tris pH 8.0, 5 mM EDTA, 0.5 M NaCl, 20 µM phenylmethylsulphonylfluoride) to 1400 ml and has a pH value of 8.0. After cooling to 5-10 °C the suspension is passed through a DYNO®-Mill (type KDL Pilot, 0.6 l) provided with polyurethane agitator disks and 500 ml glass beads of 0.5-0.75 mm diameter and having an agitation speed of 3,000 rpm and a feed rate of 10 l/h, whereby the cells are disrupted. The suspension has a pH value of 8. The suspension containing the broken cells is clarified by centrifugation. The centrifugation is performed in a Sorvall 6SA rotor et 12,000 rpm at 4 °C for 20 min.
The strain S. cerevisiae GRF18 bearing the plasmid pJDP207 PH05/IFN AM119, AM106, AM112, DM1 and DM2, respectively, can be cultured and a clarified solution containing the corresponding hybrid interferon can be produced in an analogous manner.

Example 15: Preparation of extracts of E. coli strain HB101/pAM94 and determination of IFN activity.

E. coli strain HB101/pAM94 is grown to an optical density of 8 at 650 nm in 1 liter of M 9 medium containing 0.4 % casamino acids end 2 % glucose. Cells are sedimented and resuspended in buffer (16 g cells in 160 ml buffer) containing 0.1 M Tris-HCl pH 8.0, 0.5 M NaCl, 5 mM EDTA and 100 µM PMSF. Lysozyme is added to a final concentration of 1 mg/ml. The solution is then kept on ice for 30 min. The

cells in this suspension are opened using a Sorvall Omnimix at a setting of three times for 60 seconds. The suspension containing the broken cells is clarified by centrifugation in a Sorvall 6SA rotor at 12,000 rpm at 4 °C for 20 min. The supernatant is assayed for IFN activity using the method of S. Rubinstein et al. (supra).

An IFN activity of $5 \cdot 10^7$ units/ml cell extract is found.

In an analogous manner, E. coli strains HB101/pAM90, pJC344 and pJC342 are cultured and the interferon titres are determined as above to be $5 \cdot 10^8$, $5 \cdot 10^7$ and $5 \cdot 10^6$ units/ml cell extract.

Example 16: Preparation of hybridoma cells

a) Source of immunogen: A sample of semi-purified natural human leukocyte IFN (IFNα) obtained from Dr. K Cantell (Helsinki) with a specific activity of 13 x 10^6 IU per mg protein is used for the immunizations.

b) Immunization protocol: Seven female Balb/c mice (Tierfarm Sisseln, Switzerland) 10-14 weeks old are immunized by injection into the four footpads of 4 x 10^5 IU of IFNα (Example 16a) emulsified in complete Freund's adjuvant (Difco). A further injection of 4 x 10^5 IU of IFNα in incomplete Freund's adjuvant is given at day 30, and a booster injection (i.p.) of 6 x 10^5 IU of IFNα in PBS at day 60. Three days later the spleens are taken for the fusion.

c) Cell fusion: All fusion experiments are performed according to the procedure of G. Köhler and C. Milstein (Nature 256, 495 (1975)) using the nonsecreting Sp 2/O-Ag14 myeloma line (M. Shulman, C.D. Wilde and G. Köhler, Nature 276, 269 (1978)). 10^8 spleen cells are mixed with 10^7 myeloma cells in the presence of 1 ml of 50 % polyethylene glycol (PEG 1500, Serva). After washing, the cells are resuspended in 48 ml of standard Dulbecco's minimum essential medium (Gibco No. 0422501). 3×10^6 normal mouse peritoneal exsudate cells per fusion are added as feeder cells. The cells are distributed into 48 x 1 ml costar wells and fed 3 times per week with standard HAT selection medium for 3 to 6 weeks. When the growth of hybridoma cells becomes visible, the supernatants are screened by a combined immunoprecipitation-bioassay (Example 19). Out of a total of 221 hybridomas, 10 hybridomas are found to produce anti-IFNα antibodies. The hybridoma cells are cloned by limiting dilution in microtiter plates at least once. Hybridoma 144 BS is selected for further studies because it is particularly stable and secretes large quantities of immunoglobulin.

Example 17: Isolation and purification of monoclonal antibody

Balb/c mice 8-10 weeks of age (Tierfarm Sisseln, Switzerland) are pretreated intraperitoneally with 0.3 ml pristane (Aldrich). 2-3 weeks later, $2\text{-}5 \times 10^6$ cloned hybridoma cells and 0.2 ml pristane are inoculated intraperitoneally. After 8-10 days ascites fluid is collected, centrifuged at 800 x g and stored at -20 °C.

Defrosted ascites fluid is centrifuged at 50000 x g for 60 min. A fat layer floating on the surface is carefully removed, and the protein concentration is adjusted to a concentration of 10-12 mg/ml. Crude immunoglobulin is precipitated by dropwise addition of 0.9 volume equivalents of saturated ammonium sulphate at 0 °C, then dissolved in 20 mM Tris-HCl/50 mM NaCl (pH 7.9) and dialysed against the same buffer. An immunoglobulin fraction is obtained by DEAE-D52 cellulose (Whatman) chromatography using a buffer gradient system of 20 mM Tris-HCl/25-400 mM NaCl, pH 7.9. The immunoglobulin is again precipitated with ammonium sulphate and dissolved in PBS at a concentration of 10 mg/ml.

Sodium dodecyl sulphate polyacryl amide gel electrophoresis (SDS-PAGE) demonstrates a purity grade of more than 95 percent for the monoclonal antibodies 144 BS.

Example 18: Preparation of an immunoadsorbent column

One ml of settled Affi-Gel® 10 (Bio-Rad) is coupled to 17 mg of monoclonal antibody 144 BS according to the manufacturer's instruction: Affi-Gel® 10 is washed on a sintered glass filter first with cold distilled water and then with a 0.1 M $NaHCO_3$ solution pH 8.0 (coupling buffer). A suspension of 50 % gel in coupling buffer is transferred into a plastic tube, mixed with an equal volume of purified monoclonal antibody solution and rotated for 4 hours at room temperature. After coupling the gel is washed with coupling buffer and, in order to block unreacted sites, is treated with 0.1 ml of 1 M ethanolamine-HCl (pH 8.0) for 1 to 2 hours at room temperature. The gel is washed with PBS in the presence of 10 mM NaN_3 and kept therein at 4 °C.

Example 19: Purification of recombinant IFNα by immunoaffinity chromatography

A suspension of broken E.coli cell producing recombinant IFNα, e.g. E.coli HB 101/pAM94 (cf. Example 15) is clarified by centrifugation in a Sorvall 6SA rotor at 12,000 rpm at 4°C for 20 min. Polyethyleneimine (Polyimin P®, Fluka, pH 8.0) is added to the supernatant to a final concentration of 0.25 % (w/v). The solution is stirred for 1 hour and subsequently centrifuged. The pellet is discarded and the supernatant brought to 65 % saturation with solid ammonium sulfate. This mixture is stirred for 1 hour and then centrifuged. The supernatant is discarded and the pellet suspended in one tenth volume (15-20 ml) of PBS (pH 7.2). The solution is dialysed overnight against PBS containing 0.02 % NaN_3.

The dialysed solution is mixed with 1 ml of the immunoaffinity gel of Example 18. This mixture is gently agitated for 1 hour, then packed into a column and washed successively with 5 column volumes of PBS, 5 column volumes of PBS containing 0.5 M NaCl and 0.2 % Triton X-100®, and 5 column volumes of PBS. Alternatively, the immunoaffinity gel is packed first into the column and the dialysed solution added on top, then washed as described. The recombinant hybrid IFNα ("$B_1D_2D_3D_4$") is eluted with an acid buffer pH 2.5 containing 0.1 M citric acid and 0.3 M NaCl. The active IFN fractions are neutralized with 1 M Tris, dialysed against PBS and concentrated to about 0.1 mg/ml protein using ultrafiltration flat bed membranes XM10 (Amicon Corp.).

The maximum capacity of the immunoadsorbent column of Example 18 containing 17 mg of monoclonal antibody 144 BS is about 1.2 mg of each of IFN-α-2 and IFN-α-3 polypeptides, at least 0.8 mg of IFN-α-1 type polypeptide, 1.15 mg hybrid interferon "$B_1B_2D_3B_4$", 1.25 mg hybrid interferon "$B_1D_2B_3B_4$", 1.25 mg hybrid interferon "$B_1D_2D_3D_4$", 0.44 mg hybrid interferon "$B_1B_2B_3D_4$", 1.6 mg hybrid interferon "$B_1D_2B_3D_4$", 1.6 mg hybrid interferon "$B_1D_2D_3B_4$", 1.15 mg hybrid interferon "$B_1B_2D_3D_4$" and 1.25 mg hybrid interferon "$D_1D_2B_3B_4$". The immunoadsorbent gel may be used for up to 50 separations without substantial loss of capacity. Recombinant IFN-α-2, IFN-α-3, IFN-α-1 type polypeptides and hybrid interferons purified by this method are homogenous on SDS-PAGE.

Example 20: Purification of hybrid interferons using precipitation and chromatographic methods

1 l of interferon "$B_1D_2D_3D_4$" containing solution clarified by centrifugation (cf. Examples 13-15) is acidified using 2M HCl until the solution reaches pH 2.2. The solution is stirred for 1h at 4°C and the precipitated protein is separated via centrifugation (GSA rotor Sorvall at 12,000 rpm at 4°C for 20 min). The pellet is discarded and the supernatant is brought to 30 % saturation with solid ammonium sulfate. This solution is stirred for 1h at 4°C and then centrifuged. The supernatant containing the interferon is then dialysed against 0.1M NH_4HCO_3 at pH 8.2. An interferon activity of about 10^8 IU/ml is found.

