Description

BACKGROUND OF THE INVENTION

This invention relates to a synthetic plasmid and its transformant aiming at mass-producing an interferon in which the primary structure of protein is derived from feline genetic information (hereinafter abbreviated to FeIFN) as a medicine (antiviral drug) by gene manipulation technology. This invention further relates to a gene encoding the FeIFN.

An interferon is a physiologically active substance whose main ingredient is a protein showing antiviral activity and is abbreviated to IFN. And, many literatures have been so far published on the interferon, for example, as Literature 1.

By the progress of gene manipulation technology, the mass production of not only a human IFN but also IFNs of animals such as cattle (Literature 2), horse (Literature 3), dog (Literature 3), etc. became possible. As the result, exploitative researches into the use of IFN as remedies for viral disease, tumor, etc. are carried out with respect to some animals (Literature 4).

With respect to a cat, interferon-α, -β and -γ are reported (Literature 5).

However, there has been no report yet that the mass production of a feline IFN became possible by the application of gene manipulation.

With respect to cat, it is known that there are many viral diseases including FLTV (Literature 6), feline leukemia, feline viral rhinotracheitis, feline caliciviral disease and feline infectious peritonitis (Literature 7).

In such circumstances, there has been a report on a case in which the life of a cat infected with FeLV was prolonged by the oral administration of human IFN-α or bovine IFN-β (Literature 8). If the IFN is administered not orally but by internal injection, it is readily apprehensive that the production of a neutralizing antibody against a heterologous IFN takes place though a more striking effect is expected. If a homologous IFN, that is, a feline IFN becomes readily available, it is expected that the uses of the feline IFN as an antiviral agent and an antitumor agent for a cat are opened.

SUMMARY OF THE INVENTION

In view of such circumstances as above, the present inventors exerted their originalities and ingenuities for the purpose of mass-producing an FeIFN. That is, they prepared a feline C-DNA library by using a commercially available plasmid vector, from which they successfully isolated a plasmid capable of producing an FeIFN by transient expression of simian cultured cells. Furthermore, they succeeded in preparing an FeIFN-producing Chinese hamster ovary cell by using the plasmid to establish a method for mass-producing an FeIFN simply. Whereby, they completed the present invention.

That is, an object of the present invention is to provide a plasmid making a simian cell express transiently to produce an FeIFN, a transformant of *Escherichia coli* carrying this plasmid, a Chinese hamster cell transformed with this plasmid, an FeIFN obtained from these transformants, an FeIFN gene encoding a specific amino acid sequence, an FeIFN precursor comprised of a cleavable peptide or a signal peptide being linked to the N terminal of the FeIFN, an FeIFN precursor gene encoding the FeIFN precursor and a method for producing the FeIFN.

According to the present invention, the mass production of an FeIFN becomes possible, so that an antiviral agent and an antitumor agent for a cat can be obtained readily.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a restriction map of a synthetic plasmid pFeIFN 1 according to the present invention,

Figs. 2 to 6 show preparation procedures of an expression plasmid pFeIFN 2 for *Escherichia coli* according to the present invention,

Fig. 7 shows a gene sequence and an amino acid sequence of an feline IFN, and,

Fig. 8 shows a gene sequence and an amino acid sequence of an feline IFN precursor.

DETAILED DESCRIPTION OF THE INVENTION

The present synthetic plasmid in which a DNA encoding proteins of an FeIFN can be produced, for example, as follows. That is, poly(A)$^+$ RNA is extracted from cells of a cat to prepare a C-DNA library utilizing a so called expression plasmid vector by using *Escherichia coli* as a host. From this library, a plasmid having an ability to make a simian COS cell express transiently to produce an antiviral activity can be selected. One of these plasmids having such an activity as above is pFeIFN1 and a transformant *Escherichia coli* carrying pFeIFN1 is *E. coli* (pFeIFN1) whose accession number is FERM BP-1633.

An FeIFN-producing cell obtained by the transformation with the present synthetic plasmid can be produced as follows. In case of a host cell being an eucaryotic cell, the FeIFN-producing cell can be produced, for example, by transfecting a plasmid extracted from the foregoing *E. coli* (pFeIFN1) to a DHFR defective mutant cell of CHO strain derived from a Chinese hamster. In case of a host cell

being a procaryotic cell, an FeIFN-producing *Escherichia coli* can be produced, for example, by ligating a DNA encoding protein of an FeIFN with an expression vector of a general *Escherichia coli* to transform the *Escherichia coli*.

The production of an FeIFN is carried out by incubating the foregoing FeIFN-producing cell.

Hereinafter, the present invention will be described in detail in order.

With respect to gene manipulation techniques and cell technology techniques, there are may experimentation manuals including Literatures 9 and 10, so that the conventional techniques can be applied.

A C-DNA library prepared according to an ordinary method using reverse transcriptase by making *Escherichia coli* and poly(A)$^+$ RNA extracted from a feline cell act as a host and a substrate respectively.

As a feline cell as a donor of poly (A)$^+$ RNA, for example, an established cultured cell such as LSA (Literature 5) is convenient for use. However, the feline cell to be used in the present invention is not restricted to the LSA. In case of obtaining poly-(A)$^+$ RNA from a cultured cell, it is convenient for it to investigate an interferon inducer suitable for the cell to thereby attempt increasing the yield of poly-(A)$^+$ RNA. For example, in case of an LSA cell, the use of NDV (New Castle-disease Virus), TPA (12-O-tetra-decanoylphorbol 13-acetate) or the like as an inducer at the time of incubation is advantageous for the increase in the yield of poly(A)$^+$ RNA. As a plasmid vector, it is convenient to use the one carrying an expression mechanism in an animal cell and a replicating ability in *Escherichia coli*, for example, a commercially available plasmid vector such as Okayama-Berg vectors manufactured by Pharmacia Inc. As a host microorganism, *E. coli* K-12 can be used.

The cloning of a plasmid carrying a C-DNA encoding an FeIFN can be carried out by screening a plasmid giving antiviral activity-producing ability to the simian established cell COS1 or COS7 (Literature 18) through the transient expression from a C-DNA library. The transient expression of an FeIFN with a plasmid can be carried out according to ordinary methods such as DEAE-dextran method of Literature 14 and calcium phosphate method of Literature 13. *E. coli* (pFeIFN1) (FERM BP-1633) is an example of a transformant containing plasmid which can make COS1 cells produce antiviral activity through the transient expression. The determination of antiviral activity can be carried out by using a feline cultured cell and VSV (Literature 5) and applying ordinary methods such as CPE method described in Literature 12 and the like.

An FeIFN-producing cell of an eucaryotic cell can be screened as a transformant having antiviral activity-producing ability from clones transformed to be DHFR-positive by cotransfecting the strain CHO-DUK-XB-11 having DHFR defective mutation with a plasmid pFeIFN1 extracted from an *Escherichia coli* transformant of FERM BP-1633 according to an ordinary method such as that of Literature 17, for example, together with a plasmid having DHFR-expressing ability such as pAdD26SVA (Literature 20).

An FeIFN-producing cell of a procaryotic cell can be prepared by selecting a transformant having antiviral activity-producing ability from among transformants obtained by transforming *E. coli* K-12 with an synthetic plasmid prepared according to a common gene manipulation of ligating a DNA part encoding protein of an FeIFN, that is, from a plasmid pFeIFN 1 to a so-called expression vector for *Escherichia coli*, for example, a trp promoter or the like, on the downstream of its expression regulating part.

