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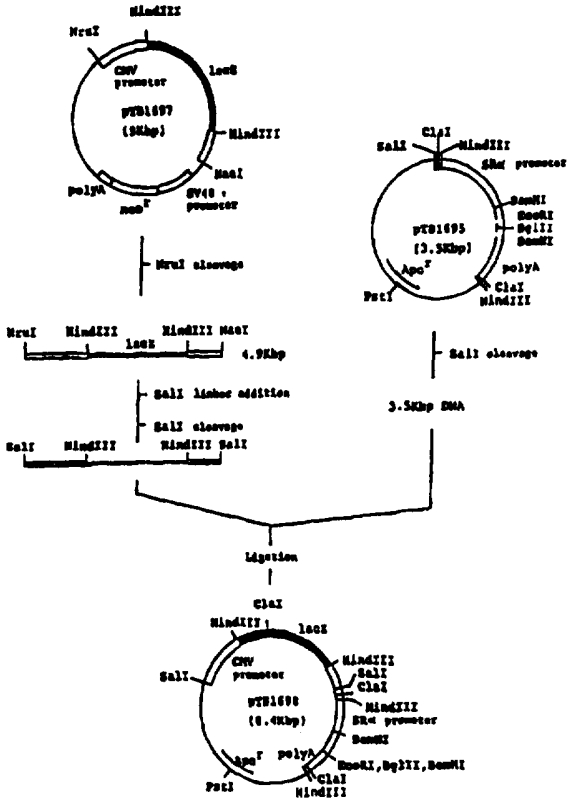
(54) Title: DNA ENCODING A CELL GROWTH INHIBITING FACTOR AND ITS PRODUCT

(57) Abstract

Disclosed are (1) a method of screening a DNA encoding a human cell growth inhibiting factor, by introducing a human DNA into a eukaryotic cell host under the control of an inducible promoter, and selecting DNA whose host cell does not grow under promoter-inducing conditions, but grows under non-promoter-inducing conditions, (2) new DNA encoding a human cell growth inhibiting factor selectable by this method, (3) a vector containing said DNA, (4) a transformant as transformed with said vector, (5) new human cell growth inhibiting factor, (6) a method for preparing said factor and a pharmaceutical composition containing said factor. According to the above screening method, a DNA encoding a human cell growth inhibiting factor can be selectively and conveniently obtained. The thus obtained DNA is useful as a probe for investigating aging, or as a reagent for diagnosing various aging-associated diseases. The human cell growth inhibiting factor can be useful as an anticancer agent, infection remedy and so on.



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DESCRIPTION

DNA ENCODING A CELL GROWTH INHIBITING FACTOR AND ITS PRODUCT

Technical Field

The present invention relates to novel DNA and a method of screening for such DNA. More specifically, the present invention relates to a method of screening for and/or selecting a human DNA encoding a eukaryotic cell growth inhibiting factor, to novel DNA encoding for the inhibiting factor obtained by the method, to novel eukaryotic cell growth inhibiting factors, method for preparing said inhibiting factor and use thereof.

Background Art

In higher multicellular organisms, there are various aging associated diseases such as dementia and arteriosclerosis. To basically clarify the causes of these diseases, the mechanism of aging must be understood. However, the mechanism of individual aging is extremely complex; there is no clue to an understanding of the mechanism. Against this background, the aging of individual-constituting cells may be analyzed as a first step toward the understanding of individual aging.

Animal tissue cells in culture lose their growth capability as the number of subculturing generations increases, eventually terminating growth and dying, although showing good growth initially. This phenomenon is called cell aging [Hayflick, L. and Moorhead, P.S., Experimental Cell Research, Vol. 25, p. 585 (1961); Hayflick, L., ibid., Vol. 37, p. 614 (1965)]. Very limited portions of such cells may become immortal (immortalized cells).

From the following experimental results, it is evident that aging at the cell level is closely associated with individual aging. (1) The maximum

possible number of divisions (division life span) of cultured cells is inversely proportional to individual age [Martin, G.M. et al., Laboratory Investigation, Vol. 23, p. 86 (1970); Schneider, E.L. and Mitsui, Y., Proceedings of the National Academy of Sciences, USA, Vol. 73, p. 3584 (1976); Goldstein, S. et al., Science, Vol. 199, p. 781 (1978)]. (2) Cells derived from patients with hereditary progeria are short in division life span while in culture [Martin, G.M. et al., ibid.; Goldstein, S., Lancet, Vol. 1, p. 424 (1969); Goldstein, S., Journal of Investigative Dermatology, Vol. 73, p. 19 (1979); Norwood, T.H. et al., ibid., Vol. 73, p. 92 (1979)]. (3) There is a correlation between the maximum life span of various animal species and the division life span of cultured cells derived therefrom [Roeme, D., Proceedings of the National Academy of Sciences, USA, Vol. 78, p. 5009 (1981)]. To summarize, cell aging is not assumed to be unique to in vitro systems.

Even if an aged cell is fused with a young or immortalized cell, DNA synthesis does not occur again in the aged cell; on the contrary, DNA synthesis in the young and immortalized cell is suppressed [Norwood, T.H. et al., Proceedings of the National Academy of Sciences, USA, Vol. 71, p. 2231 (1974); Yanishevsky, R.M. and Stein, G.H., Experimental Cell Research, Vol. 126, p. 469 (1980); Stein, G.H. and Yanishevsky, R.M., Proceedings of the National Academy of Sciences, USA, Vol. 78, p. 3025 (1981)]. This demonstrates that the phenotypes related to cellular senescence are dominant, and that the aged cell does not lack substances essential to its growth but has a substance that suppresses DNA synthesis therein. In fact, microinjection of mRNA prepared from an aged cell into a young cell is known to inhibit DNA synthesis in the latter [Lumpkin, C.K. et al., Science, Vol. 232, p. 393 (1986)]. It can therefore be held that there are some genes whose expression occurs newly or increases with

cell age, and that such genes play an important role in cell aging, directly or indirectly.

Smith et al. tested the complementation of a large number of immortalized human cells in fused pairs, demonstrating the presence of 4 groups of human aging genes [Pereira-Smith, O.M. and Smith, J., Science, Vol. 221, p. 964 (1983); Pereira-Smith, O.M. and Smith, J., Proceedings of the National Academy of Sciences, USA, Vol. 85, p. 6042 (1988)]. Also, they have recently found DNA that encodes a DNA synthesis-inhibiting protein (SDI) [WO9312251].

Clarifying the nature of such aging-associated genes is not only important in understanding aging, both at the cellular and individual levels, but is also significant in that the use of these genes or gene products would enable the diagnosis of various aging-associated diseases and the development of prophylactic/therapeutic drugs for such diseases, or their application as prophylactic/therapeutic drugs for various diseases involving uncontrollable cell growth such as cancer.

Disclosure of Invention

With the expectation that there are genes involved in the suppression of aged cell growth and showing little or no expression in young cells, there have been many attempts to clone such aging-associated genes in the form of cDNA. However, due to marked increase in the mRNA of extracellular substrates (e.g., collagen, fibronectin), which are not directly associated with aged cell growth suppression, the desired mRNA or cDNA is difficult to select and obtain. It is also known that the obtained cDNA is expressed to a considerable extent even in young cells. For these reasons, no one has succeeded in cloning cDNA specific to aged cells.

The present inventors found that human cDNA encoding a cell growth inhibiting factor can be screened

and subsequently isolated by introducing a cDNA library into a fission yeast, which is a eukaryotic cell host, wherein the cDNA library is prepared by ligating aged-cell-derived cDNA to the downstream of an inducible promoter. The present inventors conducted further investigations based on this finding, and developed the present invention.