A column containing 50 ml chelating Sepharose 6B (Pharmacia) loaded with Cu^{2+} ions according to the manufacturer's instructions is equilibrated using 250 ml 0.05M sodium acetate pH 7.0 and 0.5M NaCl. 200 ml of dialysed solution (see above) is applied at 4°C to the column. The column is washed with 150 ml equilibrating buffer. Elution of bound interferon is performed using a linear gradient of

250 ml 0.05M sodium acetate pH 7.0 and 0.5M NaCl

250 ml 0.05M sodium acetate pH 2.8 and 0.5M NaCl

and followed by a wash of 150 ml of 0.05M sodium acetate pH 2.8 and 0.5M NaCl.

The active interferon fractions are pooled and dialysed against 0.05M Tris·HCl buffer pH 8.0. Interferon activity in the dialysed solution is approximately 10^8 IU/ml. The maximum capacity of the column is about 75 mg of interferon "$B_1D_2D_3D_4$".

A column containing 50 ml Q-Sepharose anion exchanger (Pharmacia) is equilibrated using 250 ml 0.05M Tris·HCl buffer pH 8.0. 100 ml of the above dialysed interferon containing solution is applied to the column at room temperature. The column is subsequently washed with 50 ml equilibrating buffer. Bound interferon is eluted using a linear gradient of 0 to 0.7M NaCl in 200 ml 0.05 Tris·HCl pH 8.0. The pure interferon elutes at about 0.3M NaCl. The active fractions show a single band with an apparent molecular weight of 19,000 when analysed on SDS polyacrylamide gels. The maximum capacity of the anion exchange column is about 50 mg of hybrid interferon "$B_1D_2D_3D_4$".

In an analogous manner, hybrid interferons "$B_1D_2B_3B_4$", "$B_1D_2B_3D_4$", "$B_1D_2D_3B_4$", "$B_1B_2B_3D_4$" and "$B_1B_2D_3B_4$" can be purified.

Example 21: Lyophilisation of hybrid interferon "$B_1D_2D_3D_4$"

Interferon "$B_1D_2D_3D_4$" purified either via immunoaffinity chromatography using monoclonal antibody 144 BS or via conventional chromatographic techniques (cf. Exmple 20) is dialysed against 0.5M NH_4HCO_3 and subsequently lyophilised. Small volumes of water are added twice to the lyophilised sample in order to remove NH_4HCO_3 completely. The solution is then relyophilised.

Reconstitution of interferon "$B_1D_2D_3D_4$" solution is performed as follows: 0.5M NH_4HCO_3 pH 8.2 is added to the lyophilised sample to obtain an interferon concentration of about 0.1 mg/ml. The redissolved interferon has a specific antiviral activity of $2*10^8$ IU/mg which activity is identical to the corresponding value before lyophilisation.

Example 22: Physicochemical characterisation of hybrid interferons

The hybrid interferons according to the invention and the parental interferons are subjected to polyacrylamide SDS gel electrophoresis according to the method described by Laemmli l Nature 227, 680 (1970) l. The apparent molecular weights of the hybrid interferons given in Kilo-Daltons (kD) are summarized in Table 4. Table 4 contains also the isoelectric points (pI) of the hybrid interferons as determined using LKB polyacrylamide gels containing Ampholine®.

Table 4:

Apparent molecular weights and isoelectric points of hybrid interferons.		
IFN	app. molecular weight	pI
α-2	27,000	5.2
α-3	20,000	5.2
$B_1B_2D_3D_4$	27,000	5-6 (diffuse)
$D_1D_2B_3B_4$	20,000	5.0
$B_1B_2B_3D_4$	27,000	5.2
$B_1B_2D_3B_4$	26,000	5-6 (diffuse)
$B_1D_2D_3D_4$	19,000	5.4
$B_1D_2B_3D_4$	20,000	5.6
$B_1D_2D_3B_4$	20,000	5.5
$B_1D_2B_3B_4$	20,000	5.4

The amino acid sequence of the hybrid interferons according to the invention is determined using a 470 A protein sequencer from Applied Biosystems or using a 890 C Beckman sequencer. In both cases automated Edman degradation is performed. The structure found is in full accordance with the expected one. The N-terminal amino acid is cysteine.

Example 23: Effect of hybrid interferons on the level of (2'-5') oligoisoadenylate synthetase activity in Daudi cells

Daudi cells are seeded at 2 x 10^5 cells/ml in RPMI 1640 medium with 10 % foetal calf serum containing the concentration indicated (cf. Table 8) of hybrid interferons "$B_1B_2B_3D_4$", "$B_1D_2B_3B_4$", "$B_1D_2D_3D_4$" and "$B_1B_2D_3B_4$". After 24 hours treatment, 5 x 10^6 cells per sample are centrifuged (9000 rev/min) and resuspended in 500 µl of cold lysis buffer (20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer pH 7.5, 5 mM $MgCl_2$, 120 mM KCl, 7 mM dithiothreitol, 10 % glycerol, 0.5 % Nonidet P 40) and incubated for 2 min on ice. The samples are then centrifuged (9000 rev/min, 8 min., 4°C) and the supernatant is recovered and assayed for (2'-5')-oligoisoadenylate synthetase activity as follows:
The supernatant is mixed with 50 µl of poly(rI)*(rC) agarose (P-L Biochemicals) for 15 min at 30°C and the non-adsorbed material is removed by centrifugation. The poly(rI)*(rC)-adsorbed material is incubated with 2.5 mM ($\alpha^{32}P$) ATP, 400 Ci/mmol (Amersham International) for 20 h at 30°C, treated with bacterial alkaline phosphatase (Sigma) and then eluted from a column of acid alumina (Sigma) (300 µl) with 3.0 ml of 1M glycine-HCl buffer pH 2.0 as described (Merlin et al. Analyt. Biochem. 110 , 190 (1981)). Results in Table 5 are expressed as the fold increase in (2'-5')-oligoisoadenylate synthetase activity in interferon treated cells relative to the level of enzyme activity in untreated control cells (mean activity 51.6 ± 8.7 pmole/hr/10^5 cells).

Table 5

	Fold increase in (2'-5') (A) synthetase activity (pmole ATP incorporated/hr/10^5 cells)			
IFN concentration (units/ml)	1	10	100	1000
IFN α-2 ("B")	3.6	4.0	5.7	5.6
α-3 ("D")	2.2	3.2	3.2	3.9
"$B_1B_2D_3D_4$"	3.4	3.2	4.6	4.0
"$D_1D_2B_3B_4$"	2.6	2.1	4.8	4.3
"$B_1B_2B_3D_4$"	9.4	8.4	10.0	8.5
"$B_1D_2B_3B_4$"	5.6	5.7	7.8	11.4
"$B_1D_2D_3D_4$"	5.8	8.9	12.1	17.5
"$B_1B_2D_3B_4$"	5.6	7.0	9.1	9.6

Example 24: Pharmaceutical preparation (parenteral administration)

2 mg of hybrid interferon "$B_1D_2D_3D_4$" having a specific activity of $1.3 \cdot 10^8$ units/mg on human WISH cells, are dissolved in 30 ml of 5N human serum albumin. The resulting solution is passed through a bacteriological filter and the filtered solution is subdivided under aseptic conditions into 100 vials each containing $2.6 \cdot 10^6$ units of pure hybrid interferon. The vials which are suitable for parenteral administration are preferably stored in the cold, for example at -20 °C.

In the same manner, vials containing $5.2 \cdot 10^6$ or $1.04 \cdot 10^7$ units may be prepared by using 4 or 8 mg, respectively, of the hybrid interferon.

In an analogous manner, vials containing hybrid interferon "$B_1D_2B_3B_4$", "$B_1B_2D_3B_4$", "$B_1D_2B_3D_4$", "$B_1D_2D_3B_4$" or "$B_1B_2B_3D_4$" (specific activities $1.28 \cdot 10^8$, $2 \cdot 10^8$, $7.7 \cdot 10^7$, $3.8 \cdot 10^7$ and $8 \cdot 10^7$, respectively) can be prepared.

Claims

Claims for the following Contracting States : BE, DE, FR, GB, IT, NL, SE, CH, LU

1. A hybrid interferon polypeptide having an amino acid sequence composed of two to four sub-sequences corresponding in amino acid identity and number to sub-sequences of human lymphoblastoid interferons LyIFN-α-2 and LyIFN-α-3, said hybrid interferon polypeptide being selected from the group consisting of a polypeptide having an amino acid sequence consisting of amino acids 1 to 150 of LyIFN-α-2 and amino acids 151 to 166 of LyIFN-α-3, a polypeptide having an amino acid sequence consisting of amino acids 1 to 92 of LyIFN-α-2, amino acids 93 to 150 of LyIFN-α-3 and amino acids 151 to 166 of LyIFN-α-2, and a polypeptide having an amino acid sequence consisting of amino acids 1 to 60 of LyIFN-α-2, amino acids 61 to 92 of LyIFN-α-3, amino acids 93 to 150 of LyIFN-α-2 or of LyIFN-α-3 and amino acids 151 to 166 of LyIFN-α-2 or of LyIFN-α-3.

2. The hybrid interferon polypeptide "$B_1B_2B_3D_4$" having the formula

Table 5

	Fold increase in (2'-5') (A) synthetase activity [pmole ATP incorporated/hr/10^5 cells]			
IFN concentration [units/ml]	1	10	100	1000
IFN α-2 ("B")	3.6	4.0	5.7	5.6
α-3 ("D")	2.2	3.2	3.2	3.9
"$B_1B_2D_3D_4$"	3.4	3.2	4.6	4.0
"$D_1D_2B_3B_4$"	2.6	2.1	4.8	4.3
"$B_1B_2B_3D_4$"	9.4	8.4	10.0	8.5
"$B_1D_2B_3B_4$"	5.6	5.7	7.8	11.4
"$B_1D_2D_3D_4$"	5.8	8.9	12.1	17.5
"$B_1B_2D_3B_4$"	5.6	7.0	9.1	9.6

Example 24: Pharmaceutical preparation (parenteral administration)

2 mg of hybrid interferon "$B_1D_2D_3D_4$" having a specific activity of $1.3*10^8$ units/mg on human WISH cells, are dissolved in 30 ml of 5N human serum albumin. The resulting solution is passed through a bacteriological filter and the filtered solution is subdivided under aseptic conditions into 100 vials each containing $2.6*10^5$ units of pure hybrid interferon. The vials which are suitable for parenteral administration are preferably stored in the cold, for example at -20 °C.