The production of an FeIFN can be carried out by incubating a Chinese hamster transformant CHO-FeIFN (FERM BP-1634) in a medium in which an established CHO cell grows, preferably in a commercially available medium such as MEM-α medium (manufactured by GIBCO Inc., Cat. No. 410-2000) containing 5 to 10% FBS. In case of a transformant being *Escherichia coli*, an FeIFN can be produced by incubating the transformant in an ordinary medium in which *Escherichia coli* proliferates including, for example, LB medium and M9 medium and then disintegrating the bacterial cells. Furthermore, the productivity of an FeIFN can be boosted by the use of an inducer such as indoleacrylic acid or the like.

The produced FeIFN can be purified according to an ordinary method. For example, methods such as affinity chromatography and the like are used preferably. Among those methods, a method using a carrier to which a blue pigment is bonded (hereinafter abbreviated to "blue carrier"), a carrier to which a copper is bonded (hereinafter abbreviated to "copper chelate carrier"), a carrier to which a red pigment is bonded (hereinafter abbreviated to "red carrier") or the like is used particularly preferably. These carriers may be used independently. However, it is preferably to use them in combination in order to increase the purification effect. It is particularly preferable to adopt a method in which blue carrier-using chromatography, copper chelate carrier-using chromatography and red carrier-using chromatography are carried out successively.

As blue carriers, the following are used. The blue pigment is given the general name of CI reactive blue 2. As examples thereof, a blue pig-

ment marketed by Ciba-Geigy under the tradename of "Cibacron Blue F3GA" or "Cibacron Blue 3GA" and the like can be enumarated. As blue carriers to be used in chromatography, blue agarose gels marketed under the tradenames of "Blue Sepharose CL-6B" (Pharmacia Inc.), "Matrix Gel Blue A" (Amicon Inc.), "Affigel Blue" (Biorad Inc.), etc.; blue cellulose gels marketed under the tradenames of "Blue Trisacryl M" (LKB Inc.), "Blue Cellulofine" (Chisso Corp.), etc.; etc. are suitable and readily available.

As the copper chelate carrier, the ones prepared by treating carriers composed of exchangers having chelating ability, e.g., biscarboxymethylamine group [-N($CH_2COOH)_2$] and the like being bonded to agarose, cellulose, polyacrylamide gel and the like with a solution of copper salt such as copper sulfate and the like can be enumerated. Among these, an isoluble polysaccharide carrier such as "Chelating Sepharose" (manufactured by Pharmacia Inc.) or the like chelated with copper is used preferably.

As the red carrier, the following are used. The red carrier is given the general name of CI reactive red 120. As examples thereof, a red carrier marketed by ICI Inc. under the tradename of "Procion Red HE-3B", etc. can be enumerated. As carriers to which this pigment is bonded, for example, gels marketed under the tradenames of "Red Sepharose CL-6B" (Pharmacia Inc.), "Matrex Gel Red A" (Amicon Inc.), "Red Toyopearl" (Tosoh K.K.), etc. are suitable and readily available.

The purification of an FeIFN according to chromatography is carried out as follows. That is, a solution containing an FeIFN is adsorbed on the above carrier by contact firstly. The adsorption may be done by either batch method or column method. However, the column method can yield higher adsorption efficiency. Then, the adsorbed FeIFN is eluted with an eluent.

The elution of the adsorbed FeIFN from the blue carrier or the red carrier is dependent on the pH value, the ionic strength and the hydrophobicity of an eluent to be used. For example, the absorbed FeIFN is eluted at pH 6 to 7 at a higher ionic strength. The ionic strength can be increased by raising the concentration of a buffer such as phosphate buffer, acetate buffer, citrate buffer, borate buffer or the like or by the addition of neutral salt such as sodium chloride, potassium chloride or the like (0.2 to 1.0M). In case of an eluent containing a solvent such as ethylene glycol, propylene glycol or the like that weakens the hydrophobic interaction, the elution at pH 5 to 7 becomes possible.

The elution of the adsorbed FeIFN from the copper chelate carrier is usually carried out with an acidic buffer such as phosphate buffer, acetate buffer, citrate buffer or the like preferably below pH 5. However, the elution at a much higher pH becomes possible at a higher ionic strength.

The composition, the concentration and the amount of an eluent is not restricted particularly. That is, a composition effective for removing impure proteins contained in a crude FeIFN, a concentration required to maintain the pH and the amount of an eluent required to substantially recover the adsorbed FeIFN are employed.

EXAMPLES

Hereinafter, the present invention will be described more specifically, referring to examples.

Example 1

(1) Preparation of Feline C-DNA Library

A feline cell LSA-D4-K17 (Literature 5) as a donor of poly $(A)^+$ RNA was proliferated by the spinner culture in 200mℓ of MEM-L15 medium (50% Eagle's MEM - 50% Leibovitz medium) containing 10% FBS. When the cell concnetration reached 10^5 to 10^6/mℓ, TPA (12-O-tetradecanoylphorbol 13-acetate manufactured by Sigma Chemical Co.) was added to a final concentration of 5ng/mℓ. After continuing the incubation for further 20 hours, the cells were harvested by centrifugation. From the harvested cells, poly$(A)^+$ RNAs were extracted by a modification of guanidium-thiocyanate method described in Literature 15. That is, 3 to 5×10^8 cells were suspended in 20mℓ of 5mM sodium citrate-0.5% sodium sarkosyl-0.1M mercaptoethanol-6M guanidinethiocyanate and then homogenized by gettin in and out the suspension with a 18G injection needle 10 times. After pouring 1/3 vol. of 0.1M EDTA (pH 7.5)-5.7M CsCℓ into a polyaroma centrifugal tube, the cell homogenate was layered thereon. The tube and contents were then centrifuged at 35,000rpm at 20°C for 20 hours in a Hitachi RPS40T rotor. RNA fractions packed at the bottom of the tube were dissolved in 1mℓ of TE (10mM Tris•HCℓ-1mM EDTA, pH 7.5). After mixing the solution with 0.1mℓ of 3M sodium acetate solution, the mixture solution was further mixed with 2.5 vol. of cold ethanol and then allowed to stand at -20°C for 2 hours. A pellet formed at the bottom of the tube by the centrifugation was dissolved in 1mℓ of TE, incubated at 65°C for 4 minutes and then ice-cooled. After adding 1mℓ of TE to the pellet treated as above, equivalent volume of 1.0M NaCℓ was mixed thereinto. The resultant mixture was passed through a column packed with 0.5mℓ of oligo(dT) cellulose (Type 3, manufactured by Collaborative Research Inc.) equilibrated with 0.5M NaCℓ-TE to make poly$(A)^+$ RNAs adsorb on the column. After