Namely, the present invention relates to:

(1) A method of screening for a DNA encoding a eukaryotic cell growth inhibiting factor comprising :

(a) introducing a human DNA operably linked to an inducible promoter into a eukaryotic host cell ;

(b) testing said host cell for the presence of said DNA by measuring host cell growth rate under conditions in which the promoter is induced and not induced ; and

(c) determing the differential growth of these two groups at selected times whereby a host cell showing at least about 25% growth rate inhibition under the inducible condition as compared with the cell growth rate under the non-inducible condition is identified as containing the DNA encoding a eukaryotic cell growth inhibiting factor,

(2) The method according to the above paragraph (1), wherein a host cell showing at least about 50% growth rate inhibition is identified as containing the DNA,

(3) The method according to the above paragraph (1), wherein a host cell that has at least about 75% growth rate inhibition is identified as containing the DNA,

(4) The method according to the above paragraph (1), further comprising the step of isolating the DNA encoding the eukaryotic cell growth inhibiting factor from the host identified as containing the DNA,

(5) The method according to the above paragraph (1), the inducible promoter is PHO5 promoter, nmt1 promoter or hsp promoter,

(6) The method according to the above paragraph (1), the selected times is about 12 to 144 hours after culture,

(7) A method of screening for a DNA encoding a eukaryotic cell growth inhibiting factor, which comprises introducing a human DNA to be tested into a eukaryotic cell host so as to be controlled by an inducible promoter and selecting the host cell which does not grow under the inducible condition of said promoter but grows under the non-inducible condition,
(8) The method according to the above paragraph (1) or (7), wherein said eukaryotic cell host is a yeast,
(9) The method according to the above paragraph (8), wherein said yeast is a fission yeast,
(10) The method according to the above paragraph (9), wherein said fission yeast is a Shizosaccharomyces pombe,
(11) An isolated DNA encoding a eukaryotic cell growth inhibiting factor, which is screened by the method according to the above paragraph (1),
(12) A DNA which codes for a eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 11,
(13) The DNA according to the above paragraph (12), wherein said DNA comprises a nucleotide sequence at least from the 248th to the 448th residues of the nucleotide sequence represented by SEQ ID NO. 7,
(14) A DNA which codes for a eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 12,
(15) The DNA according to the above paragraph (14), wherein said DNA comprises a nucleotide sequence at least from the 279th to the 752nd residues of the nucleotide sequence represented by SEQ ID NO. 8,
(16) A DNA which codes for a eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 13,
(17) The DNA according to the above paragraph (16), wherein said DNA comprises a nucleotide sequence at least from the 201st to the 377th residues of the

nucleotide sequence represented by SEQ ID NO. 9,
(18) A DNA which codes for a eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 14,
(19) The DNA according to the above paragraph (18), wherein said DNA comprises a nucleotide sequence at least from the 296th to the 1000th residues of the nucleotide sequence represented by SEQ ID NO. 10,
(20) A DNA which codes for a eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 15,
(21) The DNA according to the above paragraph (20), wherein said DNA comprises a nucleotide sequence at least from the 51st to the 740th residues of the nucleotide sequence represented by SEQ ID NO. 16,
(22) A DNA which codes for a eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 22,
(23) The DNA according to the above paragraph (22), wherein said DNA comprises a nucleotide sequence at least from the 1062nd to the 1736th residues of the nucleotide sequence represented by SEQ ID NO. 17,
(24) A DNA which codes for a eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 23,
(25) The DNA according to the above paragraph (24), wherein said DNA comprises a nucleotide sequence at least from the 55th to the 1488th residues of the nucleotide sequence represented by SEQ ID NO. 20,
(26) A DNA coding for a eukaryotic cell growth inhibiting factor which comprises the nucleotide sequence of SEQ ID NO. 18,
(27) A DNA coding for a eukaryotic cell growth inhibiting factor which comprises the nucleotide sequence of SEQ ID NO. 19,
(28) A DNA coding for a eukaryotic cell growth inhibiting factor which comprises the nucleotide

sequence of SEQ ID NO. 21,
(29) A DNA coding for a eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 27,
(30) The DNA according to the above paragraph (29), wherein said DNA comprises a nucleotide sequence at least from the 150th to the 1004th residues of the nucleotide sequence represented by SEQ ID NO. 25,
(31) A DNA coding for a eukaryotic cell growth inhibiting factor which comprises the nucleotide sequence of SEQ ID NO. 24,
(32) A DNA coding for a eukaryotic cell growth inhibiting factor which comprises the nucleotide sequence of SEQ ID NO. 26,
(33) A vector comprising any one of DNAs according to the above paragraph (11) to (32),
(34) A transformant harboring the vector according to the above paragraph (33),
(35) A eukaryotic cell growth inhibiting factor which is coded by the DNA obtained by the method according to the above paragraph (1),
(36) A eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 11,
(37) A eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 12,
(38) A eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 13,
(39) A eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 14,
(40) A eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 15,
(41) A eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 22,
(42) A eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 23,
(43) A eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 27,

(44) A eukaryotic cell growth inhibiting factor which is encoded by the DNA according to the above paragraph (26),

(45) A eukaryotic cell growth inhibiting factor which is encoded by the DNA according to the above paragraph (27),

(46) A eukaryotic cell growth inhibiting factor which is encoded by the DNA according to the above paragraph (28),

(47) A eukaryotic cell growth inhibiting factor which is encoded by the DNA according to the above paragraph (31),

(48) A eukaryotic cell growth inhibiting factor which is encoded by the DNA according to the above paragraph (32),

(49) A method for preparing the eukaryotic cell growth inhibiting factor according to the above paragraph (35) which comprises cultivating a transformant containing a DNA encoding said factor under conditions suitable for expression of the said factor and recovering said factor,

(50) A pharmaceutical composition which comprises an effective amount of any one of eukaryotic cell growth inhibiting factors according to the above paragraph (35),

(51) Use of the eukaryotic cell growth inhibiting factor according to the above paragraph (35) for preparing an anticancer agent or infection remedy,

(52) A method for treating for a patient suffering from cancer or infection which comprises administering to said said patient an effective effective amount of the eukaryotic cell growth inhibiting factor according to the above paragraph (35) in the form of a pharmaceutical composition containing said factor as the effective component,

(53) A method for inhibiting nucleic acid synthesis in target cell comprising containing said cell with an

effective amount of a eukaryotic cell growth inhibiting factor encoded by the DNA according to the above paragraph (11).

Brief Description of Drawings

Figure 1 shows construction scheme for animal expression plasmid pTB1698.

Best Mode for Carrying Out the Invention

The screening method of the present invention can be carried out by:

1) synthesizing cDNA using mRNA prepared from a human aged cell as template,

2) ligating the cDNA to the downstream of an inducible promoter to prepare an expression cDNA library,

3) introducing the library into a eukaryotic host cell,

4) culturing the obtained transformant eukaryotic host cell under inducible promoter-inducing conditions and non-promoter-inducing conditions, and

5) screening cells for those having reduced growth rates under inducible promoter-inducing conditions as compared with growth under non-promoter-inducing conditions.

While the combination of these steps is novel, commonly known techniques are applicable to these processes 1) through 5).

Examples of the eukaryotic cell host for the present method include yeasts, fungi and animal cells. Preferred are yeasts which involve little background influence under non-inducing conditions in an expression system using an inducible promoter, with greater preference given to fission yeasts (Schizosaccharomyces). Of the fission yeasts, Schizosaccharomyces pombe is preferable.

Any inducible promoter can be used, as long as it functions as a promoter in the eukaryotic host cell used, and as long as its activity can be regulated under culturing conditions that do not affect the growth of

the host cell; an appropriate inducible promoter is selected according to the eukaryotic host cell used. When the host is a yeast, for instance, the PHO5 promoter, nmt1 promoter etc. are preferred. When the host is an animal cell, the hsp promoter, metallothionein promoter etc. are preferred.

In the present screening method, the preferred human DNA used as a sample is cDNA; it can be obtained by a common gene engineering procedure using mRNA prepared from human cells as template. Although the human cell used to prepare the subject cDNA may be any one, as long as it is of human origin, it is preferable to use normal diploid cells, such as normal human fibroblast cells MRC-5, TIG-1 [Experimental Gerontology, Vol. 15, pp. 121-133 (1980)] and TIG-3 [Journal of Gerontology, Vol. 37, pp. 33-37 (1982)], in the aging phase. It is also preferable to use aged cells prepared by subculturing relatively young cells until growth reaches a plateau. For example, RNA can be prepared from aged cells by the guanidine thiocyanate method [Chirgwin, J.M., et al., Biochemistry, Vol. 18, p. 5294 (1979)].

Using the thus-obtained RNA as template, in combination with reverse transcriptase, cDNA is synthesized by, for example, the method of Okayama, H. et al. [Molecular Cell Biology, Vol. 2, p. 161 (1982); ibid., Vol. 3, p. 280 (1983)] or the method of Gubler, U. and Hoffman, B.J. [Gene, Vol. 25, p. 263 (1983)]; the obtained cDNA is introduced into a vector such as a plasmid or phage to yield a cDNA library.