In the same manner, vials containing $5.2*10^5$ or $1.04*10^7$ units may be prepared by using 4 or 8 mg, respectively, of the hybrid interferon.

In an analogous manner, vials containing hybrid interferon "$B_1D_2B_3B_4$", "$B_1B_2D_3B_4$", "$B_1D_2B_3D_4$", "$B_1D_2D_3B_4$" or "$B_1B_2B_3D_4$" (specific activities $1.28*10^8$, $2*10^8$, $7.7*10^7$, $3.8*10^7$ and $8*10^7$, respectively) can be prepared.

Claims

Claims for the following Contracting States : BE, DE, FR, GB, IT, NL, SE, CH, LU

1. A hybrid interferon polypeptide having an amino acid sequence composed of two to four sub-sequences corresponding in amino acid identity and number to sub-sequences of human lymphoblastoid interferons LyIFN-α-2 and LyIFN-α-3, said hybrid interferon polypeptide being selected from the group consisting of a polypeptide having an amino acid sequence consisting of amino acids 1 to 150 of LyIFN-α-2 and amino acids 151 to 166 of LyIFN-α-3, a polypeptide having an amino acid sequence consisting of amino acids 1 to 92 of LyIFN-α-2, amino acids 93 to 150 of LyIFN-α-3 and amino acids 151 to 166 of LyIFN-α-2, and a polypeptide having an amino acid sequence consisting of amino acids 1 to 60 of LyIFN-α-2, amino acids 61 to 92 of LyIFN-α-3, amino acids 93 to 150 of LyIFN-α-2 or of LyIFN-α-3 and amino acids 151 to 166 of LyIFN-α-2 or of LyIFN-α-3.

2. The hybrid interferon polypeptide "$B_1B_2B_3D_4$" having the formula

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN THR PHE ASN LEU PHE
SER THR LYS ASP SER SER ALA ALA LEU ASP GLU THR LEU LEU ASP GLU PHE
TYR ILE GLU LEU ASP GLN GLN LEU ASN ASP LEU GLU SER CYS VAL MET GLN
GLU VAL GLY VAL ILE GLU SER PRO LEU MET TYR GLU ASP SER ILE LEU ALA
VAL ARG LYS TYR PHE GLN ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER SER CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER LEU SER
LEU SER THR ASN LEU GLN GLU ARG LEU ARG ARG LYS GLU

according to claim 1.

3. The hybrid interferon polypeptide "$B_1B_2D_3B_4$" having the formula

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN THR PHE ASN LEU PHE
SER THR LYS ASP SER SER ALA ALA LEU ASP GLU THR LEU LEU ASP GLU PHE
TYR ILE GLU LEU ASP GLN GLN LEU ASN ASP LEU GLU ALA CYS VAL MET GLN
GLU GLU ARG VAL GLY GLU THR PRO LEU MET ASN ALA ASP SER ILE LEU ALA
VAL LYS LYS TYR PHE ARG ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER PRO CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER PHE SER
LEU SER ILE ASN LEU GLN LYS ARG LEU LYS SER LYS GLU

according to claim 1.

4. The hybrid interferon polypeptide "$B_1D_2B_3D_4$" having the formula

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU SER CYS VAL MET GLN
GLU VAL GLY VAL ILE GLU SER PRO LEU MET TYR GLU ASP SER ILE LEU ALA
VAL ARG LYS TYR PHE GLN ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER SER CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER LEU SER
LEU SER THR ASN LEU GLN GLU ARG LEU ARG ARG LYS GLU

according to claim 1.

5. The hybrid interferon polypeptide "$B_1D_2D_3B_4$" having the formula

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU ALA CYS VAL MET GLN
GLU GLU ARG VAL GLY GLU THR PRO LEU MET ASN ALA ASP SER ILE LEU ALA
VAL LYS LYS TYR PHE ARG ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER PRO CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER PHE SER
LEU SER ILE ASN LEU GLN LYS ARG LEU LYS SER LYS GLU

according to claim 1.

6. The hybrid interferon polypeptide "$B_1D_2D_3D_4$" having the formula

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU ALA CYS VAL MET GLN
GLU GLU ARG VAL GLY GLU THR PRO LEU MET ASN ALA ASP SER ILE LEU ALA
VAL LYS LYS TYR PHE ARG ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER PRO CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER LEU SER
LEU SER THR ASN LEU GLN GLU ARG LEU ARG ARG LYS GLU

* C L M S *

according to claim 1.

7. The hybrid interferon polypeptide "$B_1D_2B_3B_4$" having the formula

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU SER CYS VAL MET GLN
GLU VAL GLY VAL ILE GLU SER PRO LEU MET TYR GLU ASP SER ILE LEU ALA
VAL ARG LYS TYR PHE GLN ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER SER CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER PHE SER
LEU SER ILE ASN LEU GLN LYS ARG LEU LYS SER LYS GLU

according to claim 1.

8. A pharmaceutical preparation that contains a therapeutically effective amount of a hybrid interferon according to claim 1.

9. Use of a hybrid interferon according to claim 1 for the manufacture of pharmaceutical preparations.

10. A hybrid interferon according to claim 1 for use in a method for the therapeutic treatment of the human or animal body.

11. A method for the preparation of a hybrid interferon according to claim 1 characterized in that host cells transformed with a recombinant vector which contains a DNA sequence coding for said hybrid interferon are cultured, and said hybrid interferon is isolated.

12. A recombinant vector comprising a DNA sequence which codes for a hybrid interferon according to claim 1.

13. A host cell transformed with a recombinant vector comprising a DNA sequence which codes for a hybrid interferon according to claim 1.

14. A host cell according to claim 13 selected from the group consisting of a strain of Escherichia coli and a strain of Saccharomyces cerevisiae.

Claims for the following Contracting State : AT

1. Process for the preparation of a hybrid interferon polypeptide having an amino acid sequence composed of two to four sub-sequences corresponding in amino acid identity and number to sub-sequences of human lymphoblastoid interferons LyIFN-α-2 and LyIFN-α-3, said hybrid interferon polypeptide being selected from the group consisting of a polypeptide having an amino acid sequence consisting of amino acids 1 to 150 of LyIFN-α-2 and amino acids 151 to 166 of LyIFN-α-3, a polypeptide having an amino acid sequence consisting of amino acids 1 to 92 of LyIFN-α-2, amino acids 93 to 150 of LyIFN-α-3 and amino acids 151 to 166 of LyIFN-α-2 and a polypeptide having an amino acid sequence consisting of amino acids 1 to 60 of LyIFN-α-2, amino acids 61 to 92 of LyIFN-α-3, amino acids 93 to 150 of LyIFN-α-2 or of LyIFN-α-3 and amino acids 151 to 166 of LyIFN-α-2 or of LyIFN-α-3, characterized in that host cells transformed with a recombinant vector which contains a DNA sequence coding for said hybrid interferon polypeptide are cultured, and said hybrid interferon is

isolated.

2. Process according to claim 1 characterized in that the hybrid interferon polypeptide is selected from the group consisting of hybrid interferon polypeptide "$B_1B_2B_3D_4$" having the formula

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN THR PHE ASN LEU PHE
SER THR LYS ASP SER SER ALA ALA LEU ASP GLU THR LEU LEU ASP GLU PHE
TYR ILE GLU LEU ASP GLN GLN LEU ASN ASP LEU GLU SER CYS VAL MET GLN
GLU VAL GLY VAL ILE GLU SER PRO LEU MET TYR GLU ASP SER ILE LEU ALA
VAL ARG LYS TYR PHE GLN ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER SER CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER LEU SER
LEU SER THR ASN LEU GLN GLU ARG LEU ARG ARG LYS GLU,

hybrid interferon polypeptide "$B_1B_2D_3B_4$" having the formula

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN THR PHE ASN LEU PHE
SER THR LYS ASP SER SER ALA ALA LEU ASP GLU THR LEU LEU ASP GLU PHE
TYR ILE GLU LEU ASP GLN GLN LEU ASN ASP LEU GLU ALA CYS VAL MET GLN
GLU GLU ARG VAL GLY GLU THR PRO LEU MET ASN ALA ASP SER ILE LEU ALA
VAL LYS LYS TYR PHE ARG ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER PRO CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER PHE SER
LEU SER ILE ASN LEU GLN LYS ARG LEU LYS SER LYS GLU,

hybrid interferon polypeptide "$B_1D_2B_3D_4$" having the formula

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU SER CYS VAL MET GLN
GLU VAL GLY VAL ILE GLU SER PRO LEU MET TYR GLU ASP SER ILE LEU ALA
VAL ARG LYS TYR PHE GLN ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER SER CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER LEU SER
LEU SER THR ASN LEU GLN GLU ARG LEU ARG ARG LYS GLU,

hybrid interferon polypeptide "$B_1D_2D_3B_4$" having the formula

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU ALA CYS VAL MET GLN
GLU GLU ARG VAL GLY GLU THR PRO LEU MET ASN ALA ASP SER ILE LEU ALA
VAL LYS LYS TYR PHE ARG ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER PRO CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER PHE SER
LEU SER ILE ASN LEU GLN LYS ARG LEU LYS SER LYS GLU,

hybrid interferon polypeptide "$B_1D_2D_3D_4$" having the formula

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU ALA CYS VAL MET GLN
GLU GLU ARG VAL GLY GLU THR PRO LEU MET ASN ALA ASP SER ILE LEU ALA
VAL LYS LYS TYR PHE ARG ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER PRO CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER LEU SER
LEU SER THR ASN LEU GLN GLU ARG LEU ARG ARG LYS GLU,

and hybrid interferon polypeptide "$B_1D_2B_3B_4$" having the formula

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU SER CYS VAL MET GLN
GLU VAL GLY VAL ILE GLU SER PRO LEU MET TYR GLU ASP SER ILE LEU ALA
VAL ARG LYS TYR PHE GLN ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER SER CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER PHE SER
LEU SER ILE ASN LEU GLN LYS ARG LEU LYS SER LYS GLU.