washing the column with 10mℓ of 0.5M NaCℓ-TE, the adsorbed poly(A)$^+$ RNAs were eluted with 5mℓ of TE. The poly(A)$^+$ RNAs pelleted according to the ethanolic precipition method were dissolved in 30μℓ of TE and preserved at -80°C. From 7×10^8 cells was obtained 300μg of poly(A)$^+$ RNA. The connection of a poly(A)$^+$ RNA to a plasmid vector and the synthesis of C-DNA were carried out by using commercially available plasmid primers and linkers in the light of Literature 14. That is, 5μℓ of 5mg/mℓ poly(A)$^+$ RNA was poured into a 1.5-mL Eppendorf tube, to which water was then so added that the total volume might reach 20μℓ. After incubating the resultant solution at 65°C for 3 minutes, the incubated solution was tempered back to room temperature. To this incubated solution, were added 4μℓ of 0.3M Tris•HCℓ buffer (pH 8.3)-80mM $MgC\ell_2$-0.3M KCℓ-3mM dithiothreitol, 2μg (3μℓ) of oligo(dT)-tailed pcDV1 plasmid primer (manufactured by Pharmacia Inc.), 4μℓ of mixture of each 25mM dATP, dTTP, dGTP and dCTP, 2μℓ of [α-^{32}P]dCTP, 3μℓ of water and 4μℓ of 18 unit/μℓ reverse transcriptase (manufactures by Seikagaku Kogyo K.K.) in order. Thus prepared solution was incubated at 42°C for 1 hour to carry out the enzymatic reaction. After terminating the reaction by the addition of 4μℓ of 0.25M EDTA and 2μℓ of 10% SDS, phenol-chloroform extraction was carried out. 40μℓ of 4M ammomium acetate and 160μℓ of ethanol was added to the separated aqueous later after phenol-chloroform extraction procedure, which was then cooled in dry ice for 15 minutes. Thus treated aqueous layer was tempered back to room temperature and then centrifuged in a microcentrifuge for 10 minutes. After decanting the supernatant, the pellet was dissolved in 20μℓ of water. To this solution, were added 20μℓ of 4M ammonium acetate and 80μℓ of ethanol to carry out the ethanol precipitation again. The resultant pellet was washed with ethanol, dried and then dissolved in 10μℓ of water.

To this solution, were added 2μℓ of 1.4M sodium cacodylate-0.3M Tris•HCℓ buffer (pH 6.8)-1mM dithiothreitol, 1μℓ of 200μg/mℓ polyadenylic acid (manufactured by Seikagaku Kogyo K.K.), 1μℓ of 20mM $CoC\ell_2$, 1.4μℓ of 1mM dCTP, 0.5μℓ of 400Ci/mmol [α-^{32}P]dCTP in order. After making the total volume of the solution reach 20μℓ by adding water, 0.8μℓ of 27 unit/μℓ terminal nucleotidyl transferase was added thereto. The mixture solution was incubated at 37°C for 5 minutes and the enzymatic reaction was terminated by placing the culture in ice. The number of dCMP residue added to the terminal was calculated to 12 on the average according to Literature 14. From the reaction solution, nucleic acids were recovered according to phenol-chloroform extraction method and double ethanol precipitation method.

The nucleic acids were dissolved in 40μℓ of 10mM Tris•HCℓ (pH 8.0)-60mM NaCℓ-10mM $MgC\ell_2$-1mM 2-mercaptoethanol solution, to which 10 units of Hind III restriction enzyme was added. After incubating thus prepared solution at 37°C for 3 hours, DNAs were recovered by the phenol-chloroform extraction and double ethanol precipitation. The recovered DNAs were washed with ethanol, dried and then dissolved in 10μℓ of TE buffer.

To this solution were added 5μℓ of 2M NaCℓ, 81μℓ of TE buffer and 4μℓ of commercially available 3'-oligo(dG)-tailed pL1 linker (manufactured by Pharmacia Inc.) in order. After heating the mixture solution firstly at 65°C for 5 minutes and then at 42°C for 1 hour, this solution was ice-cooled. To the ice-cooled solution, 100μℓ of 0.2mM Tris•HCℓ buffer (pH 7.5)-40mM $MgC\ell_2$-0.1M ammonium sulfate-1M KCℓ, 7μℓ of 14mM β-NAD, 50μℓ of 1mg/mℓ bovine serum albumin solution and 6μℓ of 1mg/mℓ *E. coli* DNA ligase were added in order, to which water was so added that the total volume might reach 1mℓ. The resultant solution was incubated overnight at 12°C.

To this reaction solution, 2μℓ of mixed solution of each 25mM dATP, dGTP, dTTP and dCTP, 3μℓ of 14mM β-NAD, 0.7μℓ of 35 unit/μℓ *E. coli* DNA polymerase (manufactured by Takara Shuzo Co., Ltd.), 2.4μℓ of 2.5 units/μℓ *E. coli* RNase H (manufactured by Takara Shuzo Co., Ltd.) and 4μℓ of 1mg/mℓ *E. coli* DNA ligase in order. After incubating thus prepared solution firstly at 12°C for 1 hour and then at 25°C for 1 hour, the reaction solution was preserved at -20°C.

After carrying out the transformation reaction by adding 100μℓ of the preserved reaction solution to 1mℓ of suspension of *E. coli* MC1061 (Literature 16) which was made to be competent according to the method of Literature 15, this reaction solution was poured into 250mℓ of LB medium containing 100μg/mℓ ampicillin and then incubated overnight at 37°C. To 10mℓ of this culture, 0.7mℓ of DMSO was added. This portion was preserved at -80°C as a C-DNA library.

(2) Cloning

A portion of thus prepared C-DNA library solution was so sprayed on ten 9-cm diam. LB plates that 1,000 to 2,000 colonies might be formed in each plate. After incubating these plates overnight at 37°C, the grown colonies were scraped off in every petri dish and respectively suspended in each 10mℓ of LB media. 3mℓ of this suspension was mixed with 0.21mℓ of DMSO and then cryopreserved. The remaining suspensions were respectively mixed with each 100mℓ of LB media each containing 100μg/mℓ ampicillin and then in-

cubated overnight at 37°C. Cells were harvested from respective culture media, and plasmids were extracted and purified from the harvested cells according to the method of Literature 17. Each 30μg of these plasmids were subjected to the transient expression of COS1 cells proliferated to the confluent state in 9-cm petri dishes by applying the DEAE dextrantransfection method of Literature 14, whereby the FeIFN-producing ability of respective plasmid DNA samples was determined.

That is, after proliferating COS1 cells to the confluent state in 20mℓ of RPMI1640 (manufactured by GIBCO Inc.) medium containing 10% FBS in a 9-cm diam. petri dish, the medium was removed therefrom and the 4mℓ of RPMI1640 medium containing 5μg/mℓ plasmid DNA sample, 50mM Tris•HCℓ buffer (pH 7.4), 400μg/mℓ DEAE-dextran (manufactured by Pharmacia Inc.) was poured in the petri dish to continue the incubation at 37°C for 4 hours. The medium was exchanged with 4mℓ of RPMI1640 containing 150μM chloroquine. After a 3-hour incubation at 37°C, the medium was further exchanged with an RPMI1640 medium containing 10% FBS. After the incubation at 37°C for 3 days, the antiviral activity in the medium was determined. All of the RPMI1640 media mentioned above were used by adding 100 unit/mℓ penicillin and 100μg/mℓ streptomycin.

As the result, 3 out of 10 culture media showed antiviral activity of 20 unit/mℓ or more, so that the concerned cryopreserved C-DNA library solutions were screened for *Escherichia coli* carrying a plasmid giving antiviral activity-producing ability to the COS1 cell in the following manner.