Examples of the plasmid for cDNA insertion include plasmids derived from Escherichia coli such as pBR322 [Gene, Vol. 2, p. 95 (1977)], pBR325 [Gene, Vol. 4, p. 121 (1978)], pUC12 [Gene, Vol. 19, p. 259 (1982)] and pUC13 [Gene, Vol. 19, p. 259 (1982)] and those derived from Bacillus subtilis such as pUB110 [Biochemical and Biophysical Research Communications, Vol. 112, p. 678

(1983)], but any other can be used for this purpose, as long as it is replicable in the host. Also included is λ gt 11 [Young, R. and Davis. R., Proceedings of the National Academy of Sciences, USA, Vol. 80, p. 1194 (1983)], but any other can be used, as long as it is capable of growing in the host. From the viewpoint of procedural simplicity, it is particularly preferable that cDNA is ligated to the downstream of an inducible promoter of a eukaryotic host cell, using a plasmid into which the promoter is inserted in advance.

Example methods for inserting a cDNA into the plasmid include that described by T. Maniatis et al. in Molecular Cloning, Cold Spring Harbor Laboratory, page 239 (1982). Example methods for inserting a cDNA into the phage vector include the method of Hyunh, T.V. et al. [DNA Cloning, A Practical Approach, Vol. 1, p. 49 (1985)]. The thus-obtained plasmid or phage vector is introduced into an appropriate host, such as Escherichia coli, and stored.

Example strains of Escherichia coli include Escherichia coli K12 DH1 [Proceedings of the National Academy of Sciences, USA, Vol. 60, p. 160 (1968)], JM103 [Nucleic Acids Research, Vol. 9, p. 309 (1981)], JA221 [Journal of Molecular Biology, Vol. 120, p. 517 (1978)], HB101 [Journal of Molecular Biology, Vol. 41, p. 459 (1969)] and C600 [Genetics, Vol. 39, p. 440 (1954)].

Example strains of Bacillus subtilis include Bacillus subtilis MI 114 [Gene, Vol. 24, p. 255 (1983)] and 207-21 [Journal of Biochemistry, Vol. 95, p. 87 (1984)].

Example methods for transforming a host cell with a plasmid include the calcium chloride method described by T. Maniatis et al. [ibid., p. 249 (1982)], the calcium chloride/rubidium chloride method and the electroporation method. The phage vector can, for example, be introduced into cultured Escherichia coli by the in vitro packaging method.

Next, the plasmid or phage vector is isolated from the transformant microorganism thus obtained to prepare subject cDNA for the present selection method. The isolation method is exemplified by the alkali-SDS method [Birmboim, H.C., et al., Nucleic Acids Research, Vol. 1, p. 1513 (1979)]. The subject cDNA, as such or, if desired, after digestion with restriction enzymes, can be ligated to the downstream of the inducible promoter, to yield an expression vector.

The above-described eukaryotic host cells can be transformed with the thus-obtained vector containing the desired DNA as follows: Yeasts can be transformed in accordance with the method described in the Proceedings of the National Academy of Science, USA, Vol. 75, p. 1929 (1978), for instance. Animal cells can be transformed in accordance with the method described in Virology, Vol. 52, 456 (1973), for instance.

In the present selection method, transformants carrying the subject cDNA are cultured in accordance with the culturing method for the eukaryotic host cell used, with medium composition (metal ions, nitrogen sources, inorganic or organic acids, bases and other components), pH, culturing temperature and other culturing conditions changed as appropriate. When the host is a yeast, for instance, the promoter can be induced in the absence of phosphate ions in the medium, using the PHO5 promoter under the culturing conditions described later, and promoter activity can be adjusted by changing between the presence and absence of thiamine in the medium as described in Examples below, using the nmt1 promoter. For observing cell growth in an animal host system using the hsp promoter, promoter activity can be induced by incubation at a temperature (41 - 42℃) slightly higher than ordinary culturing temperature (36 - 37℃) for a given period of time.

A transformant is thus selected whose cell growth is inhibited under the promoter activity-inducing

conditions, and which shows normal growth, according to the host cell used, under non-promoter-activity-inducing conditions.

Here, "cell growth is inhibited" means that the cell growth under promoter-inducible-conditions is reduced as comparing with the cell growth under non-promoter-inducible-conditions (control). For example, the cell growth rate inhibition is at least 25%, preferably 50%, more preferably 75%. And "cell does not grow" means that the cell hardly grow and the cell growth inhibition is about 75 - 100%.

For observing cell growth inhibition, it is preferable to do when the cell growth reaches plateau. For example, when the host cell is a fission yeast, it is preferable to observe the cell growth after cultivation is carried at about 20 to 40℃ for about 12 to 144 hours, more preferably at 20 to 35 ℃ for about 24 to 72 hours.

For observing cell growth inhibition, it is preferable to use a solid medium. Cells undergoing growth inhibition can easily be selected from the subject cell group by comparing the sizes of colonies formed. In confirming the growth inhibitory activity of the cells thus screened for, it is also effective to culture the cells in a liquid medium, in addition to the assay system using a solid medium, and determine growth inhibition rate by measuring a culture broth turbidity or uptake of tritium thymidine by cultured cell as an index.

The sizes of colonies formed are observed by naked eys or using a microscope. The culture broth turbidity is determined by measuring the transmittance of visible radiation of culture broth. The uptake of tritium thymidine by cultured cell is measured by known method.

In the present invention, a eukaryotic cell growth inhibiting factor is defined as a peptide or protein that suppresses or terminates the growth of eukaryotic

cells such as yeasts and animal cells, under ordinary culturing conditions as shown below, and may be any one, as long as it is capable of reversibly or irreversibly inhibiting the growth of at least one kind of eukaryotic cell. Such inhibiting factors include peptides or proteins having a partial or full-length portion of the amino acid sequence of SEQUENCE ID NOS. 11, 12, 13, 14, 15, 22, 23 or 27, including peptides or proteins whose sequences lack the N-terminal methionine residue.

DNA encoding the above-described eukaryotic cell growth inhibitor can be obtained by the method of the present invention for screening DNA and so on. The DNA of the present invention is exemplified by DNA containing the nucleotide sequences shown by NUCLEIC ACID RESIDUE NOS. 279-752 of SEQUENCE ID NO. 8 encoding the amino acid sequenceof SEQ ID No. 12, NUCLEIC ACID RESIDUE NOS. 201-377 of SEQUENCE ID NO. 9 encoding the amino acid sequence of SEQ ID No. 13 , NUCLEIC ACID RESIDUE NOS. 296-1000 of SEQUENCE ID NO. 10 encoding the amino acid sequence of SEQ ID No. 14, NUCLEIC ACID RESIDUE NOS. 248-448 of SEQUENCE ID NO. 7 encoding the amino acid sequence of SEQ ID No. 11, NUCLEIC ACID RESIDUE NOS. 51-740 of SEQUENCE ID NO. 16 encoding the amino acid sequence of SEQ ID No. 15, NUCLEIC ACID RESIDUE NOS. 1062-1736 of SEQUENCE ID NO. 17 encoding the amino acid sequence of SEQ ID No. 22, NUCLEIC ACID RESIDUE NOS. 55-1488 of SEQUENCE ID NO. 20 encoding the amino acid sequence of SEQ ID No. 23, NUCLEIC ACID RESIDUE NOS. 150-1004 of SEQUENCE ID NO. 25 encoding the amino acid sequence of SEQ ID No. 27 and SEQUENCE ID NOS. 18 19, 21, 24 and 26. For obtaining eukaryotic cell growth inhibiting factor encoded by the DNA of the present invention using that DNA, N-terminal Met may be added to the inhibitor polypeptide; the cell growth factor may be a glycoprotein (sugar chain added) or a fused protein with another polypeptide.

An expression vector containing the DNA of the

present invention that encodes eukaryotic cell growth inhibiting factor can be produced by, for example, 1) preparing cell growth inhibiting factor-encoding mRNA from an aged cell, 2) synthesizing cDNA and then double-stranded DNA from the mRNA to yield a cDNA library, 3) selecting cDNA encoding a polypeptide that inhibits host cell growth from the cDNA library, and 4) ligating the cDNA to the downstream of the promoter in the vector.

The cDNA library obtained from an aged cell in accordance with the above-described method may be treated to concentrate the desired cDNA by known methods, e.g., the differential hybridization method [Sambrook, J. et al., Molecular Cloning, A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory, (1989)], the subtraction method [Molecular Cloning, ibid.] and the cell suicide selection method. The screening method of the present invention is also preferred. In such a case, the cell suicide selection method [Stetten, G. et al., Experimental Cell Research, Vol. 108, p. 447 (1977), Brdurd-Hoechst] may be used to concentrate cells whose growth has been suppressed by the cDNA introduced, followed by selection of the desired cDNA. The desired cDNA can also be selected by the present selection method using an inducible promoter.