3. Process according to claim 1 for the preparation of hybrid interferon "$B_1B_2B_3D_4$" characterized in that the recombinant vector contains a DNA sequence coding for said hybrid interferon.

4. Process according to claim 1 for the preparation of hybrid interferon "$B_1B_2D_3B_4$" characterized in that the recombinant vector contains a DNA sequence coding for said hybrid interferon.

5. Process according to claim 1 for the preparation of hybrid interferon "$B_1D_2B_3D_4$" characterized in that the recombinant vector contains a DNA sequence coding for said hybrid interferon.

6. Process according to claim 1 for the preparation of hybrid interferon "$B_1D_2D_3B_4$" characterized in that the recombinant vector contains a DNA sequence coding for said hybrid interferon.

7. Process according to claim 1 for the preparation of hybrid interferon "$B_1D_2D_3D_4$" characterized in that the recombinant vector contains a DNA sequence coding for said hybrid interferon.

8. Process according to claim 1 for the preparation of hybrid interferon "$B_1D_2B_3B_4$" characterized in that the recombinant vector contains a DNA sequence coding for said hybrid interferon.

9. Process according to claim 1 comprising the steps of
a) preparing a DNA comprising the structural gene coding for said hybrid interferon by in vitro recombination at the sites corresponding to amino acids 60, 92 and 150, respectively, of interferons LyIFN-α-2 and LyIFN-α-3 or by chemical DNA synthesis and incorporating the obtained DNA into an appropriate expression vector,
b) transferring the obtained recombinant vector carrying the hybrid IFN structural gene into a recipient host cell,
c) selecting transformed host cells from untransformed host cells by culturing under such conditions where only the transformed host cells survive,
d) culturing the transformed host cells under conditions which allow expression of the hybrid IFN structural gene, and
e) isolating the expressed hybrid IFN.

10. Process for the preparation of a recombinant vector comprising a DNA sequence coding for a hybrid interferon polypeptide having an amino acid sequence composed of two to four sub-sequences corresponding in amino acid identity and number to sub-sequences of human lymphoblastoid interferons LyIFN-α-2 and LyIFN-α-3, said hybrid interferon polypeptide being selected from the group consisting of a polypeptide having an amino acid sequence consisting of amino acids 1 to 150 of LyIFN-α-2 and amino acids 151 to 166 of LyIFN-α-3, a polypeptide having an amino acid sequence consisting of amino acids 1 to 92 of LyIFN-α-2, amino acids 93 to 150 of LyIFN-α-3 and amino acids 151 to 166 of LyIFN-α-2, and a polypeptide having an amino acid sequence consisting of amino acids 1 to 60 of LyIFN-α-2, amino acids 61 to 92 of LyIFN-α-3, amino acids 93 to 150 of LyIFN-α-2 or of LyIFN-

α-3 and amino acids 151 to 166 of LyIFN-α-2 or of LyIFN-α-3, comprising preparing a DNA comprising the structural gene coding for said hybrid interferon by in vitro recombination at the sites corresponding to amino acids 60, 92 and 150, respectively, of interferons LyIFN-α-2 and LyIFN-α-3 or by chemical DNA synthesis and incorporating the obtained DNA into an appropriate expression vector.

11. Process for the preparation of a host cell transformed with a recombinant vector comprising a DNA sequence coding for a hybrid interferon polypeptide having an amino acid sequence composed of two to four sub-sequences corresponding in amino acid identity and number to sub-sequences of human lymphoblastoid interferons LyIFN-α-2 and LyIFN-α-3, said hybrid interferon polypeptide being selected from the group consisting of a polypeptide having an amino acid sequence consisting of amino acids 1 to 150 of LyIFN-α-2 and amino acids 151 to 166 of LyIFN-α-3, a polypeptide having an amino acid sequence consisting of amino acids 1 to 92 of LyIFN-α-2, amino acids 93 to 150 of LyIFN-α-3 and amino acids 151 to 166 of LyIFN-α-2, and a polypeptide having an amino acid sequence consisting of amino acids 1 to 60 of LyIFN-α-2, amino acids 61 to 92 of LyIFN-α-3, amino acids 93 to 150 of LyIFN-α-2 or of LyIFN-α-3 and amino acids 151 to 166 of LyIFN-α-2 or of LyIFN-α-3, comprising transforming a host cell with a recombinant vector obtainable according to the method of claim 10.

12. Process for the preparation of transformed host cells according to claim 11 selected from the group consisting of a transformed Escherichia coli strain and a transformed Saccharomyces cerevisiae strain.

13. Process for the preparation of a pharmaceutical composition characterized in that a hybrid interferon polypeptide obtainable according to the method of claim 1 is admixed with a pharmaceutically acceptable carrier.

Revendications

Revendications pour les Etats contractants suivants : BE, DE, FR, GB, IT, NL, SE, CH, LU

1. Polypeptide d'interféron hybride ayant une séquence d'acides aminés composée de deux à quatre sous-séquences correspondantes en identité d'acides aminés et en nombre aux sous-séquences d'interférons lymphoblastoïdes humains LyIFN-α-2 et LyIFN-α-3, ledit polypeptide d'interféron hybride étant choisi dans le groupe constitué par un polypeptide ayant une séquence d'acides aminés constituée des acides aminés 1 à 150 de LyIFN-α-2 et des acides aminés 151 à 166 de LyIFN-α-3, un polypeptide ayant une séquence d'acides aminés constituée des acides aminés 1 à 92 de LyIFN-α-2, des acides aminés 93 à 150 de LyIFN-α-3, et des acides aminés 151 à 166 de LyIFN-α-2, et un polypeptide ayant une séquence d'acides aminés constituée des acides aminés 1 à 60 de LyIFN-α-2, des acides aminés 61 à 92 de LyIFN-α-3, des acides aminés 93 à 150 de LyIFN-α-2 ou de LyIFN-α-3, et des acides aminés 151 à 166 de LyIFN-α-2 ou de LyIFN-α-3.

2. Polypeptide d'interféron hybride "$B_1B_2B_3D_4$" ayant la formule

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN THR PHE ASN LEU PHE
SER THR LYS ASP SER SER ALA ALA LEU ASP GLU THR LEU LEU ASP GLU PHE
TYR ILE GLU LEU ASP GLN GLN LEU ASN ASP LEU GLU SER CYS VAL MET GLN
GLU VAL GLY VAL ILE GLU SER PRO LEU MET TYR GLU ASP SER ILE LEU ALA
VAL ARG LYS TYR PHE GLN ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER SER CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER LEU SER
LEU SER THR ASN LEU GLN GLU ARG LEU ARG ARG LYS GLU

selon la revendication 1.

3. Le polypeptide d'interféron hybride "$B_1B_2D_3B_4$" ayant la formule

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN THR PHE ASN LEU PHE
SER THR LYS ASP SER SER ALA ALA LEU ASP GLU THR LEU LEU ASP GLU PHE
TYR ILE GLU LEU ASP GLN GLN LEU ASN ASP LEU GLU ALA CYS VAL MET GLN
GLU GLU ARG VAL GLY GLU THR PRO LEU MET ASN ALA ASP SER ILE LEU ALA
VAL LYS LYS TYR PHE ARG ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER PRO CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER PHE SER
LEU SER ILE ASN LEU GLN LYS ARG LEU LYS SER LYS GLU

selon la revendication 1.

4. Le polypeptide d'interféron hybride "$B_1D_2B_3D_4$" ayant la formule

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU SER CYS VAL MET GLN
GLU VAL GLY VAL ILE GLU SER PRO LEU MET TYR GLU ASP SER ILE LEU ALA
VAL ARG LYS TYR PHE GLN ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER SER CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER LEU SER
LEU SER THR ASN LEU GLN GLU ARG LEU ARG ARG LYS GLU

selon la revendication 1.

5. Le polypeptide d'interféron hybride "$B_1D_2D_3B_4$" ayant la formule

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU ALA CYS VAL MET GLN
GLU GLU ARG VAL GLY GLU THR PRO LEU MET ASN ALA ASP SER ILE LEU ALA
VAL LYS LYS TYR PHE ARG ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER PRO CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER PHE SER
LEU SER ILE ASN LEU GLN LYS ARG LEU LYS SER LYS GLU

selon la revendication 1.

6. Le polypeptide d'interféron hybride "$B_1D_2D_3D_4$ ayant la formule

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU ALA CYS VAL MET GLN
GLU GLU ARG VAL GLY GLU THR PRO LEU MET ASN ALA ASP SER ILE LEU ALA
VAL LYS LYS TYR PHE ARG ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER PRO CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER LEU SER
LEU SER THR ASN LEU GLN GLU ARG LEU ARG ARG LYS GLU

selon la revendication 1.

7. Le polypeptide d'interféron hybride "$B_1D_2B_3B_4$" ayant la formule

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU SER CYS VAL MET GLN
GLU VAL GLY VAL ILE GLU SER PRO LEU MET TYR GLU ASP SER ILE LEU ALA
VAL ARG LYS TYR PHE GLN ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER SER CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER PHE SER
LEU SER ILE ASN LEU GLN LYS ARG LEU LYS SER LYS GLU

* C L M S *

selon la revendication 1.