That is, 1 out of 3 cryopreserved C-DNA library solutions carrying plasmids producing the activity was diluted, so sprayed on 10 LB plates each containing 100μg/mℓ ampicillin that approx. 600 colonies might be formed per plate and then incubated overnight at 37°C. After preparing replicas thereof as preservation plates, cells were scraped off in every plate, suspended in each 5mℓ of LB media and then mixed with each 100mℓ of LB media respectively containing 100μg/mℓ ampicillin. After incubating thus treated cells overnight at 37°C, the resultant cells were harvested to extract and purify plasmids therefrom. Each 20μg per petri dish of these 10 kinds of plasmids were subjected to the transient expression of COS1 cells according to the DEAE-dextran method, whereby the FeIFN-producing ability was determined.

As the result, 1 out of 10 plasmid samples were recognized to have the FeIFN-producing ability, so that 593 colonies in the concerned preservation plate were transplanted to fresh LB plates containing ampicillin by using tooth picks in the ratio of approx. 100 colonies per fresh plate. After an overnight incubation at 37°C, cells scraped off from every plate and then incubated overnight in each 100mℓ of ampicillin containing-LB medium. From the harvested cells plasmids were extracted and purified. The antiviral activity-producing ability of each plasmid was determined according to the transient expression method.

As the result, one plasmid sample was recognized to have the antiviral activity-producing ability, so that each 100 colonies of the concerned preservation plate were incubated in each 2mℓ of LB medium. From these media, plasmids were extracted. The antiviral activity-producing ability of each extracted plasmid was determined according to the transient expression method. A plasmid having the highest antiviral activity-producing ability and an *Escherichia coli* carrying the plasmid were respectively designated as pFeIFN1 and *E. coli* - (pFeIFN1), and this strain was deposited in the Fermentation Research Institute (FERM BP-1633).

(3) Method for Antiviral Activity Determination

The antiviral activity was determined by using Vesicular Stomatitis Virus as a virus and a feline Fc9 cell (Literature 5) as a sensitive cell according to CPE method. As a standard reference, an HuIFN-α calculated in terms of NIH's human natural αIFN was used.

(4) FeIFN Production by CHO Cell

A CHO cell strain DUK-XB-11 (Literature 18) as a DHFR defective mutant, which was subcultured at a dilution rate of 1/10 in a 12-well plate by using MEMα medium (manufactured by GIBCO Inc., Cat. No. 410-1900) containing 10% FBS and then cultured for 3 days, was cotransfected with 5μg of pFeINF1 and 0.5μg of pAdD26SVA carrying a DHFR gene (Literature 20) by applying the calcium phosphate method of Literature 13. After a 1-day incubation, the culture was transplanted to a selective medium of nucleic acid constituent-free MEMα (manufactured by GIBCO Inc., Cat. No. 410-2000) containing 10% FBS. In the course of incubation, the medium was exchanged twice. After a 10-day incubation in a 9-cm diam. petri dish, 147 colonies were obtained. From among these colonies, 16 colonies were transplanted to 24-well plates and then incubated for 3 to 4 days until the plates became confluent. As a result of determining the antiviral activity of the culture media, 8 culture media were recognized to have an activity of 10,000 unit/mℓ or more. Active clones were purified according to the single colony isolation method. One of purified colonies was designated as CHO-FeIFN and deposited in the Fermentation Research Institute (FERM BP-1634).

(5) pFeIFN1

The pFeIFN1 has a size of 4.3Kb, and the restriction map thereof was shown in Fig. 1.

Example 2

(1) Preparation of Fragment Including FeIFN Structure Gene

From the plasmid pFeIFN1 shown in Fig. 1, the fragment containing a part of FeIFN structure genes was prepared according to the method shown in Fig. 2.

That is, 100μg of plasmid pFeIFN1 was completely decomposed with restriction enzymes BamHI and Eco0109. The obtained plural DNA fragments were separated by agarose gel electrophoresis and DNA fragment of approx. 1Kb was extracted by the electroelution to recover approx. 20μg of DNA fragment.

Then, the recovered 20μg of DNA fragment was completely decomposed with the restriction enzyme HincII. Among the obtained DNA fragments, those of approx. 630bp were treated in the same manner as above to recover approx. 15μg of DNA fragment. In such manners as above, Eco0109-HincII fragment containing a downstream part of FeIFN to the Eco0109 was obtained.

(2) Preparation of Plasmid pMT1

A vector pMT1 was prepared which has SD sequence required for translation downstream to a tryptophan promoter and into which a synthetic oligomer containing translation initiation codon ATG and a recognition site for 5 kinds of restriction enzymes were inserted downstream to the SD sequence instead of human interferon-β structure gene

After digesting a human interferon-β expression plasmid pKM6 (Jap. Pat. Appln. Laid-open No. 19487/1986) with BglII, the digested site was made to be a blunt end with an *E. coli* DNA polymerase I large fragment (Klenow) enzyme. A pHindIII linker, i.e., d(pC-A-A-G-C-T-T-G) was ligated to the blunt end. After the digestion with ClaI and HindIII, the larger fragment was separated by agarose gel electrophoresis.

On the other hand, 2 pieces of oligomers, each of which was so designed that it might contain a translation initiation codon ATG, ClaI site at 5'-end, HindIII site at 3'-end and KpnI, SmaI and BamHI sites inside and was synthesized according to the solid phase method, were heated at 60°C for 5 minutes and then gradually cooled so as to anneal them. The larger fragment and the synthesized fragment were ligated with a T4DNA ligase to obtain pMT1.

(3) Preparation of ClaI-SmaI fragment from pMT1

As shown in Fig. 4, 50μg of plasmid pMT1 was completely digested with restriction enzymes ClaI and SmaI, subjected to agarose gel electrophoresis to remove smaller DNA fragments. As the result, approx. 40μg of the desired DNA fragment was recovered to obtain ClaI-EcoRI fragments containing a tryptophan promoter as an *E. coli* expression promoter.

(4) Preparation of ClaI-EcoRI Fragment at N Terminal

This part was synthesized on the basis of the results of the determination of base sequences of DNA on the upstream of Eco0109 site in an FeIFN structure gene integrated in the plasmid pFeIFN1.

That is, a DNA fragment which contains initiation codon ATG at its N terminal, ClaI site next to the initiation codon and Eco0109 site was synthesized by annealing 43mer and 44mer DNAs as shown in Fig. 5.

(5) Preparation of Plasmid pFeIFN2

As shown in Fig. 6, the ligation with T4-DNA ligase was carried out by using 3 DNA fragments obtained in the above (1), (3) and (4). Here, the ligation was possible because the SmaI and the HincII both had blunt ends. The reaction solution was mixed with *E. coli* MC1061 which was made to be competent to carry out the transformation reaction. Clones grown in an LB plate containing 100μg/mℓ ampicillin were incubated overnight at 37°C in 2mℓ of LB medium containing 100μg/mℓ ampicillin. From the culture, plasmids were extracted according to alkaline miniscreen method. The extracted plasmids were decomposed with ClaI and HindIII to obtain clones in which DNA fragments having the desired size were integrated. 3 clones were seclected from among the obtained clones and subjected to the DNA sequencing with respect to approx. 150 bases containing ClaI-Eco0109 fragments to confirm that the desired plasmids were obtained.