Next, the plasmid or phage vector is isolated from the microorganism by a known method such as the above-described alkali-SDS method. The obtained plasmid or phage vector, harboring DNA containing a base sequence encoding a cell growth inhibitor, can be used as such or, if desired, after digestion with restriction enzymes, according to the purpose of use.

The cloned gene is ligated to the downstream of the promoter, in a vector suitable for its expression, to yield an expression vector. The gene may have ATG as a translational initiation codon at its 5'-terminal and TAA, TGA or TAG as a translational termination codon at

its 3'-terminal. To express the gene, a promoter is ligated to its upstream. Any promoter can be used for the present invention, as long as it is appropriate for the host used to express the gene. Example vectors include the above-mentioned plasmids derived from Escherichia coli (e.g., pBR322, pBR325, pUC12, pUC13, ptrp 781), plasmids derived from Bacillus subtilis (e.g., pUB110, pTM5, pC194), yeast-derived plasmids (e.g., pSH19, pSH15), bacteriophages such as λ phage, and animal viruses such as retrovirus and vaccinia virus.

Examples of preferred promoters include the T7 promoter, trp promoter, lac promoter, rec promoter, λ PL promoter and lpp promoter when the transformation host is Escherichia coli, the SPO1 promoter, SPO2 promoter and pen P promoter when the host is Bacillus subtilis, and the PHO5 promoter, PGK promoter, GAP (GLD) promoter, ADH promoter and nmt1 promoter when the host is a yeast. Preference is given to the case in which Escherichia coli is used as host in combination with the T7 promoter, trp promoter or λ PL promoter.

When the host is an animal cell, preferable promoters include the SV40-derived promoter, retrovirus promoter, metallothionein promoter and hsp promoter, with preference given to the SV40-derived promoter.

The thus-constructed vector, harboring DNA containing a nucleotide sequence such as one of the sequences of SEQUENCE ID NOS. 7-10, 16-21 and 24-26 is used to produce a transformant. Examples of the host include prokaryotes such as Escherichia coli, Bacillus subtilis and actinomycetes, and eukaryotes such as yeasts, fungi and animal cells.

Examples of the strains of Escherichia coli and Bacillus subtilis are the same as those mentioned above.

Examples of the yeasts include Saccharomyces cerevisiae AH22, AH22R$^-$, NA87-11A, DKD-5D, Schizosaccharomyces pombe and mutants thereof.

Examples of the animal cells include simian cells COS-7, Vero, Chinese hamster cells CHO and mouse L cells. The strains of Escherichia coli can be transformed in accordance with the method described in the Proceedings of the National Academy of Sciences, USA, Vol. 69, p. 2110 (1972), Gene, Vol. 17, p. 107 (1982) and other publications, for instance.

Strains of Bacillus subtilis can be transformed in accordance with the method described in Molecular and General Genetics, Vol. 168, p. 111 (1979) and other publications, for instance.

Yeasts can be transformed in accordance with the method described in the Proceedings of the National Academy of Sciences, USA, Vol. 75, p. 1929 (1978), for instance.

Animal cells can be transformed in accordance with the method described in Virology, Vol. 52, p. 456 (1973), for instance.

A transformant as transformed with a vector harboring the desired DNA is thus obtained. When the host is a eukaryotic cell, the transformant is subcultured under non-promoter-inducing conditions using an inducible promoter.

For culturing a transformant whose host is Escherichia coli, Bacillus subtilis, an actinomycete, yeast or fungus, it is appropriate to use liquid medium supplemented with carbon sources, nitrogen sources, minerals and other substances necessary for the growth of the transformant. Examples of carbon sources include glucose, dextrin, soluble starch and sucrose; examples of nitrogen sources include organic or inorganic substances such as ammonium salts, nitrates, corn steep liquor, peptone, casein, meat extracts, soybean cake and potato extracts; examples of minerals include calcium chloride, sodium dihydrogen phosphate and magnesium chloride. The pH of the medium is preferably about 5 to 8.

Examples of media preferably used to culture Escherichia coli include the M9 medium containing glucose and casamino acid [Miller, Journal of Experimental Molecular Genetics, p. 431, Cold Spring Harbor Laboratory, New York (1972)]. Cultivation is normally carried out at about 14 to 43℃ for about 3 to 24 hours, with aeration and/or stirring as necessary.

When the host is Bacillus subtilis, cultivation is normally carried out at about 30 to 40℃ for about 6 to 24 hours, with aeration and/or stirring as necessary.

Examples of media for culturing a transformant whose host is a yeast include Burkholder's minimal medium [Bostian, K.L. et al., Proceedings of the National Academy of Sciences, USA, Vol. 77, p. 4505 (1980)], preferably adjusted to a pH of about 5 to 8.

For culturing a transformant to express the desired gene using an inducible promoter, when an nmt1 promoter, for instance, is used, the transformant is cultured under promoter-inducing conditions, e.g., in a thiamine-free medium.

When an inducible promoter is used, cultivation is normally carried out at about 20 to 35℃ for 24 to 72 hours, with aeration and/or stirring as necessary, until cell growth reaches a plateau.

Example media for culturing a transformant whose host is an animal cell include MEM containing about 5 to 20% fetal bovine serum [Science, Vol. 122, p. 501 (1952)], DMEM [Virology, Vol. 8, p. 396 (1959)], RPMI 1640 medium [Journal of the American Medical Association, Vol. 199, p. 519 (1967)] and 199 medium [Proceedings of the Society of Experimental Biological Medicine, Vol. 73, p. 1 (1950)]. For culturing a transformant to express the desired gene using an inducible promoter, the transformant is cultured under promoter-inducing conditions, e.g., in a medium supplemented with heavy metal ions when a metallothionein promoter is used. The pH is preferably

about 6 to 8. Cultivation is normally carried out at about 30 to 40℃ for 15 to 60 hours. When an inducible promoter is used, aeration and/or stirring is conducted as necessary, until cell growth reaches a plateau.

The eukaryotic cell growth inhibiting factor of the present invention is produced and accumulated intracellularly or extracellularly. For extracting the intracellular cell growth inhibitor from the culture, cultured cells collected by a known method are suspended in a buffer containing a protein denaturant such as guanidine hydrochloride or urea, or a surfactant such as Triton X-100, and then centrifuged to obtain a supernatant containing the cell growth inhibitor, or cells are disrupted by ultrasonication, treatment with an enzyme such as lysozyme, or freeze-thawing, followed by centrifugation to obtain a supernatant containing the cell growth inhibiting factor.

For separating and purifying the cell growth inhibiting factor produced and accumulated in the supernatant or extracellularly, known methods of separation and purification can be used in combination, as appropriate. Such known methods of separation and purification include those based on solubility differences, such as salting-out and solvent precipitation, those based mainly on molecular weight differences, such as dialysis, ultrafiltration, gel filtration and SDS-polyacrylamide gel electrophoresis, those based on charge differences, such as ion exchange chromatography, those based on specific affinity, such as affinity chromatography, those based on hydrophobicity differences, such as reverse-phase high performance liquid chromatography, and those based on isoelectric point differences, such as isoelectric focusing.

A eukaryotic cell growth inhibiting factor containing substantially no pyrogen or endotoxin is thus obtained in substantially pure form. The substantially

pure cell growth inhibiting factor of the present invention contains the cell growth inhibiting factor protein at not lower than 95% (w/w), preferably not lower than 98% (w/w). Here, "containing substantially no pyrogen or endotoxin" means that the cell growth inhibiting factor is negative in, for example, the known limulus test or pyrogen test.

The DNA of the present invention that encodes a eukaryotic cell growth inhibiting factor can be used as a probe for examining individual aging at the RNA level. Specifically, the DNA of the present invention can be used as a diagnostic reagent for various aging-associated diseases. The gene of the present invention may also be introduced into cells of a target tissue, such as skin or vascular endothelium, to establish an in vitro aged cell line of the target tissue. Such a line is useful as a screening system for clarifying the mechanisms of onset and action of various aging-associated diseases, or for seeking therapeutic drugs for these diseases.

The eukaryotic cell growth inhibiting factor encoded by the DNA of the present invention can be used as a pharmaceutical, such as an anticancer agent or infection remedy, as described later. In such case, the eukaryotic cell growth inhibiting factor can be safely administered parenterally or orally, preferably topically, in the form of powder as such, or in the form of pharmaceutical compositions (e.g., injections, tablets, capsules, solutions, ointments) together with pharmacologically acceptable carriers, excipients and diluents, to warm-blooded animals (e.g., humans, mice, rats, hamsters, rabbits, dogs, cats). The cell growth inhibiting factor can also be used as a skin drug.