8. Préparation pharmaceutique contenant une quantité thérapeutiquement efficace d'un interféron hybride selon la revendication 1.

9. Application d'un interféron hybride selon la revendication 1 à la préparation de préparations pharmaceutiques.

10. Interféron hybride selon la revendication 1 pour application dans un procédé de traitement thérapeutique du corps humain ou animal.

11. Procédé de préparation d'un interféron hybride selon la revendication 1, caractérisé en ce qu'on cultive des cellules hôtes transformées avec un vecteur recombinant qui contient une séquence d'ADN codant pour ledit interféron hybride, et en ce qu'on isole ledit interféron hybride.

12. Vecteur recombinant comprenant une séquence d'ADN qui code pour un interféron hybride selon la revendication 1.

13. Cellule hôte transformée avec un vecteur recombinant comprenant une séquence d'ADN qui code pour un interféron hybride selon la revendication 1.

14. Cellule hôte selon la revendicatin 13, choisie dans le groupe constitué par une souche d'Escherichia coli et une souche de Saccharomyces cerevisiae.

Revendications pour l'Etat contractants suivant : AT

1. Procédé de préparation d'un polypeptide d'interféron hybride ayant une séquence d'acides aminés composée de deux à quatre sous-séquences correspondantes en identité d'acides aminés et en nombre aux sous-séquences d'interférons lymphoblastoïdes humains LyIFN-α-2 et LyIFN-α-3, ledit polypeptide d'interféron hybride étant choisi dans le groupe constitué par un polypeptide ayant une séquence d'acides aminés constituée des acides aminés 1 à 150 de LyIFN-α-2 et des acides aminés 151 à 166 de LyIFN-α-3, un polypeptide ayant une séquence d'acides aminés constituée des acides aminés 1 à 92 de LyIFN-α-2, des acides aminés 93 à 150 de LyIFN-α-3, et des acides aminés 151 à 166 de LyIFN-α-2, et un polypeptide ayant une séquence d'acides aminés constituée des acides aminés 1 à 60 de LyIFN-α-2, des acides aminés 61 à 92 de LyIFN-α-3, des acides aminés 93 à 150 de LyIFN-α-2 ou de LyIFN-α-3, et des acides aminés 151 à 166 de LyIFN-α-2 ou de LyIFN-α-3, caractérisé en ce qu'on cultive des cellules hôtes transformées avec un vecteur recombinant qui contient une séquence d'ADN codant pour ledit polypeptide d'interféron hybride, et en ce qu'on isole ledit interféron hybride.

2. Procédé selon la revendication 1, caractérisé en ce que le polypeptide d'interféron hybride est choisi dans le groupe constitué par le polypeptide d'interféron hybride "$B_1B_2B_3D_4$" ayant la formule

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN THR PHE ASN LEU PHE
SER THR LYS ASP SER SER ALA ALA LEU ASP GLU THR LEU LEU ASP GLU PHE
TYR ILE GLU LEU ASP GLN GLN LEU ASN ASP LEU GLU SER CYS VAL MET GLN
GLU VAL GLY VAL ILE GLU SER PRO LEU MET TYR GLU ASP SER ILE LEU ALA
VAL ARG LYS TYR PHE GLN ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER SER CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER LEU SER
LEU SER THR ASN LEU GLN GLU ARG LEU ARG ARG LYS GLU,

le polypeptide d'interféron hybride "$B_1B_2D_3B_4$" ayant la formule

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN THR PHE ASN LEU PHE
SER THR LYS ASP SER SER ALA ALA LEU ASP GLU THR LEU LEU ASP GLU PHE
TYR ILE GLU LEU ASP GLN GLN LEU ASN ASP LEU GLU ALA CYS VAL MET GLN
GLU GLU ARG VAL GLY GLU THR PRO LEU MET ASN ALA ASP SER ILE LEU ALA
VAL LYS LYS TYR PHE ARG ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER PRO CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER PHE SER
LEU SER ILE ASN LEU GLN LYS ARG LEU LYS SER LYS GLU,

le polypeptide d'interféron hybride "$B_1D_2B_3D_4$" ayant la formule

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU SER CYS VAL MET GLN
GLU VAL GLY VAL ILE GLU SER PRO LEU MET TYR GLU ASP SER ILE LEU ALA
VAL ARG LYS TYR PHE GLN ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER SER CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER LEU SER
LEU SER THR ASN LEU GLN GLU ARG LEU ARG ARG LYS GLU,

le polypeptide d'interféron hybride "$B_1D_2D_3B_4$" ayant la formule

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU ALA CYS VAL MET GLN
GLU GLU ARG VAL GLY GLU THR PRO LEU MET ASN ALA ASP SER ILE LEU ALA
VAL LYS LYS TYR PHE ARG ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER PRO CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER PHE SER
LEU SER ILE ASN LEU GLN LYS ARG LEU LYS SER LYS GLU,

le polypeptide d'interféron hybride "$B_1D_2D_3D_4$" ayant la formule

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU ALA CYS VAL MET GLN
GLU GLU ARG VAL GLY GLU THR PRO LEU MET ASN ALA ASP SER ILE LEU ALA
VAL LYS LYS TYR PHE ARG ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER PRO CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER LEU SER
LEU SER THR ASN LEU GLN GLU ARG LEU ARG ARG LYS GLU,

et le polypeptide d'interféron hybride "$B_1D_2B_3B_4$" ayant la formule

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU SER CYS VAL MET GLN
GLU VAL GLY VAL ILE GLU SER PRO LEU MET TYR GLU ASP SER ILE LEU ALA
VAL ARG LYS TYR PHE GLN ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER SER CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER PHE SER
LEU SER ILE ASN LEU GLN LYS ARG LEU LYS SER LYS GLU.

3. Procédé selon la revendication 1 de préparation de l'interféron hybride "$B_1B_2B_3D_4$" caractérisé en ce que le vecteur recombinant contient une séquence d'ADN codant pour ledit interféron hybride.

4. Procédé selon la revendication 1 de préparation de l'interféron hybride "$B_1B_2D_3B_4$" caractérisé en ce que le vecteur recombinant contient une séquence d'ADN codant pour ledit interféron hybride.

5. Procédé selon la revendication 1 de préparation de l'interféron hybride "$B_1D_2B_3D_4$" caractérisé en ce que le vecteur recombinant contient une séquence d'ADN codant pour ledit interféron hybride.

6. Procédé selon la revendication 1 de préparation de l'interféron hybride "$B_1D_2D_3B_4$" caractérisé en ce que le vecteur recombinant contient une séquence d'ADN codant pour ledit interféron hybride.

7. Procédé selon la revendication 1 de préparation de l'interféron hybride "$B_1D_2D_3D_4$" caractérisé en ce que le vecteur recombinant contient une séquence d'ADN codant pour ledit interféron hybride.

8. Procédé selon la revendication 1 de préparation de l'interféron hybride "$B_1D_2B_3B_4$" caractérisé en ce que le vecteur recombinant contient une séquence d'ADN codant pour ledit interféron hybride.

9. Procédé selon la revendication 1, comprenant les étapes de
   a) préparation d'un ADN comprenant le gène de structure codant pour ledit interféron hybride par recombinaison in vitro aux sites correspondants aux acides aminés 60, 92 et 150, respectivement des interférons LyIFN-α-2 et LyIFN-α-3 ou par synthèse d'ADN chimique et incorporation de l'ADN obtenu dans le vecteur d'expression approprié,
   b) transfert du vecteur recombinant obtenu portant le gène de structure de l'IFN hybride dans une cellule hôte réceptrice,
   c) sélection des cellules hôtes transformées à partir de cellules hôtes non transformées par culture dans des conditions telles que celles des cellules transformées survivent,
   d) culture des cellules hôtes transformées dans des conditions qui permettent l'expression du gène de structure d'IFN hybride, et
   e) isolement de l'IFN hybride exprimé.

10. Procédé de préparation d'un vecteur recombinant comprenant une séquence d'ADN codant pour un polypeptide d'interféron hybride ayant une séquence d'acides aminés composée de 2 à 4 sous-séquences correspondant en identité d'acides aminés et en nombres aux sous-séquences des interférons lymphoblastoïdes humains LyIFN-α-2 et LyIFN-α-3, ledit polypeptide d'interféron hybride étant choisi dans le groupe constitué par un polypeptide ayant une séquence d'acides aminés constituée des acides aminés 1 à 150 de LyIFN-α-2 et des acides aminés 151 à 166 de LyIFN-α-3, un polypeptide ayant une séquence d'acides aminés constituée des acides aminés 1 à 92 de LyIFN-α-2, des acides aminés 93 à 150 de LyIFN-α-3 et des acides aminés 151 à 166 de LyIFN-α-2, et un polypeptide ayant une séquence d'acides aminés constituée des acides aminés 1 à 60 de LyIFN-α-2, des acides aminés 61 à 92 de LyIFN-α-3, des acides aminés 93 à 150 de LyIFN-α-2, ou de LyIFN-α-3, et des acides aminés 151 à 166 de LyIFN-α-2 ou de LyIFN-α-3, dans lequel on prépare un ADN comprenant le gène de structure codant pour ledit interféron hybride par une recombinaison in vitro aux sites correspondants aux acides aminés 60, 92 et 150, respectivement, des interférons de LyIFN-α-2 et LyIFN-α-3 ou par synthèse d'ADN chimique et incorporation de l'ADN obtenu dans un vecteur d'expression approprié.