(6) Expression of Plasmid pFeIFN2

The strain HB101 transformed with plasmid pFeIFN2 was incubated in 10mℓ of LB medium containing 100μg/mℓ ampicillin at 30°C for 8 hours. Then, the culture was planted in 50mℓ of 2×M9 medium (0.6% KH_2PO_4, 1.2% Na_2HPO_4, 0.2% $NH_4Cℓ$, 0.1% NaCℓ, 1% casamino acid, 1% glucose, 0.25μg/mℓ $MgSO_4 \cdot 7H_2O$, 0.01μg/mℓ

thiamine) at the inoculum size 5% and aerobically incubated overnight at 25°C. When the OD_{550} reached 8 to 10 and then 1% glucose was added to the medium, the pH of the medium was adjusted to 7.0 and 20μg/mℓ indole-acrylic acid was added thereto. After an 8-hour cultivation, cells were harvested and lysed by freeze-thawing and lyzozyme treatment and the lysate was subjected to centrifugation to remove cell debris. As a result of determining the antiviral activity of the supernatant, it was found that 1.2×10^4 unit/mℓ (culture medium) of FeIFN was produced.

Example 3

Determination of C-DNA Base Sequence of FeIFN

A BamHI fragment isolated from the pFeIFN1 was inserted into a sequencing vector pUC 18 (manufactured by Takara Shuzo Co., Ltd.). Thus processed vector was subjected to the determination of C-DNA base sequence of FeIFN according to dideoxy sequencing method using a 7-DEAZA sequencing kit (manufactured by Takara Shuzo Co., Ltd.). Parts having indiscernible autoradiographic bands were confirmed according to Maxam-Gilbert method. Whereby, the DNA base sequence shown in Fig. 8 were determined.

Example 4

After proliferating COS1 cells in 20mℓ of RPMI1640 medium (manufactured by GIBCO inc.) containing 10% FBS in a 15-cm petri dish so that the dish might become confluent, the medium was removed therefrom and then 4mℓ of RPMI1640 medium containing 7.5μg/mℓ plasmid pFeIFN1 obtained in Example 1, 50mM Tris•HCℓ buffer (pH 7.4) and 300μg/mℓ DEAE-dextran (manufactured by Pharmacia Inc.) was poured into the dish to continue the incubation at 37°C for 4 hours. Then, the medium was changed with 4mℓ of RPMI1640 medium containing 150μM chloroquine. After continuing the incubation at 37°C for 3 hours, the medium was changed with a RPMI1640 medium containing 10% FBS and the incubation was continuted at 37°C for 3 days. All of the above RPMI1640 media were used by adding thereto 100 unit/mℓ penicillin and 100μg/mℓ streptomycin.

After the completion of the incubation, the supernatant was obtained. This crude FeIFN solution contained the FeIFN activity of 2.6×10^4 U/mℓ and had the specific activity of 2.3×10^4 U/mg protein. 18ℓ of this solution was loaded to a column containing 500mℓ of Blue Sepharose (fast flow type). After washing the column with 5ℓ of 50mM phosphate buffer (pH 7.0) containing 0.5M sodium chloride, the adsorbed FeIFN was eluted with 0.5ℓ of 50mM phosphate buffer (pH 7.0) containing 1M sodium chloride and 1.0ℓ of 50mM phosphate buffer (pH7.0) containing 1M sodium chloride and 20% ethylene glycol. The eluted FeIFN contained the FeIFN activity of 2.3×10^5 U/mℓ and had the specific activity of 2.8×10^5 U/mg protein. The recovery of FeIFN activity reached 75% and the specific activity was raised by 121 times.

Then, 1.5ℓ of FeIFN eluate from the blue carrier was directly loaded to a column containing 70mℓ of Sepharose chelated with copper. After washing this column with 20mM acetate buffer (pH 3.9) containing 0.5M sodium chloride, the adsorbed FeIFN was eluted with 210mℓ of 20mM acetate buffer (pH 3.6) containing 0.5M sodium chloride. The eluted FeIFN containing the FeIFN activity of 1.2×10^5 U/mℓ and had the specific activity of 7.1×10^7 U/mg protein. The recovery of FeIFN activity reached 77% and the specific activity was raised by 23 times.

Furthermore, 210mℓ of FeIFN eluate from the copper chelate carrier was loaded to a column containing 15mℓ of Red Sepharose (fast flow type). After washing this column with 200mℓ of phosphate-buffered saline solution (pH 7.0), the adsorbed FeIFN was eluted with 15mℓ of 50mM phosphate buffer (pH 7.0) containing 1M sodium chloride and 40% ethylene glycol. The eluted FeIFN contained the FeIFN activity of 2.0×10^7 U/mℓ and had the specific activity of 5.9×10^8 U/mg protein. The recovery of FeIFN activity reached 95% and the specific activity was raised by 11 times.

REFERENTIAL LITERATURES

1. Shigeyasu Kobayashie (ed.), 1985. Science of Interferon, Kodansha Scientifics. Kodansha, Tokyo.
2. Official Gazette of Jap. Pat. Appln. Laid-open No. 224690/1983.
3. Official Gazette of Jap. Pat. Appln. Laid-open No. 501469/1987.
4. A. J. Liberson and A. J. Spiliotes, 1987. Spectrum, Feb. 1-21~1-23. In Arthur D. Little's Decision Resources, U. S. A.
5. J. K. Yamamoto et al, 1986. *Vet. Immunol.* and *Immunopathol.* 11, pp. 1~9
6. N. Pedersen et al, 1987. *Science* 235, pp. 790~793.
7. Shinichiro Konishi, 1985. Viral Disease of Dogs and Cats. Gakusousha, Tokyo.
8. V. P. Steed, 1987. *Feline Practice* 17, pp. 24~30.
9. T. Maniatis et al (eds.), 1982. Molecular Cloning, A Laboratory Manual. Cold Spring Harbor Laboratory, New York.

10. The Japanese Biochemical Society (eds.), 1986. Genetic Research Method II in Lectures on Biochemical Experiment, 2nd series, vol. 1. Tokyo Kagakudojin, Tokyo.
11. The Japanese Biochemical Society (eds.), 1987. Genetic Research Method III in Lectures on Biochemical Experiment, 2nd series, vol. 1. Tokyo Kagakudojin, Tokyo.
12. The Japanese Biochemical Society (eds.), 1987. Lectures on Biochemical Experiment, 2nd series, vol. 5, pp. 250~256. Tokyo Kagakudojin, Tokyo.
13. F. L. Grabam et al, (1973). *Virology* 54, pp. 536~.
14. Pages 221~236 of Literature 11.
15. Katsuya Shigesada, 1983. *Saibo Kogaku* - (Cell Technology) 2, pp. 616~629.
16. M. J. Casadaban et al., 1980. *J. Mol. Biol.* 138, pp. 179~207.
17. Pages 86~96 of Literature 9.
18. G. Urlaub and L. A. Chasin, 1980. *ProNAS*, 77, pp. 4216~4220.
19. Y. Gluzman, 1981. *Cell*, 23, pp. 175~182.
20. R. J. Kaufman and P. A. Sharp, 1982. *Mol. Cell. Biol.* 2, pp. 1304~1319.

Claims

1. A synthetic plasmid comprising the DNA shown in figure 7 encoding feline interferon integrated therein.

2. A transformant, **characterized in that** it is obtainable by transforming a host cell with a synthetic plasmid in which the DNA shown in figure 7, encoding the protein of a feline interferon is integrated.