An injectable preparation is prepared in accordance with a conventional method using physiological saline or an aqueous solution containing glucose and other auxiliaries. Other pharmaceutical compositions, such as

tablets and capsules, can also be prepared in accordance with conventional methods.

When using the cell growth inhibiting factor of the present invention as a pharmaceutical as described above in mammals, it is administered at daily doses of about 0.2 μg/kg to 20 mg/kg, preferably about 2 μg/kg to 0.2 mg/kg. The cell growth inhibiting factor obtained according to the present invention is thought of as terminating cell division, and can therefore be used as a reagent for terminating the cell cycle of cultured cells at a given time point, e.g., a reagent for synchronizing cell division. By making constant the cell cycle of cells in an in vitro experimental system, it is possible to improve assay precision or establish an experimental system of a particular cell cycle. When using the cell growth inhibiting factor of the present invention as such a reagent, it is preferable to add it to the medium to a final concentration of 1 ng/ml to 1 mg/ml, more preferably 1 ng/ml to 10 μg/ml.

The factor encoded by the DNA of the present invention acts on young cells capable of division, or infinitely growing cancer cells, to prevent their growth. The DNA of the present invention can therefore be used for gene therapy for cancer patients or as a probe for the diagnosis of aging-associated diseases. The factor encoded by the DNA of the present invention can also be used as an anticancer agent. It is also effective against fungal infections (e.g., cutaneous mycosis, deep mycosis). Moreover, the DNA of the present invention can be used as a system for clarifying the mechanism of onset of aging-associated diseases or seeking therapeutic drugs, to establish an in vitro aged cell line of the target tissue. Substances that inhibit the cell growth inhibiting factor appear to be applicable as prophylactic/therapeutic drugs for aging and various aging-associated diseases, such as dementia and arteriosclerosis. Accordingly, the DNA of the

present invention and the factor encoded thereby can be used to seek such drugs. The factor can also be used as a reagent for terminating the cell cycle of cultured cells at a given time point.

Antibodies or antiserum to the eukaryotic cell growth inhibiting factor or the partial peptides thereof of the present invention can be produced by the methods known per se in the art, using the factor or the partial peptides thereof as antigens. The antibodies or antiserum can be used for inhibiting the activity of the eukaryotic cell growth inhibiting factor to rejuvenate the aged cell or tissues. The antibodies or antiserum can also be used for quantitative analysis or detection of the factor or the partial peptides thereof by methods known per se in the art.

Antisense oligonucleotides complementary to the cDNA encoding the eukaryotic cell growth inhibiting factor can be synthesized by the known methods. The oligonucleotides hybridize to the mRNA, inhibit the production of the eukaryotic cell growth inhibiting factor and induce rejuvevation of aged cell or tissues. These oligonucleotides can be used for in vivo and ex vivo treatment of diseases caused by cellular senescence or aging-associated diseases, such as arteriosclerosis and dementia. These oligonucleotides are also effective for a normal tissue or cell, such as skin cell, a cell present in wound or burn tissue, lymphocyte, vascular tissue, liver, kidney, heart, bone, spleen, etc.

Abbreviations for bases, amino acids and others used in the present specification and attached drawings are based on abbreviations specified by the IUPAC-IUB Commission on Biochemical Nomenclature or abbreviations in common use in relevant fields. Some examples are given below. When an optical isomer may be present in amino acid, it is of the L-configuration, unless otherwise stated.

PBS : Phosphate-buffered saline
DNA : Deoxyribonucleic acid
cDNA: Complementary deoxyribonucleic acid
A : Adenine
T : Thymine
G : Guanine
C : Cytosine
RNA : Ribonucleic acid
mRNA: Messenger ribonucleic acid
dATP: Deoxyadenosine triphosphate
dTTP: Deoxythymidine triphosphate
dGTP: Deoxyguanosine triphosphate
dCTP: Deoxycytidine triphosphate
ATP : Adenosine triphosphate
EDTA: Ethylenediaminetetraacetic acid
SDS : Sodium dodecyl sulfate
Gly : Glycine
Ala : Alanine
Val : Valine
Leu : Leucine
Ile : Isoleucine
Ser : Serine
Thr : Threonine
Cys : Cysteine
Met : Methionine
Glu : Glutamic acid
Asp : Aspartic acid
Lys : Lysine
Arg : Arginine
His : Histidine
Phe : Phenylalanine
Tyr : Tyrosine
Trp : Tryptophan
Pro : Proline
Asn : Asparagine
Gln : Glutamine

Examples

The present invention is hereinafter described in more detail by means of the following reference example and working examples, which are not to be construed as limitative to the present invention.

The transformants obtained in the following examples, that carry the DNA of the present invention, have been deposited as follows:

Transformant	IFO (IFO No.)	NIBH (FERM No.)
E. coli MC1061/pTB1617	15627 January 21, 1994	BP-4551 February 7, 1994
E. coli MC1061/pTB1618	15628 January 21, 1994	BP-4552 February 7, 1994
E. coli DH1/pTB1668	15756 October 27, 1994	BP-4890 November 14, 1994
E. coli DH1/pTB1671	15757 October 27, 1994	BP-4891 November 14, 1994
E. coli DH1/pTB1673	15758 October 27, 1994	BP-4892 November 14, 1994
E. coli DH1/pTB1848	15807 March 16, 1995	BP-5126 June 9, 1995
E. coli MC1061/pTB1689	15821 May 30, 1995	BP-5127 June 9, 1995
E. coli MC1061/pTB1721	15822 May 30, 1995	BP-5128 June 9, 1995
E. coli MC1061/pTB1756	15823 May 30, 1995	BP-5129 June 9, 1995
E. coli MC1756/pTB1761	15824 May 30, 1995	BP-5130 June 9, 1995
E. coli MC1061/pTB1786	———	BP-5268 October 27, 1995
E. coli MC1061/pTB1810	———	BP-5269 October 27, 1995
E. coli MC1061/pTB1819	———	BP-5270 October 27, 1995

IFO : Institute for Fermentation, Osaka (foundation)
NIBH: National Institute for Bioscience and Human-Technology, Agency of Industrial Science and Technology, Ministry of International Trade and Industry
Dates in parentheses are dates of accession.

Reference Example 1 Construction of vector for animal cells

Plasmid pTB399 (Cell Struct. Funct., Vol. 12, pp. 205-217) was cleaved with EcoRI and reacted with the Klenow fragment, followed by addition of BglII linker (CAGATCTG) and cleavage with BglII, to yield a 3.8 kb DNA fragment deleting the interleukin-2 (IL-2) cDNA region. The fragment was then cyclized using T4 ligase. The MuLV-LTR portion was then replaced with an SRα promoter derived from pME18S [Maruyama, K. and Takebe, Y., Medical Immunology, Vol. 20, pp. 27-32 (1990)] in accordance with a conventional method, to yield plasmid pTB1695.

Example 1 Preparation of cDNA from aged normal human diploid fibroblast

A relatively young line (22PDL) of normal diploid fibroblast MRC-5 of human male fetal lung origin [Jacobs, J.P. et al., Nature, Vol. 227, p. 168 (1970)] was purchased from 3the Institute for Fermentation, Osaka (IFO 50073). The cell line was subcultured in Eagle MEM medium (produced by Nissui Pharmaceutical Co., Ltd.) containing 10% (v/v) fetal bovine serum (FBS) until its growth terminated (cell aging) at 44.5 PDL, to yield aged normal human diploid fibroblasts (hereinafter aged cells). Aged cells growing on 25 petri dishes(10cm in diameter) at confluences of 75% or more were scraped using a cell scraper. After washing with Dulbecco's PBS (Dainippon Pharmaceutical), the cells were treated to extract an RNA fraction using an RNA extraction kit (Pharmacia-LKB), as directed in the kit protocol.

After 0.2 g of oligo-dT cellulose (type 3) (Collaborative Biomedical), previously swollen with 10 mM Tris-HCl buffer containing 0.1 M sodium chloride and 1 mM EDTA (pH 7.4) (TE buffer), was packed in a ECONO-COLUMN (17 mm in diameter, 15 cm in length) (Nippon Bio-Rad Laboratories), the column was equilibrated with TE

buffer containing 0.5 M sodium chloride. The RNA fraction, previously adjusted to a final concentration of 0.5 M sodium chloride, was heated at 65℃ for 5 minutes then immediately quenched in ice, after which it was applied to the equilibrated column. The coupled RNA fraction was eluted with TE buffer to yield an mRNA fraction (yield 115 μg).