11. Procédé de préparation d'une cellule hôte transformée avec un vecteur recombinant comprenant une séquence d'ADN codant pour un polypeptide d'interféron hybride ayant une séquence d'acides aminés composée de 2 à 4 sous-séquences correspondantes en identité d'acides aminés et en nombres aux sous-séquences des interférons lymphoblastoïdes humains LyIFN-α-2 et LyIFN-α-3, ledit polypeptide d'interféron hybride étant choisi dans le groupe constitué par un polypeptide ayant une séquence d'acides aminés constituée des acides aminés 1 à 150 de LyIFN-α-2 et des acides aminés 151 à 166 de LyIFN-α-3, un polypeptide ayant une séquence d'acides aminés constituée des acides aminés 1 à 92 de LyIFN-α-2, les acides aminés 93 à 150 de LyIFN-α-3 et les acides aminés 151 à 166 de LyIFN-α-2, et un polypeptide ayant une séquence d'acides aminés constituée des acides aminés 1 à 160 de LyIFN-α-2, des acides aminés 61 à 92 de LyIFN-α-3, des acides aminés 93 à 150 de LyIFN-α-2 ou de LyIFN-α-3 et des acides aminés 151 à 166 de LyIFN-α-2 ou de LyIFN-α-3, dans lequel on transforme une cellule hôte avec un vecteur recombinant que l'on peut obtenir selon le procédé de la revendication 10.

12. Procédé de préparation de cellules hôtes transformées selon la revendication 11, choisies dans le groupe constitué par une souche d'Escherichia coli transformée et une souche de Saccharomyces cerevisiae transformée.

13. Procédé de préparation d'une composition pharmaceutique caractérisé en ce qu'on mélange un polypeptide d'interféron hybride que l'on peut obtenir selon le procédé de la revendication 1 avec un support pharmaceutiquement acceptable.

Patentansprüche

Patentansprüche für folgende Vertragstaaten : BE, DE, FR, GB, IT, NL, SE, CH, LU

1. Hybrid-Inteferon-Polypeptid mit einer Aminosäuresequenz bestehend aus zwei bis vier Subsequenzen, die hinsichtlich der Identität und Zahl der Aminosäuren Subsequenzen Von Human-Lymphoblastoid-Interferonen LyIFN-α-2 und LyIFN-α-3 entsprechen, wobei besagtes Hybrid-Interferon-Polypeptid ausgewählt wird aus der Gruppe bestehend einem Polypeptid mit einer Aminosäuresequenz bestehend aus Aminosäuren 1 bis 150 von LyIFN-α-2 und Aminosäuren 151 bis 166 von LyIFN-α-3, einem Polypeptid mit einer Aminosäuresequenz bestehend aus Aminosäuren 1 bis 92 von LyIFN-α-2, Aminosäuren 93 bis 150 von LyIFN-α-3 und Aminosäuren 151 bis 166 von LyIFN-α-2, und einem Polypeptid mit einer Aminosäuresequenz bestehend aus Aminosäuren 1 bis 60 von LyIFN-α-2, Aminosäuren 61 bis 92 von LyIFN-α-3, Aminosäuren 93 bis 150 von LyIFN-α-2 oder von LyIFN-α-3 und Aminosäuren 151 bis 166 von LyIFN-α-2 oder von LyIFN-α-3.

2. Hybrid-Interferon-Polypeptid "$B_1B_2B_3D_4$", mit der Formel

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN THR PHE ASN LEU PHE
SER THR LYS ASP SER SER ALA ALA LEU ASP GLU THR LEU LEU ASP GLU PHE
TYR ILE GLU LEU ASP GLN GLN LEU ASN ASP LEU GLU SER CYS VAL MET GLN
GLU VAL GLY VAL ILE GLU SER PRO LEU MET TYR GLU ASP SER ILE LEU ALA
VAL ARG LYS TYR PHE GLN ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER SER CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER LEU SER
LEU SER THR ASN LEU GLN GLU ARG LEU ARG ARG LYS GLU

nach Anspruch 1.

3. Hybrid-Interferon-Polypeptid "$B_1B_2D_3B_4$" mit der Formel

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN THR PHE ASN LEU PHE
SER THR LYS ASP SER SER ALA ALA LEU ASP GLU THR LEU LEU ASP GLU PHE
TYR ILE GLU LEU ASP GLN GLN LEU ASN ASP LEU GLU ALA CYS VAL MET GLN
GLU GLU ARG VAL GLY GLU THR PRO LEU MET ASN ALA ASP SER ILE LEU ALA
VAL LYS LYS TYR PHE ARG ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
CER PRO CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER PHE SER
LEU SER ILE ASN LEU GLN LYS ARG LEU LYS SER LYS GLU

nach Anspruch 1.

4. Hybrid-Interferon-Polypeptid "$B_1D_2B_3D_4$" mit der Formel

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU SER CYS VAL MET GLN
GLU VAL GLY VAL ILE GLU SER PRO LEU MET TYR GLU ASP SER ILE LEU ALA
VAL ARG LYS TYR PHE GLN ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER SER CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER LEU SER
LEU SER THR ASN LEU GLN GLU ARG LEU ARG ARG LYS GLU

nach Anspruch 1.

5. Hybrid-Interferon-Polypeptid "$B_1D_2D_3B_4$" mit der Formel

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU ALA CYS VAL MET GLN
GLU GLU ARG VAL GLY GLU THR PRO LEU MET ASN ALA ASP SER ILE LEU ALA
VAL LYS LYS TYR PHE ARG ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER PRO CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER PHE SER
LEU SER ILE ASN LEU GLN LYS ARG LEU LYS SER LYS GLU

nach Anspruch 1.

6. Hybrid-Interferon-Polypeptid "$B_1D_2D_3D_4$", mit der Formel

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU ALA CYS VAL MET GLN
GLU GLU ARG VAL GLY GLU THR PRO LEU MET ASN ALA ASP SER ILE LEU ALA
VAL LYS LYS TYR PHE ARG ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER PRO CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER LEU SER
LEU SER THR ASN LEU GLN GLU ARG LEU ARG ARG LYS GLU

nach Anspruch 1.

7. Hybrid-Interferon-Polypeptid "$B_1D_2B_3B_4$", mit der Formel

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU SER CYS VAL MET GLN
GLU VAL GLY VAL ILE GLU SER PRO LEU MET TYR GLU ASP SER ILE LEU ALA
VAL ARG LYS TYR PHE GLN ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER SER CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER PHE SER
LEU SER ILE ASN LEU GLN LYS ARG LEU LYS SER LYS GLU

nach Anspruch 1.

8. Pharmazeutische Zubereitung, die eine therapeutisch wirksame Menge eines Hybrid-Interferons nach Anspruch 1 enthält.

9. Verwendung eines Hybrid-Interferons nach Anspruch 1 zur Herstellung pharmazeutischer Zubereitungen.

10. Hybrid-Interferon nach Anspruch 1 zur Verwendung bei einer Methode zur therapeutischen Behandlung des menschlichen oder tierischen Körpers.

11. Verfahren zur Herstellung eines Hybrid-Interferons nach Anspruch 1, dadurch gekennzeichnet, daß Wirtszellen, die mit einem rekombinanten Vektor transformiert wurden, der eine für das Hybrid-Interferon codierende DNA-Sequenz enthält, kultiviert werden und besagtes Hybrid-Interferon isoliert wird.

12. Rekombinanter Vektor, umfassend eine für ein Hybrid-Interferon nach Anspruch 1 codierende DNA-

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Sequenz.

13. Wirtszelle, die transformiert ist mit einem rekombinanten Vektor, der eine für ein Hybrid-Interferon nach Anspruch 1 codierende DNA-Sequenz enthält.

14. Wirtszelle nach Anspruch 13, ausgewählt aus der Gruppe bestehend aus einem Stamm von Escherichia coli und einem Stamm von Saccharomyces cerevisiae.

Patentansprüche für folgenden Vertraggstaaten : AT

1. Verfahren zur Herstellung eines Hybrid-Interferon-Polypeptids mit einer Aminosäuresequenz bestehend aus zwei bis vier Subsequenzen, die hinsichtlich der Identität und Zahl der Aminosäuren Subsequenzen von Human-Lymphoblastoid-Interferonen LyIFN-α-2 und LyIFN-α-3-entsprechen, wobei besagtes Hybrid-Interferon-Polypeptid ausgewählt wird aus der Gruppe bestehend aus einem Polypeptid mit einer Aminosäuresequenz bestehend aus Aminosäuren 1 bis 150 von LyIFN-α-2 und Aminosäuren 151 bis 166 von LyIFN-α-3, einem Polypeptid mit einer Aminosäuresequenz bestehend aus Aminosäuren 1 bis 92 von LyIFN-α-2, Aminosäuren 93 bis 150 von LyIFN-α-3 und Aminosäuren 151 bis 166 von LyIFN-α-2, und einem Polypeptid mit einer Aminosäuresequenz bestehend aus Aminosäuren 1 bis 60 von LyIFN-α-2, Aminosäuren 61 bis 92 von LyIFN-α-3, Aminosäuren 93 bis 150 von LyIFN-α-2 oder von LyIFN-α-3 und Aminosäuren 151 bis 166 von LyIFN-α-2 oder von LyIFN-α-3, dadurch gekennzeichnet, daß mit einem rekombinanten Vektor, der eine für besagtes Hybrid-Interferon-Polypeptid codierende DNA-Sequenz enthält, transformierte Wirtszellen kultiviert werden und besagtes Hybrid-Interferon isoliert wird.

2. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß das Hybrid-Interferon-Polypeptid ausgewählt wird aus der Gruppe bestehend aus Hybrid-Interferon-Polypeptid "$B_1B_2B_3D_4$" mit der Formel

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN THR PHE ASN LEU PHE
SER THR LYS ASP SER SER ALA ALA LEU ASP GLU THR LEU LEU ASP GLU PHE
TYR ILE GLU LEU ASP GLN GLN LEU ASN ASP LEU GLU SER CYS VAL MET GLN
GLU VAL GLY VAL ILE GLU SER PRO LEU MET TYR GLU ASP SER ILE LEU ALA
VAL ARG LYS TYR PHE GLN ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER SER CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER LEU SER
LEU SER THR ASN LEU GLN GLU ARG LEU ARG ARG LYS GLU,

Hybrid-Interferon-Polypeptid "$B_1B_2D_3B_4$" mit der Formel



CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN THR PHE ASN LEU PHE
SER THR LYS ASP SER SER ALA ALA LEU ASP GLU THR LEU LEU ASP GLU PHE
TYR ILE GLU LEU ASP GLN GLN LEU ASN ASP LEU GLU ALA CYS VAL MET GLN
GLU GLU ARG VAL GLY GLU THR PRO LEU MET ASN ALA ASP SER ILE LEU ALA
VAL LYS LYS TYR PHE ARG ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER PRO CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER PHE SER
LEU SER ILE ASN LEU GLN LYS ARG LEU LYS SER LYS GLU,

Hybrid-Interferon-Polypeptid "$B_1D_2B_3D_4$" mit der Formel

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU SER CYS VAL MET GLN
GLU VAL GLY VAL ILE GLU SER PRO LEU MET TYR GLU ASP SER ILE LEU ALA
VAL ARG LYS TYR PHE GLN ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER SER CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER LEU SER
LEU SER THR ASN LEU GLN GLU ARG LEU ARG ARG LYS GLU,

Hybrid-Interferon-Polypeptid "$B_1D_2D_3B_4$" mit der Formel

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU ALA CYS VAL MET GLN
GLU GLU ARG VAL GLY GLU THR PRO LEU MET ASN ALA ASP SER ILE LEU ALA
VAL LYS LYS TYR PHE ARG ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER PRO CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER PHE SER
LEU SER ILE ASN LEU GLN LYS ARG LEU LYS SER LYS GLU,

Hybrid-Interferon-Polypeptid "$B_1D_2D_3D_4$" mit der Formel

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU ALA CYS VAL MET GLN
GLU GLU ARG VAL GLY GLU THR PRO LEU MET ASN ALA ASP SER ILE LEU ALA
VAL LYS LYS TYR PHE ARG ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER PRO CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER LEU SER
LEU SER THR ASN LEU GLN GLU ARG LEU ARG ARG LYS GLU.

und Hybrid-Interferon-Polypeptid "$B_1D_2B_3B_4$" mit der Formel

CYS ASP LEU PRO GLN THR HIS SER LEU GLY ASN ARG ARG ALA LEU ILE LEU
LEU ALA GLN MET ARG ARG ILE SER PRO PHE SER CYS LEU LYS ASP ARG HIS
ASP PHE GLU PHE PRO GLN GLU GLU PHE ASP ASP LYS GLN PHE GLN LYS ALA
GLN ALA ILE SER VAL LEU HIS GLU MET ILE GLN GLN ILE PHE ASN LEU PHE
THR THR LYS ASP SER SER ALA ALA TRP ASP GLU ASP LEU LEU ASP LYS PHE
CYS THR GLU LEU TYR GLN GLN LEU ASN ASP LEU GLU SER CYS VAL MET GLN
GLU VAL GLY VAL ILE GLU SER PRO LEU MET TYR GLU ASP SER ILE LEU ALA
VAL ARG LYS TYR PHE GLN ARG ILE THR LEU TYR LEU THR GLU LYS LYS TYR
SER SER CYS ALA TRP GLU VAL VAL ARG ALA GLU ILE MET ARG SER PHE SER
LEU SER ILE ASN LEU GLN LYS ARG LEU LYS SER LYS GLU.

3. Verfahren nach Anspruch 1 zur Herstellung von Hybrid-Interferon "$B_1B_2B_3D_4$", dadurch gekennzeichnet, daß der rekombinante Vektor eine für dieses Hybrid-Interferon codierende DNA-Sequenz enthält.

4. Verfahren nach Anspruch 1 zur Herstellung von Hybrid-Interferon "$B_1B_2D_3B_4$", dadurch gekennzeichnet, daß der rekombinante Vektor eine für dieses Hybrid-Interferon codierende DNA-Sequenz enthält.

5. Verfahren nach Anspruch 1 zur Herstellung von Hybrid-Interferon "$B_1D_2B_3D_4$", dadurch gekennzeichnet, daß der rekombinante Vektor eine für dieses Hybrid-Interferon codierende DNA-Sequenz enthält.

6. Verfahren nach Anspruch 1 zur Herstellung von Hybrid-Interferon "$B_1D_2D_3B_4$", dadurch gekennzeichnet, daß der rekombinante Vektor eine für dieses Hybrid-Interferon codierende DNA-Sequenz enthält.

7. Verfahren nach Anspruch 1 zur Herstellung von Hybrid-Interferon "$B_1D_2D_3D_4$", dadurch gekennzeichnet, daß der rekombinante Vektor eine für dieses Hybrid-Interferon codierende DNA-Sequenz enthält.

8. Verfahren nach Anspruch 1 zur Herstellung von Hybrid-Interferon "$B_1D_2B_3B_4$", dadurch gekennzeichnet, daß der rekombinante Vektor eine für dieses Hybrid-Interferon codierende DNA-Sequenz enthält.

9. Verfahren nach Anspruch 1, umfassend die Schritte a) Herstellung einer DNA, die das für besagtes Hybrid-Interferon codierende Strukturgen enthält, durch in vitro-Rekombination an den Stellen, die den Aminosäuren 60, 92 bzw. 150 von Interferonen LyIFN-α-2 und LyIFN-α-3 entsprechen, oder durch chemische DNA-Synthese und Einbringen der erhaltenen DNA in einen geeigneten Expressionsvektor, b) Transferieren des erhaltenen rekombinanten Vektors, der das Hybrid-IFN-Strukturgen trägt, in eine

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Rezipienten-Wirtszelle, c) Selektion transformierter Wirtszellen von untransformierten Wirtszellen durch Kultivieren unter solchen Bedingungen, wo nur die transformierten Wirtszellen überleben, d) Kultivieren der transformierten Wirtszellen unter Bedingungen, die Expression des Hybrid-IFN-Strukturgens erlauben, und e) Isolierung des exprimierten Hybrid-IFN.

10. Verfahren zur Herstellung eines rekombinanten Vektors, der eine DNA-Sequenz enthält, die für ein Hybrid-Interferon-Polypeptid codiert, das eine Aminosäuresequenz hat, bestehend aus zwei bis vier Subsequenzen, die hinsichtlich der Identität und Zahl der Aminosäuren Subsequenzen von Human-Lymphoblastoid-Interferonen LyIFN-α-2 und LyIFN-α-3 entsprechen, wobei besagtes Hybrid-Interferon-Polypeptid ausgewählt wird aus der Gruppe bestehend einem Polypeptid mit einer Aminosäuresequenz bestehend aus Aminosäuren 1 bis 150 von LyIFN-α-2 und Aminosäuren 151 bis 166 von LyIFN-α-3, einem Polypeptid mit einer Aminosäuresequenz bestehend aus Aminosäuren 1 bis 92 von LyIFN-α-2, Aminosäuren 93 bis 150 von LyIFN-α-3 und Aminosäuren 151 bis 166 von LyIFN-α-2, und einem Polypeptid mit einer Aminosäuresequenz bestehend aus Aminosäuren 1 bis 60 von LyIFN-α-2, Aminosäuren 61 bis 92 von LyIFN-α-3, Aminosäuren 93 bis 150 von LyIFN-α-2 oder von LyIFN-α-3 und Aminosäuren 151 bis 166 von LyIFN-α-2 oder von LyIFN-α-3, umfassend die Herstellung einer DNA, umfassend das Strukturgen, das für dieses Hybrid-Interferon codiert, durch in vitro-Rekombination an den Stellen, die den Aminosäuren 60, 92 bzw. 150 von Interferonen LyIFN-α-2 und LyIFN-α-3 entsprechen, oder durch chemische DNA-Synthese und Einbringen der erhaltenen DNA in einen geeigneten Expressionsvektor.

11. Verfahren zur Herstellung einer Wirtszelle, die transformiert ist mit einem rekombinanten Vektor, der eine DNA-Sequenz umfaßt, die für ein Hybrid-Interferon-Polypeptid codiert, das eine Aminosäuresequenz hat, bestehend aus zwei bis vier Subsequenzen, die hinsichtlich der Identität und Zahl der Aminosäuren Subsequenzen von Human-Lymphoblastoid-Interferonen LyIFN-α-2 und LyIFN-α-3 entsprechen, wobei besagtes Hybrid-Interferon-Polypeptid ausgewählt wird aus der Gruppe bestehend einem Polypeptid mit einer Aminosäuresequenz bestehend aus Aminosäuren 1 bis 150 von LyIFN-α-2 und Aminosäuren 151 bis 166 von LyIFN-α-3, einem Polypeptid mit einer Aminosäuresequenz bestehend aus Aminosäuren 1 bis 92 von LyIFN-α-2, Aminosäuren 93 bis 150 von LyIFN-α-3 und Aminosäuren 151 bis 166 von LyIFN-α-2, und einem Polypeptid mit einer Aminosäuresequenz bestehend aus Aminosäuren 1 bis 60 von LyIFN-α-2, Aminosäuren 61 bis 92 von LyIFN-α-3, Aminosäuren 93 bis 150 von LyIFN-α-2 oder von LyIFN-α-3 und Aminosäuren 151 bis 166 von LyIFN-α-2 oder von LyIFN-α-3, umfassend das Transformieren einer Wirtszelle mit einem rekombinanten Vektor, der nach dem Verfahren von Anspruch 10 erhältlich ist.

12. Verfahren zur Herstellung transformierter Wirtszellen nach Anspruch 11, die ausgewählt werden aus der Gruppe bestehend aus einem transformierten Escherichia coli-Stamm und einem transformierten Saccharomyces cerevisiae-Stamm.

13. Verfahren zur Herstellung einer pharmazeutischen Zusammensetzung, dadurch gekennzeichnet, daß ein nach dem Verfahren von Anspruch 1 erhältliches Hybrid-Interferon-Polypeptid mit einem pharmazeutisch annehmbaren Träger vermischt wird.