3. A transformant according to claim 2, wherein the host cell is an eucaryotic cell.

4. A transformant according to claim 3, wherein the eucaryotic cell is a CHO cell.

5. A transformant according to claim 3, wherein the CHO cell is CHO-DUK-XB-1.

6. A transformant according to claim 2, wherein the host cell is a procaryotic cell.

7. A transformant according to claim 6, wherein the procaryotic cell is Escherichia coli.

8. A transformant according to claim 7, wherein the Escherichia coli is E. coli K-12.

9. A transformant according to claim 2, wherein the transformant is CHO-FeIFN (FERM BP-1634).

10. A transformant according to claim 2, wherein the transformant is E. coli (pFeIFN1) (FERM BP-1633).

11. A feline interferon carrying a sugar chain, **characterized in that** it is obtainable through the incubation of the synthetic plasmid according to claim 1 and an eucaryotic cell.

12. A feline interferon carrying a sugar chain according to claim 11, wherein the eucaryotic cell is a COS cell.

13. A feline interferon carrying a sugar chain according to claim 12, wherein the COS cell is COS1 or COS7.

14. A feline interferon carrying a sugar chain according to any one of claims 11 to 13, wherein the specific activity thereof is 1×10^8 U/mg protein or more and the molecular weight is approx. 24,000.

15. A feline interferon carrying a sugar chain, **characterized in that** it is obtainable through the incubation of an eucaryotic cell transformed with the synthetic plasmid according to claim 1.

16. A feline interferon carrying a sugar chain according to claim 15, wherein the eucaryotic cell is a CHO cell.

17. A feline interferon carrying a sugar chain according to claim 16, wherein the CHO cell is CHO-DUK-XB-1.

18. A feline interferon carrying a sugar chain according to any one of claims 15 to 17, wherein the specific activity thereof is $1\text{-}10^8$ U/mg protein or more and the molecular weight is approx. 24,000.

19. A feline interferon not carrying a sugar chain, **characterized in that** it is obtainable through the incubation of a procaryotic cell transformed with the synthetic plasmid according to claim 1.

20. A feline interferon not carrying a sugar chain according to claim 19, wherein the procaryotic cell is Escherichia coli.

21. A feline interferon not carrying a sugar chain according to claim 20, wherein the Escherichia coli is E. coli K-12.

10. The Japanese Biochemical Society (eds.), 1986. Genetic Research Method II in Lectures on Biochemical Experiment, 2nd series, vol. 1. Tokyo Kagakudojin, Tokyo.
11. The Japanese Biochemical Society (eds.), 1987. Genetic Research Method III in Lectures on Biochemical Experiment, 2nd series, vol. 1. Tokyo Kagakudojin, Tokyo.
12. The Japanese Biochemical Society (eds.), 1987. Lectures on Biochemical Experiment, 2nd series, vol. 5, pp. 250~256. Tokyo Kagakudojin, Tokyo.
13. F. L. Grabam et al, (1973). *Virology* 54, pp. 536~.
14. Pages 221~236 of Literature 11.
15. Katsuya Shigesada, 1983. *Saibo Kogaku* - (Cell Technology) 2, pp. 616~629.
16. M. J. Casadaban et al., 1980. *J. Mol. Biol.* 138, pp. 179~207.
17. Pages 86~96 of Literature 9.
18. G. Urlaub and L. A. Chasin, 1980. *ProNAS*, 77, pp. 4216~4220.
19. Y. Gluzman, 1981. *Cell*, 23, pp. 175~182.
20. R. J. Kaufman and P. A. Sharp, 1982. *Mol. Cell. Biol.* 2, pp. 1304~1319.

Claims

1. A synthetic plasmid comprising the DNA shown in figure 7 encoding feline interferon integrated therein.

2. A transformant, **characterized in that** it is obtainable by transforming a host cell with a synthetic plasmid in which the DNA shown in figure 7, encoding the protein of a feline interferon is integrated.

3. A transformant according to claim 2, wherein the host cell is an eucaryotic cell.

4. A transformant according to claim 3, wherein the eucaryotic cell is a CHO cell.

5. A transformant according to claim 3, wherein the CHO cell is CHO-DUK-XB-1.

6. A transformant according to claim 2, wherein the host cell is a procaryotic cell.

7. A transformant according to claim 6, wherein the procaryotic cell is Escherichia coli.

8. A transformant according to claim 7, wherein the Escherichia coli is E. coli K-12.

9. A transformant according to claim 2, wherein the transformant is CHO-FeIFN (FERM BP-1634).

10. A transformant according to claim 2, wherein the transformant is E. coli (pFeIFN1) (FERM BP-1633).

11. A feline interferon carrying a sugar chain, **characterized in that** it is obtainable through the incubation of the synthetic plasmid according to claim 1 and an eucaryotic cell.

12. A feline interferon carrying a sugar chain according to claim 11, wherein the eucaryotic cell is a COS cell.

13. A feline interferon carrying a sugar chain according to claim 12, wherein the COS cell is COS1 or COS7.

14. A feline interferon carrying a sugar chain according to any one of claims 11 to 13, wherein the specific activity thereof is 1×10^8 U/mg protein or more and the molecular weight is approx. 24,000.

15. A feline interferon carrying a sugar chain, **characterized in that** it is obtainable through the incubation of an eucaryotic cell transformed with the synthetic plasmid according to claim 1.

16. A feline interferon carrying a sugar chain according to claim 15, wherein the eucaryotic cell is a CHO cell.

17. A feline interferon carrying a sugar chain according to claim 16, wherein the CHO cell is CHO-DUK-XB-1.

18. A feline interferon carrying a sugar chain according to any one of claims 15 to 17, wherein the specific activity thereof is $1\text{-}10^8$ U/mg protein or more and the molecular weight is approx. 24,000.

19. A feline interferon not carrying a sugar chain, **characterized in that** it is obtainable through the incubation of a procaryotic cell transformed with the synthetic plasmid according to claim 1.

20. A feline interferon not carrying a sugar chain according to claim 19, wherein the procaryotic cell is Escherichia coli.

21. A feline interferon not carrying a sugar chain according to claim 20, wherein the Escherichia coli is E. coli K-12.

* C L M S *

22. A feline interferon not carrying a sugar chain according to any one of claims 19 to 21, wherein the specific activity thereof is $1x10^8$ U/mg protein or more and the molecular weight thereof is approx. 20,000.

23. A method for producing a feline interferon carrying a sugar chain, **characterized in that** the feline interferon is obtainable through the incubation of the synthetic plasmid according to claim 1 and an eucaryotic cell.

24. A method for producing a feline interferon carrying a sugar chain according to claim 23, wherein the eucaryotic cell is a COS cell.

25. A method for producing a feline interferon carrying a sugar chain according to claim 24, wherein the COS cell is COS1 or COS7.

26. A method for producing a feline interferon carrying a sugar chain, **characterized in that** the feline interferon is obtained by transforming an eucaryotic cell with the synthetic plasmid according to claim 1 and then incubating the transformant.

27. A method for producing a feline interferon carrying a sugar chain according to claim 26, wherein the eucaryotic cell is a CHO cell.

28. A method for producing a feline interferon carrying a sugar chain according to claim 27, wherein the CHO is CHO-DUK-XB-1.

29. A method for producing a feline interferon not carrying a sugar chain, **characterized in that** the feline interferon is obtained by transforming a procaryotic cell with the synthetic plasmid according to claim 1 and then incubating the transformant.

30. A method for producing a feline interferon not carrying a sugar chain according to claim 29, wherein the procaryotic cell is Escherichia coli.