Using a ZAP-cDNA synthesis kit (Stratagene), cDNA was synthesized by the method of Nojima et al. [Nojima, H., Development and Application of New Vector System in an Attempt to Catalog All Human cDNA Banks (Research Subject No. 02557098), 1992 Grant-in-Aid for Scientific Research from the Ministry of Education (Investigation B(1)) Final Report, p. 29 (1993)]. After 5 μg of mRNA was subjected to reverse transcription with an oligo-dT primer linker having an NotI recognition sequence (GCGGCCGC) as a template, to synthesize a first strand, RNase H and Escherichia coli DNA polymerase I were simultaneously reacted to remove the mRNA region and synthesize a second strand at the same time. Both ends of the thus-obtained double-stranded DNA were blunted using T4 DNA polymerase, followed by T4 DNA ligase action to bind a dephosphorylation BamHI adapter to both ends; NotI was then reacted to yield a cDNA fragment having a dephosphorylated BamHI site on the 5'-terminal side and a phosphorylated NotI site on the 3'-terminal side (yield about 5 μg).

Example 2 Modification of fission yeast expression vector

The following six DNA oligomers (SEQUENCE ID NOS. (1) to (6)) were synthesized.

(1) 5'-ACGCGTCCAGGATCCTGGTCGACGC-3' (SEQUENCE ID NO:1)
(2) 5'-GGCCGCCCTTTAGTGAGGGTTAA-3' (SEQUENCE ID NO:2)
(3) 5'-CGCGTCCCTATAGTGAGTCGTATTAC-3' (SEQUENCE ID NO:3)
(4) 5'-GGCGGCCGCGTCGACCAGGATCCTGGA-3' (SEQUENCE ID NO:4)
(5) 5'-GATCTTAACCCTCACTAAAG-3' (SEQUENCE ID NO:5)

(6) 5'-TCGAGTAATACGACTCACTATAGGG-3' (SEQUENCE ID NO:6)

DNA oligomers (1), (2), (4) and (5) were mixed in an amount of 10 μg each, followed by 5'-terminal phosphorylation by the action of T4 polynucleotide kinase. After the reaction mixture was kept standing at 65℃ for 15 minutes, DNA oligomers (3) and (6), 10 μg each, were added and ligated using T4 DNA ligase. The reaction mixture was subjected to 4% agarose electrophoresis to recover a 73 bp DNA fragment; the 5'-terminal was then phosphorylated by the action of T4 polynucleotide kinase. Next, to the fission yeast expression vector pREP1 [Maundrell, K., Gene, Vol. 123, p. 127 (1993)], previously cleaved at the SalI-BamHI site, the above 73 bp DNA fragment was ligated by the action of T4 DNA ligase to yield pTB1589. This plasmid is a fission yeast expression vector having a T7 RNA polymerase recognition sequence, MluI site, BamHI site, BstXI site, SalI site, NotI site, T3 RNA polymerase recognition sequence and nmt1 terminator in that order, and an LEU2 gene as a selection marker, downstream of the nmt1 promoter [Maundrell, K., Journal of Biological Chemistry, Vol. 265, p. 10857 (1989)].

Example 3 cDNA insersion into fission yeast expression vector pTB1589

The fission yeast expression vector prepared in Example 2 (pTB1589) was digested with NotI, treated with alkaline phosphatase, and further digested with BamHI, followed by 0.7% agarose gel electrophoresis to recover a vector fraction. 1 μg of the cDNA of aged normal human diploid fibroblast origin prepared in Example 1, and 100 ng of the above-described linearized vector, were ligated by the action of T4 DNA ligase; the ligation product was introduced into Escherichia coli MC1061 (electro-competent cell MC1061, Nippon Bio-Rad Laboratories) by electroporation using a Gene Pulser (Nippon Bio-Rad Laboratories). The cDNA library thus

obtained comprised 1.7 x 10^6 independent transformant cells of 1.5 kbp mean cDNA length. The plasmid was purified from the transformant, diluted with TE buffer to a concentration of 1μ g per 15μl, and stored at -20℃ until use.

Example 4 Transformation of fission yeast and screening for transformants showing cDNA-dependent growth inhibition

The reagents used to transform a fission yeast were prepared in accordance with the formulation of Moreno, S. et al. [Methods in Enzymology, Vol. 194, p. 795 (1991)] unless otherwise stated, with the same designations. Fission yeast cells (Schizosaccharomyces pombe h^- $leu1^-$) growing on YEA plate were inoculated to 100 ml of MB medium containing 0.25% (w/v) L-leucine at a density of 10^6 cells/ml, and cultured at 30℃ until the cell density reached 5 x 10^6 to 1 x 10^7 cells/ml. Cells were harvested at room temperature and washed with sterile water, after which they were suspended in a 0.1 M lithium acetate solution (pH 4.9-5.0) to 10^9 cells/ml. This suspension was dispensed to Eppendorf tubes at 100μl per tube, and kept standing at 30℃ for 1 hour. To each tube, 1μg (15μl) of the plasmid prepared in Example 5 and 290μl of a 50% (w/v) polyethylene glycol 4000 solution were added. After thorough mixing, the mixture was kept standing at 30℃ for 50 minutes, then heated at 43℃ for 15 minutes, then kept standing at room temperature for 10 minutes, followed by cell harvest. The cells were suspended in 1 ml of 1/2 YEL medium containing 0.25% (w/v) L-leucine; the suspension was then shaken at 30℃ for 1 to 2 hours. After dilution with 9 ml of 1/2 YEL medium, the suspension was applied to MMA plates containing 2μM thiamine (MMAT plates of medium that inhibits nmt1 promoter transcription) at 100μl per plate, and cultured at 30℃ for 2 days. The MMAT plates on which minute colonies appeared were

replicated to new MMA plates (medium that promotes the same promoter transcription as above) and MMAT plates, followed by culturing at 30℃ for 3 to 4 days. As a result of screening of 8.25×10^4 transformant cells by the above-described method, 18 transformants (candidate strains) that grow on MMAT plates but not on MMA plates were found (cell growth rate inhibiting is more than about 75%).

Example 5 Recovery of plasmid having cDNA from candidate strains

Each candidate strain was inoculated over the entire surface of an MMAT plate and cultured at 30℃ for 2 to 3 days. The cells on the plate were recovered into an Eppendorf tube using 1.5 ml of TES solution (TE buffer containing 10 mM sodium sulfite), followed by cell harvest. The cells were then suspended in 1 ml of a sorbitol solution (1M sorbitol, 100 mM EDTA, 10 mM sodium sulfite, 100 mM lithium acetate); the suspension was kept standing at 30℃ for 1 hour in the presence of 20 units of Lyticase (Boehringer). After centrifugal recovery, the protoplast was suspended in 300 l of 100 mM Tris-HCl buffer (pH 7.5) containing 0.5% (w/v) SDS and 50 mM EDTA, and kept standing at 65℃ for 1 hour. After the suspension was thoroughly mixed with 150µl of a 3 M potassium acetate solution, the mixture was kept standing on ice for 30 minutes. After centrifugation at 15,000 rpm at room temperature for 5 minutes, the supernatant was extracted with an equal amount of phenol/chloroform mixture (v/v = 1/1). This extraction was repeated 2 to 3 times; after addition of a 2-fold volume of ethanol, the supernatant was kept standing at -20℃ for not less than 4 hours, to precipitate a crude DNA fraction.

The crude DNA fraction was dissolved in 50µl of TE buffer; after addition of 100µl of a sodium iodide solution (GENECLEAN II Kit, BIO101 Company) and 5µl of a

glass milk suspension (provided with the kit), the mixture was kept standing at room temperature for 5 minutes. The glass milk fraction was centrifugally recovered, and washed with 3 portions of 400µl of an ice-cooled NEW solution (provided with the kit). The washed precipitate was treated with 10µl of TE buffer at 55℃ for 3 minutes; this operation was repeated in two cycles to yield a purified DNA fraction. Using 3µl of the extracted purified DNA fraction, Escherichia coli MC1061 was transformed by the electroporation method described in Example 3; the plasmid having cDNA was recovered from the resulting transformant.