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Fig. 1: Comparison of the amino acid sequences of LyIFN-α-2 and LyIFN-α-3

	1														16
α-2	CYS	ASP	LEU	PRO	GLN	THR	HIS	SER	LEU	GLY	ASN	ARG	ARG	ALA	LEU	ILE
α-3					GLU					ASP				THR		MET
																32
α-2	LEU	LEU	ALA	GLN	MET	ARG	ARG	ILE	SER	PRO	PHE	SER	CYS	LEU	LYS	ASP
α-3						SER					SER				MET	
																48
α-2	ARG	HIS	ASP	PHE	GLU	PHE	PRO	GLN	GLU	GLU	PHE	ASP	ASP	LYS	GLN	PHE
α-3					GLY								GLY	ASN		
												60				64
α-2	GLN	LYS	ALA	GLN	ALA	ILE	SER	VAL	LEU	HIS	GLU	MET	ILE	GLN	GLN	THR
α-3				PRO								LEU				ILE
																80
α-2	PHE	ASN	LEU	PHE	SER	THR	LYS	ASP	SER	SER	ALA	ALA	LEU	ASP	GLU	THR
α-3					THR								TRP			ASP
												92				96
α-2	LEU	LEU	ASP	GLU	PHE	TYR	ILE	GLU	LEU	ASP	GLN	GLN	LEU	ASN	ASP	LEU
α-3				LYS		CYS	THR			TYR						
																112
α-2	GLU	SER	CYS	VAL	MET	GLN	GLU	VAL	GLY	VAL	ILE	GLU	SER	PRO	LEU	MET
α-3		ALA						GLU	ARG		GLY		THR			
																128
α-2	TYR	GLU	ASP	SER	ILE	LEU	ALA	VAL	ARG	LYS	TYR	PHE	GLN	ARG	ILE	THR
α-3	ASN	ALA							LYS				ARG			
																144
α-2	LEU	TYR	LEU	THR	GLU	LYS	LYS	TYR	SER	SER	CYS	ALA	TRP	GLU	VAL	VAL
α-3										PRO						
						150										160
α-2	ARG	ALA	GLU	ILE	MET	ARG	SER	PHE	SER	LEU	SER	ILE	ASN	LEU	GLN	LYS
α-3								LEU			THR					GLU
						166										
α-2	ARG	LEU	LYS	SER	LYS	GLU										
α-3			ARG	ARG												

Fig. 2: Nucleotide sequence of the coding regions of LyIFN-α-2 and LyIFN-α-3 (coding triplets are numbered)

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α-2      ...GAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTAATG
α-3      ...GAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTAATG
     1   Sau3A                                                  16
α-2  TGT GAT CTG CCT CAG ACT CAC AGC CTG GGT AAC AGG AGG GCC TTG ATA
α-3  TGT GAT CTC CCT GAG ACC CAC AGC CTG GAT AAC AGG AGG ACC TTG ATG
                                                                32
α-2  CTC CTG GCA CAA ATG CGA AGA ATC TCT CCT TTC TCC TGC CTG AAG GAC
α-3  CTC CTG GCA CAA ATG AGC AGA ATC TCT CCT TCC TCC TGT CTG ATG GAC
                                                                48
α-2  AGA CAT GAC TTT GAA TTC CCC CAG GAG GAG TTT GAT GAT AAA CAG TTC
α-3  AGA CAT GAC TTT GGA TTT CCC CAG GAG GAG TTT GAT GGC AAC CAG TTC
                                                 Sau3A          64
α-2  CAG AAG GCT CAA GCC ATC TCT GTC CTC CAT GAG ATG ATC CAG CAG ACC
α-3  CAG AAG GCT CCA GCC ATC TCT GTC CTC CAT GAG CTG ATC CAG CAG ATC
                                                                80
α-2  TTC AAC CTC TTC AGC ACA AAG GAC TCA TCT GCT GCT TTG GAT GAG ACC
α-3  TTC AAC CTC TTT ACC ACA AAA GAT TCA TCT GCT GCT TGG GAT GAG GAC
                                                 PvuII          96
α-2  CTT CTA GAT GAA TTC TAC ATC GAA CTT GAC CAG CAG CTG AAT GAC CTG
α-3  CTC CTA GAC AAA TTC TGC ACC GAA CTC TAC CAG CAG CTG AAT GAC TTG
                                                               112
α-2  GAG TCC TGT GTG ATG CAG GAA GTG GGG GTG ATA GAG TCT CCC CTG ATG
α-3  GAA GCC TGT GTG ATG CAG GAG GAG AGG GTG GGA GAA ACT CCC CTG ATG
                                                               128
α-2  TAC GAG GAC TCC ATC CTG GCT GTG AGG AAA TAC TTC CAA AGA ATC ACT
α-3  AAT GCG GAC TCC ATC TTG GCT GTG AAG AAA TAC TTC CGA AGA ATC ACT
                                                               144
α-2  CTA TAT CTG ACA GAG AAG AAA TAC AGC TCT TGT GCC TGG GAG GTT GTC
α-3  CTC TAT CTG ACA GAG AAG AAA TAC AGC CCT TGT GCC TGG GAG GTT GTC
                          Sau3A                                160
α-2  AGA GCA GAA ATC ATG AGA TCC TTC TCT TTA TCA ATC AAC TTG CAA AAA
α-3  AGA GCA GAA ATC ATG AGA TCC CTC TCT TTA TCA ACA AAC TTG CAA GAA
                          166
α-2  AGA TTG AAG AGT AAG GAA TGA GACCTGGTACAACACGGAAATGATTCTTATAGACT
α-3  AGA TTA AGG AGG AAG GAA TAA CACCTGGTCCAACATGAAACAATTCTTATTGACTC

α-2  AATACAGCAGCTCACACTTCGACAAGTTGTGCTCTTTCAAAGACCCTTGTTTCTGCCAAAACC
α-3  ATATACCAGGTCACGCTTTCATGAATTCTGCCATTTCAAAGACTCTCACTTCTGCTATAACTA

α-2  ATGCTATGTTTTGAATCAAATGTGTCAAGTGTTTTCAGGAGTGTTAAGCAACATCCTGTT
α-3  TGACCATGCTGATAAACTGATTTATCTATTTAAATATTTATTTAGCTATTCATAAGATTT
     PvuII                                          Sau3A
α-2  CAGCTGTATGGGCACTAGTCCCTTACAGATGACCATGCTGATGGATCTATTCATCTATTTATT
α-3  AAATTATTTTTGTTCATATAACATCATGTGCATCTTTACACTGTGGTTAGTGTAATAAAACAT

α-2  TAAATCTTTATTTAGTTAACTATCTATAGGGCTTAAATTAGTTTTGTTCATATTATATTATGT
α-3  GTTCCTTATATTTACTCAAATTCATTATTTT.....

α-2  GAACTTTTACATTGTGAATTGTGTAACAAAAACATGTTCTTTATATTTATTATTTTGCCTTGT

α-2  TTATTAAATTTTTACTATAG...
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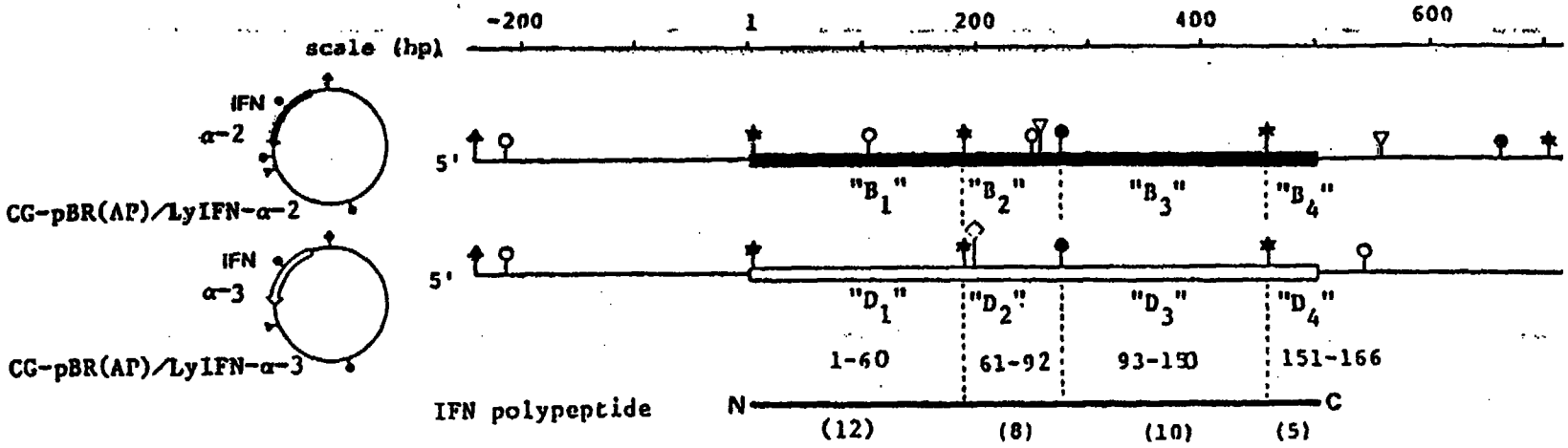


Figure 3: Restriction maps of the Hind-Pst small segments of plasmids CG-pBR(AP)/LyIFN-α CG-pBR(AP)/LyIFN-α-3. The white bars ▭ (IFN α-3 bars ▬ (IFN α-2) delimit the coding regions of the mature IFNs. Below is th peptide (166 amino acids). In brackets the number of amino acid residues is gi which the IFNs α-2 and α-3 differ from each other in each section.

Legend of restriction sites: ◆ Hind III ▼ Pst I ○ Eco RI
● Pvu II ✱ Sau 3A ◇ Bgl II

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