31. A method for producing a feline interferon not carrying a sugar chain according to claim 30, wherein the Escherichia coli is E. coli K-12.

32. A feline interferon, **characterized in that** it has an amino acid sequence shown in Fig.7.

33. A feline interferon gene, **characterized in that** it encodes a protein of the feline interferon according to claim 1.

34. A feline interferon gene according to claim 33, wherein the DNA sequence shown in figure 7 is contained.

35. A feline interferon according to claim 32, wherein a sugar chain is linked to a protein carrying the amino acid sequence shown in figure 7.

36. A feline interferon precursor, **characterized in that** a cleavable peptide or a signal peptide is linked to the N-terminal of the feline interferon according to claim 32.

37. A feline interferon precursor according to claim 36, wherein the amino acid sequence shown in figure 8 is contained.

38. A feline interferon precursor gene, **characterized in that** it encodes the feline interferon precursor according to claim 36.

39. A feline interferon precursor gene according to claim 38, wherein the DNA sequence shown in figure 8 is contained.

Patentansprüche

1. Synthetisches Plasmid, das darin integriert die in Fig. 7 gezeigte DNA umfaßt, die Katzen-Interferon kodiert.

2. Transformante, dadurch gekennzeichnet, daß sie durch Transformation einer Wirtszelle mit einem synthetischen Plasmid erhalten werden kann, in das die in Fig. 7 gezeigte DNA integriert ist, die das Protein von Katzen-Interferon kodiert.

3. Transformante nach Anspruch 2, wobei die Wirtszelle eine eukaroytische Zelle ist.

4. Transformante nach Anspruch 3, wobei die eukaroytische Zelle eine CHO-Zelle ist.

5. Transformante nach Anspruch 3, wobei die CHO-Zelle CHO-DUK-XB-1 ist.

6. Transformante nach Anspruch 2, wobei die Wirtszelle ein prokaroytische Zelle ist.

7. Transformante nach Anspruch 6, wobei die prokaroytische Zelle Escherichia coli ist.

8. Transformante nach Anspruch 7, wobei Escherichia coli E. coli K-12 ist.

9. Transformante nach Anspruch 2, wobei die Transformante CHO-FeIFN (FERM BP-1634) ist.

10. Transformante nach Anspruch 2, wobei die Transformante E. coli (pFeIFN1) (FERM BP-1633) ist.

11. Katzen-Interferon, das eine Zuckerkette trägt, dadurch gekennzeichnet, daß es durch Inkubieren des synthetischen Plasmids nach Anspruch 1 und einer eukaroytischen Zelle erhalten werden kann.

12. Katzen-Interferon, das eine Zuckerkette trägt, nach Anspruch 11, wobei die eukaroytische Zelle eine COS-Zelle ist.

13. Katzen-Interferon, das eine Zuckerkette trägt, nach Anspruch 12, wobei die COS-Zelle COS1 oder COS7 ist.

14. Katzen-Interferon, das eine Zuckerkette trägt, nach einem der Ansprüche 11 bis 13, wobei dessen spezifische Aktivität $1x10^8$ E/mg Protein oder mehr und das Molekulargewicht etwa 24000 betragen.

15. Katzen-Interferon, das einen Zuckerkette trägt, dadurch gekennzeichnet, daß es durch Inkubieren einer eukaroytischen Zelle erhalten werden kann, die mit dem synthetischen Plasmid nach Anspruch 1 transformiert wurde.

16. Katzen-Interferon, das eine Zuckerkette trägt, nach Anspruch 15, wobei die eurkaroytische Zelle eine CHO-Zelle ist.

17. Katzen-Interferon, das eine Zuckerkette trägt, nach Anspruch 16, wobei die CHO-Zelle CHO-DUK-XB-1 ist.

18. Katzen-Interferon, das eine Zuckerkette trägt, nach einem der Ansprüche 15 bis 17, wobei dessen spezifische Aktivität $1x10^8$ E/mg Protein oder mehr und dessen Molekulargewicht etwa 24000 betragen.

19. Katzen-Interferon, das keine Zuckerkette trägt, dadurch gekennzeichnet, daß es durch Inkubieren einer prokaroytischen Zelle erhalten werden kann, die mit dem synthetischen Plasmid nach Anspruch 1 transformiert wurde.

20. Katzen-Interferon, das keine Zuckerkette trägt, nach Anspruch 19, wobei die prokaroytische Zelle Escherichia coli ist.

21. Katzen-Interferon, das keine Zuckerkette trägt, nach Anspruch 20, wobei Escherichia coli E. coli K-12 ist.

22. Katzen-Interferon, das keine Zuckerkette trägt, nach einem der Ansprüche 19 bis 21, wobei dessen spezifische Aktivität $1x10^8$ E/mg Protein oder mehr und dessen Molekulargewicht etwa 20000 betragen.

23. Verfahren zur Herstellung von Katzen-Interferon, das eine Zuckerkette trägt, dadurch gekennzeichnet, daß das Katzen-Interferon durch Inkubieren eines synthetischen Plasmids nach Anspruch 1 und einer eukaroytischen Zelle erhalten werden kann.

24. Verfahren zur Herstellung von Katzen-Interferon, das eine Zuckerkette trägt, nach Anspruch 23, wobei die eukaroytische Zelle eine COS-Zelle ist.

25. Verfahren zur Herstellung von Katzen-Interferon, das eine Zuckerkette trägt, nach Anspruch 24, wobei die COS-Zelle COS1 oder COS7 ist.

26. Verfahren zur Herstellung von Katzen-Interferon, das eine Zuckerkette trägt, dadurch gekennzeichnet, daß das Katzen-Interferon durch Transformation einer eukaroytischen Zelle mit dem synthetischen Plasmid nach Anspruch 1 und anschließendes Inkubieren der Transformante erhalten wird.

27. Verfahren zur Herstellung von Katzen-Interferon, das eine Zuckerkette trägt, nach Anspruch 26, wobei die eukaroytische Zelle eine CHO-Zelle ist.

28. Verfahren zur Herstellung von Katzen-Interferon, das eine Zuckerkette trägt, nach Anspruch 27, wobei CHO CHO-DUK-XB-1 ist.

29. Verfahren zur Herstellung von Katzen-Interferon, das keine Zuckerkette trägt, dadurch gekennzeichnet, daß das Katzen-Interferon durch Transformation einer prokaroytischen Zelle mit dem synthetischen Plasmid nach Anspruch 1 und anschließendes Inkubieren der Transformante erhalten wird.

30. Verfahren zur Herstellung von Katzen-Interferon, das keine Zuckerkette trägt, nach Anspruch 29, wobei die prokaroytische Zelle Escherichia coli ist.

31. Verfahren zur Herstellung von Katzen-Interferon, das keine Zuckerkette trägt, nach An-

* C L M S *

spruch 30, wobei Escherichia coli E. coli K-12 ist.

32. Katzen-Interferon, dadurch gekennzeichnet, daß es die in Fig. 7 gezeigte Aminosäuresequenz aufweist.

33. Katzen-Interferon-Gen, dadurch gekennzeichnet, daß es ein Protein von Katzen-Interferon nach Anspruch 1 kodiert.

34. Katzen-Interferon-Gen nach Anspruch 33, worin die in Fig. 7 gezeigte DNA-Sequenz enthalten ist.

35. Katzen-Interferon nach Anspruch 32, wobei eine Zuckerkette an das Protein gebunden ist, das die in Fig. 7 gezeigte Aminosäuresequenz trägt.