Example 6 Analysis of candidate strains

The cDNA plasmids recovered from the 20 candidate strains were used to transform fission yeasts by the method described in Example 4. The resulting transformants were again replicated to MMA plates; 10 clones showing cDNA expression-dependent growth inhibition were found. For the cDNAs in plasmids of these reproducible clones, base sequences were determined using the Sequenase Ver. 2.0 DNA sequencing kit (Amersham Medical Ltd., US70777) with [^{35}S]dCTPS, in accordance with the kit protocol. The thus-obtained base sequences were examined for homology on the current DNA data base (GeneBank Release 84.0; 196703 entries); 4 new clones were found (Table 1). The plasmids harbored by the respective clones were designated pTB1617, pTB1668, pTB1671 and pTB1673, respectively; the entire base sequences of these cDNAs are shown in SEQUENCE ID NOS. 7, 8, 9 and 10, respectively.

The amino acid sequences of the polypeptides or proteins encoded by the cDNAs harbored by pTB1617, pTB1668, pTB1671 and pTB1673 are shown in SEQUENCE ID NOS. 11, 12, 13 and 14, respectively.

The expression plasmid of sdi-1 gene [WO9312251; Noda, A et. al., Experimental Cell Research, Vol. 211 p.

90-98 (1994)] was also constructed, in which sdi-1 gene was ligated downstream of the nmt1 promoter as shown in Example 3, and introduced into fission yeast. The yeast transformant thus obtained could form colonies on MMA plate as same as on MMAT plate (cell growth rate inhibiting is about 0%), indicating that sdi-1 type gene can not be obtained by the screening method using fission yeast as described.

[Table 1]

Plasmid	cDNA Length
pTB1617	0.6Kbp
pTB1668	0.9Kbp
pTB1671	0.6Kbp
pTB1673	1.6Kbp

Example 7 Analysis of candidate strains

The cDNA plasmids recovered from 10 candidate strains were used to transform fission yeasts by the method described in Example 4. The resulting transformants were again replicated to MMA plates; 3 clones showing cDNA expression-dependent growth inhibition were found. For the cDNA plasmids of these reproducible clones, base sequences were determined using the Sequenase Ver. 2.0 DNA sequencing kit (Amersham Medical Ltd., US70777) with [^{35}S]dCTPS, in accordance with the kit protocol. The thus-obtained base sequences were examined on the current DNA data base (GeneBank Release 86.0; 237775 entries); 1 new clone was found. The plasmid having the clone was designated pTB1848; the entire base sequence of its cDNA is shown in SEQUENCE ID NO. 16. The amino acid sequence of the protein encoded by the cDNA harbored by plasmid pTB1848 is shown in SEQUENCE ID NO. 15.

Example 8 Construction of animal cell expression plasmids

From plasmid pTB1697 obtained by introducing an E. coli lacZ gene into the HindIII site of pRc/CMV (Invitrogen, USA), a 4.9 kbp NruI-NaeI fragment was cut out and inserted into the SalI site of the plasmid pTB1695 prepared in Reference Example 1 to yield the plasmid pTB1698 (Figure 1). Next, the plasmids pTB1668, pTB1671, pTB1673 and pTB1848, obtained in Examples 6 and 7, were each cleaved with BamHI-NotI; the resulting cDNA portions were each inserted into the BglII site of pTB1698, located downstream of the SRα promoter, to yield animal cell expression plasmids.

Example 9 Determination of DNA synthesis inhibitory activity

The animal cells used to determine DNA synthesis inhibitory activity were normal diploid fibroblasts

(purchased from Cell System; defined primary human dermal fibroblast cell system, hereinafter Fb cells) at the growth stage, subcultured in 20-35 generations under the same culturing conditions as in Example 1. After being sown over Lab-Tek chamber slides (Nunc, USA) at 5 x 10^4 cells per plate and cultured at 37℃ for 1 day, Fb cells were transfected with the pTB1848-derived cDNA expression plasmid prepared in Example 8, by the calcium phosphate method [Chen, C. and Okayama, H., Molecular Cell Biology, Vol. 7, pp. 2745-2752 (1987)]. After the obtained transformant cells were cultured at 37℃ for 1 day, the medium was replaced with fresh one, followed by cultivation for 1 more day. Next, 37 KBq/ml (925 GBq/mmol) tritiated thymidine (^{3}H-thymidine) was added, followed by 48 hours of cultivation to label the cells. After glutaraldehyde fixation, the cells were stained with X-gal. After further fixation in methanol, an emulsion was applied; the plate was kept standing in a dark room for 4-5 days, followed by development. The blue-stained-galactosidase expression cells were counted under a microscope; the ratio of cells showing black particles in their nuclei due to ^{3}H-thymidine uptake was determined.

DNA synthesis inhibitory rates (%) were calculated with the labeling index, taking plasmid pTB1698 as 0%. For positive control, Fb cells were used which had been transformed with the expression plasmid pTB1699 constructed by introducing the sdi-1 gene [Noda, A. et al., Experimental Cell Research, Vol. 211, pp. 90-98 (1994)] into the BglII site of pTB1698 in accordance with the method described in Example 8. The results are shown in Table 2.

As shown in Table 2, the cells incorporating the pTB1848-derived cDNA underwent thymidine uptake inhibition in 3 experiments, as with the positive control.

[Table 2]

Ex.	Clone	Number of Labeled Nuclei per Blue strained Cell		Labeling Index (%)	DNA Synthesis Inhibitory Rates (%)
1	pTB1698	77/257	56/178	30.8	0
	pTB1699	59/229	26/151	21.5	30.1
	pTB1848	29/134	26/110	22.6	26.5
2	pTB1698	59/126	63/133	47.1	0
	pTB1699	62/225	56/139	34.0	27.9
	pTB1848	50/130	32/96	35.9	23.8
3	pTB1698	73/143	89/170	51.7	0
	pTB1699	49/161	60/145	35.9	30.6
	pTB1848	36/88	29/66	42.4	18.0

Example 10 Analysis of candidate strains

The plasmids having cDNA recovered from 25 candidate strains were used to transform fission yeasts by the method described in Example 4. The resulting transformants were again replicated to MMA plates; 10 clones showing cDNA expression-dependent growth inhibition were found. For the cDNA in plasmids of these reproducible clones, base sequences were determined using the Sequenase Ver. 2.0 DNA sequencing kit (Amersham Medical Ltd., US70777) with [^{35}S]dCTPS, in accordance with the kit protocol. The thus-obtained base sequences were examined on the current DNA data base (GeneBank Release 86.0; 237775 entries); 5 new clones were found (Table 3). The plasmids having the clones were designated as pTB1618, pTB1689, pTB1756, pTB1761 and pTB1721; the entire base sequences of their cDNAs are shown in SEQUENCE ID NOS. 17, 18, 19, 20 and 21, respectively. The amino acid sequences of the polypeptides or proteins encoded by the cDNAs harbored by the plasmids pTB1618 and pTB1761 are shown in SEQUENCE ID NOS. 22 and 23, respectively.

[Table 3]

Plasmid	cDNA Length
pTB1618	2.5Kbp
pTB1689	1.1Kbp
pTB1756	0.7Kbp
pTB1761	1.9Kbp
pTB1721	0.5Kbp

Example 11 Determination of DNA synthesis inhibitory activity

Expression plasmids for animal cell were constructed by cleaving the plasmids pTB1618, pTB1689, pTB1721, pTB1756 or pTB1761 obtained in Example 10 with BamHI-NotI, and introducing into the BglII site of pTB1698 each of the obtained cDNA portions by the method described in Example 8. Using these cDNA expression plasmids, DNA synthesis inhibitory activity was determined by the method described in Example 9. As shown in Table 4, the cells incorporating the pTB1689-derived cDNA underwent thymidine uptake inhibition in 3 experiments, as with the positive control. Similarly, all cells incorporating pTB1618, pTB1721, pTB1756 or pTB1761 underwent thymidine uptake inhibition in 3 experiments, as shown in Table 5.