36. Katzen-Interferon-Präkursor, dadurch gekennzeichnet, daß ein abspaltbares Peptid oder ein Signalpeptid mit dem N-Terminus des Katzen-Interferons nach Anspruch 32 verbunden ist.

37. Katzen-Interferon-Präkursor nach Anspruch 36, worin die in Fig. 8 gezeigten Aminosäuresequenz enthalten ist.

38. Katzen-Interferon-Präkursor-Gen, dadurch gekennzeichnet, daß es den Katzen-Interferon-Präkursor nach Anspruch 36 kodiert.

39. Katzen-Interferon-Präkursor-Gen nach Anspruch 38, worin die in Fig. 8 gezeigte DNA-Sequenz enthalten ist.

Revendications

1. Plasmide synthétique dans lequel est intégré l'ADN représenté sur la figure 7, qui code un interféron de félin.

2. Cellule transformée caractérisée en ce qu'on peut l'obtenir en transformant une cellule hôte avec un plasmide synthétique dans lequel est intégré l'ADN représenté sur la figure 7, qui code une protéine qui est un interféron de félin.

3. Cellule transformée conforme à la revendication 2, dans laquelle la cellule hôte est une cellule eucaryote.

4. Cellule transformée conforme à la revendication 3, dans laquelle la cellule eucaryote est une cellule CHO.

5. Cellule transformée conforme à la revendication 4, dans laquelle la cellule CHO est une cellule CHO-DUK-XB-1.

6. Cellule transformée conforme à la revendication 2, dans laquelle la cellule hôte est une cellule procaryote.

7. Cellule transformée conforme à la revendication 6, dans laquelle la cellule procaryote est Escherichia coli.

8. Cellule transformée conforme à la revendication 7, dans laquelle Escherichia coli est E. coli K-12.

9. Cellule transformée conforme à la revendication 2, qui est CHO-FeIFN (FERM BP- 1634).

10. Cellule transformée conforme à la revendication 2, qui est E. coli-(pFeIFN1) (FERM BP-1633).

11. Interféron de félin portant une chaîne glucidique, caractérisé en ce qu'on peut l'obtenir par incubation d'un plasmide synthétique conforme à la revendication 1 et d'une cellule eucaryote.

12. Interféron de félin portant une chaîne glucidique, conforme à la revendication 11, pour lequel la cellule eucaryote est une cellule COS.

13. Interféron de félin portant une chaîne glucidique, conforme à la revendication 12, pour lequel la cellule COS est une cellule COS1 ou COS7.

14. Interféron de félin portant une chaîne glucidique, conforme à l'une des revendications 11 à 13, dont l'activité spécifique vaut 1.10^8 U/mg de protéine ou plus, et dont la masse moléculaire vaut environ 24 000.

15. Interféron de félin portant une chaîne glucidique, caractérisé en ce qu'on peut l'obtenir par incubation d'une cellule eucaryote transformée avec un plasmide synthétique conforme à la revendication 1.

16. Interféron de félin portant une chaîne glucidique conforme à la revendication 15, pour lequel la cellule eucaryote est une cellule CHO.

17. Interféron de félin portant une chaîne glucidique, conforme à la revendication 16, pour lequel la cellule CHO est une cellule CHO-DUK-XB-1.

* C L M S *

18. Interféron de félin portant une chaîne glucidique conforme à l'une des revendications 15 à 17, dont l'activité spécifique vaut 1.10^8 U/mg de protéine ou plus, et dont la masse moléculaire vaut environ 24 000.

19. Interféron de félin ne portant pas de chaîne glucidique, caractérisé en ce qu'on peut l'obtenir par incubation d'une cellule procaryote transformée avec un plasmide synthétique conforme à la revendication 1.

20. Interféron de félin ne portant pas de chaîne glucidique, conforme à la revendication 19, pour lequel la cellule procaryote est Escherichia coli.

21. Interféron de félin ne portant pas de chaîne glucidique, conforme à la revendication 20, pour lequel Escherichia coli est E. coli K-12.

22. Interféron de félin ne portant pas de chaîne glucidique conforme à l'une des revendications 19 à 21, dont l'activité spécifique vaut 1.10^8 U/mg de protéine ou plus, et dont la masse moléculaire vaut environ 20 000.

23. Procédé de production d'un interféron de félin portant une chaîne glucidique, caractérisé en ce qu'on peut obtenir l'interféron de félin par incubation d'un plasmide synthétique conforme à la revendication 1 et d'une cellule eucaryote.

24. Procédé de production d'un interféron de félin portant une chaîne glucidique, conforme à la revendication 23, dans lequel la cellule eucaryote est une cellule COS.

25. Procédé de production d'un interféron de félin portant une chaîne glucidique, conforme à la revendication 24, dans lequel la cellule COS est une cellule COS1 ou COS7.

26. Procédé de production d'un interféron de félin portant une chaîne glucidique, caractérisé en ce qu'on peut obtenir l'interféron de félin en transformant une cellule eucaryote avec un plasmide synthétique conforme à la revendication 1, et en faisant ensuite incuber la cellule ainsi transformée.

27. Procédé de production d'un interféron de félin portant une chaîne glucidique, conforme à la revendication 26, dans lequel la cellule eucaryote est une cellule CHO.

28. Procédé de production d'un interféron de félin portant une chaîne glucidique, conforme à la revendication 27, dans lequel la cellule CHO est une cellule CHO-DUK-XB-1.

29. Procédé de production d'un interféron de félin ne portant pas de chaîne glucidique, caractérisé en ce qu'on peut obtenir l'interféron de félin en transformant une cellule procaryote avec un plasmide synthétique conforme à la revendication 1, et en faisant ensuite incuber la cellule ainsi transformée..

30. Procédé de production d'un interféron de félin ne portant pas de chaîne glucidique, conforme à la revendication 29, dans lequel la cellule procaryote est Escherichia coli.

31. Procédé de production d'un interféron de félin ne portant pas de chaîne glucidique, conforme à la revendication 30, dans lequel Escherichia coli est E. coli K-12.

32. Interféron de félin, caractérisé en ce qu'il présente la séquence d'acides aminés indiquée sur la figure 7.

33. Gène d'interféron de félin, caractérisé en ce qu'il code une protéine qui est un interféron de félin conforme à la revendication 32.

34. Gène d'interféron de félin, conforme à la revendication 33, qui contient la séquence d'ADN indiquée sur la figure 7.

35. Interféron de félin, conforme à la revendication 32, dans lequel une chaîne glucidique est liée à une protéine présentant la séquence d'acides aminés indiquée sur la figure 7.

36. Précurseur d'interféron de félin, caractérisé en ce qu'il comporte un peptide séparable ou un peptide signal lié à l'extrémité N-terminale de l'interféron de félin conforme à la revendication 32.

37. Précurseur d'interféron de félin, conforme à la revendication 36, qui contient la séquence d'acides aminés indiquée sur la figure 8.

38. Gène de précurseur d'interféron de félin, caractérisé en ce qu'il code un précurseur d'interféron de félin conforme à la revendication 36.

39. Gène de précurseur d'interféron de félin, conforme à la revendication 38, qui contient la séquence d'ADN indiquée sur la figure 8.