[Table 4]

Ex.	Clone	Number of Labeled Nuclei per Blue strained Cell		Labeling Index (%)	DNA Synthesis Inhibitory Rates (%)
1	pTB1698	77/257	56/178	30.8	0
	pTB1699	59/229	26/151	21.5	30.1
	pTB1689	42/224	44/161	23.1	25.0
2	pTB1698	59/126	63/133	47.1	0
	pTB1699	62/225	56/139	34.0	27.9
	pTB1689	55/178	71/170	36.4	22.8
3	pTB1698	73/143	89/170	51.7	0
	pTB1699	49/161	60/145	35.9	30.6
	pTB1689	21/85	31/88	30.0	42.1

[Table 5]

Ex.	Clone	Number of Labeled Nuclei per Blue strained Cell		Labeling Index (%)	DNA Synthesis Inhibitory Rates (%)
1	pTB1698	114/219	84/168	51.0	0
	pTB1699	101/311	83/237	33.8	34.3
	pTB1618	56/157	44/141	33.5	34.3
	pTB1721	87/245	62/188	34.3	32.7
	pTB1756	64/184	72/195	35.9	29.6
	pTB1761	76/239	52/199	29.0	43.1
2	pTB1698	140/300	100/264	42.3	0
	pTB1699	83/325	63/274	24.3	42.6
	pTB1618	71/197	65/201	34.2	19.1
	pTB1721	52/160	51/142	34.2	19.1
	pTB1756	74/214	53/145	35.6	15.8
	pTB1761	76/225	76/190	36.9	12.8
3	pTB1698	156/386	91/287	36.1	0
	pTB1699	69/281	78/290	25.8	28.6
	pTB1721	58/181		32.0	11.2
	pTB1756	68/221	43/184	27.1	24.8
	pTB1761	42/141	27/123	25.9	28.2
4	pTB1698	43/105	54/102	47.0	0
	pTB1699	54/177	47/181	28.3	39.8
	pTB1618	37/132	29/120	26.1	44.4

Example 12 Analysis of candidate strains

The plasmids having cDNA recovered from 22 candidate strains were used to transform fission yeasts by the method described in Example 4. The resulting transformants were again replicated to MMA plates; 9 clones showing cDNA expression-dependent growth inhibition were found. For the cDNA in plasmids of these reproducible clones, base sequences were determined using the Sequenase Ver. 2.0 DNA sequencing kit (Amersham Medical Ltd., US70777) with [^{35}S]dCTPS, in accordance with the kit protocol. The thus-obtained base sequences were examined on the current DNA data base (GeneBank Release 86.0; 237775 entries); 3 new clones were found (Table 6). The plasmids having the clones were designated as pTB1786, pTB1810 and pTB1819; the entire base sequences of their cDNAs are shown in SEQUENCE ID NOS. 24, 25 and 26, respectively. The amino acid sequence of the polypeptide or protein encoded by the cDNA harbored by the plasmid pTB1810 is shown in SEQUENCE ID NO. 27.

[Table 6]

Plasmid	cDNA Length
pTB1786	0.4Kbp
pTB1810	1.4Kbp
pTB1819	0.6Kbp

Example 13 Determination of DNA synthesis inhibitory activity

Expression plasmids for animal cell were constructed by cleaving the plasmids pTB1786, pTB1810 or pTB1819 obtained in Example 12 with BamHI-NotI, and introducing into the BglII site of pTB1698 each of the obtained cDNA portions by the method described in Example 8. Using these cDNA expression plasmids, DNA synthesis inhibitory activity was determined by the method described in Example 9. As shown in Tables 7 and 8, the cells incorporating the pTB1689-derived cDNA underwent thymidine uptake inhibition in more than 2 experiments, as with the positive control.

[Table 7]

Ex.	Clone	Number of Labeled Nuclei per Blue strained Cell		Labeling Index (%)	DNA Synthesis Inhibitory Rates (%)
1	pTB1698	60/88	38/72	60.5	0
	pTB1699	12/25	11/22	49.0	19.0
	pTB1786	9/17	10/23	48.2	20.3
	pTB1810	23/52	31/62	47.1	22.1
2	pTB1698	74/156	33/89	42.3	0
	pTB1699	36/143	28/126	23.7	44.0
	pTB1786	24/73	36/116	32.0	24.5
	pTB1819	18/58	24/82	30.2	28.7
3	pTB1698	48/132	35/118	33.0	0
	pTB1699	24/72	29/105	30.0	9.0
	pTB1786	35/127	38/135	27.9	15.6
	pTB1810	28/89	23/93	26.4	20.0
	pTB1819	22/79	21/80	27.0	18.0

[Table 8]

Ex.	Clone	Number of Labeled Nuclei per Blue strained Cell		Labeling Index (%)	DNA Synthesis Inhibitory Rates (%)
1	pTB1698	60/161		37.3	0
	pTB1699	33/130	46/176	25.8	30.9
	pTB1810	14/71	17/64	23.1	38.3

SEQUENCE LISTING

INFORMATION FOR SEQ ID NO:1

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 25

(B) TYPE : Nucleic acid

(C) STRANDEDNESS : Single

(D) TOPOLOGY : Linear

(ii) MOLECULE TYPE : Other nucleic acid (synthetic DNA)

(iv) ANTI-SENSE : No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ACGCGTCCAG GATCCTGGTC GACGC 25

INFORMATION FOR SEQ ID NO:2

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 23

(B) TYPE : Nucleic acid

(C) STRANDEDNESS : Single

(D) TOPOLOGY : Linear

(ii) MOLECULE TYPE : Other nucleic acid (synthetic DNA)

(iv) ANTI-SENSE : No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GGCCGCCCTT TAGTGAGGGT TAA 23

INFORMATION FOR SEQ ID NO:3

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 26

(B) TYPE : Nucleic acid

(C) STRANDEDNESS : Single

(D) TOPOLOGY : Linear

(ii) MOLECULE TYPE : Other nucleic acid (synthetic DNA)

(iv) ANTI-SENSE : No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CGCGTCCCTA TAGTGAGTCG TATTAC 26

INFORMATION FOR SEQ ID NO:4

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 27

(B) TYPE : Nucleic acid

(C) STRANDEDNESS : Single

(D) TOPOLOGY : Linear

(ii) MOLECULE TYPE : Other nucleic acid (synthetic DNA)

(iv) ANTI-SENSE : Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GGCGGCCGCG TCGACCAGGA TCCTGGA 27

INFORMATION FOR SEQ ID NO:5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 20

(B) TYPE : Nucleic acid

(C) STRANDEDNESS : Single

(D) TOPOLOGY : Linear

(ii) MOLECULE TYPE : Other nucleic acid (synthetic DNA)

(iv) ANTI-SENSE : Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GATCTTAACC CTCACTAAAG 20

INFORMATION FOR SEQ ID NO:6

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 25

(B) TYPE : Nucleic acid

(C) STRANDEDNESS : Single

(D) TOPOLOGY : Linear

(ii) MOLECULE TYPE : Other nucleic acid (synthetic DNA)

(iv) ANTI-SENSE : Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TCGAGTAATA CGACTCACTA TAGGG 25

INFORMATION FOR SEQ ID NO:7

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 637

(B) TYPE : Nucleic acid

(C) STRANDEDNESS : Single

(D) TOPOLOGY : Linear

(ii) MOLECULE TYPE : cDNA

(iv) ANTI-SENSE : No

(vi) ORIGINAL SOURCE :

(A) ORGANISM : Human

(F) TISSUE TYPE : Lung

(G) CELL TYPE : Fibroblast

(H) CELL LINE : MRC-5

(ix) FETURE :

(A) NAME KEY : CDS

(B) LOCATION : 248..448

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GAATTCAGAT CCCCGGGGAG CTTCTGCCAG GGGTGGATGT ACTCCTGGAG GTGTTCCCTA 60
CCTGTTCGGT GGAGCAGGCC CAGTGGGTGC TGGCCAAAGC TCGGGGGGAC TTGGAAGAAG 120
CTGTGCAGAT GCTGGTAGAG GGAAAGGAAG AGGGCCTGCA GCCTGGGAGG GCCCCAACCA 180
GGACCTGCCC AGACGCCTCA GAGGCCCCCA AAAGGATGAG CTGAAGTCCT TCATCCTGCA 240
GAAGTACATG ATGGTGGATA GCGCAGAGGA TCAGAAGATT CACCGGCCCA TGGCTCCCAA 300
GGAGGCCCCC AAGAAGCTGA TCCGATACAT CGACAACCAG GTAGTGAGCA CCAAAGGGGA 360
GCGATTCAAA GATGTGCGGA ACCCTGAGGC CGAGGAGATG AAGGCCACAT ACATCAACCT 420
CAAGCCAGCC AGAAAGTACC GCTTCCATTG AGGCACTCGC CGGACTCTGC CCGAGCCTTC 480
TAGGCTCAGA TCCCAGAGGG ATGCAGGAGC CCTATACCCC TACACAGGGG CCCCCTAACT 540
CCTGTCCCCC TTCTCTACTC CTTTGCTCCA TAGTGTTAAC CTACTCTCGG AGCTGCCTCC 600
ATGGGCACAG TAAAGGTGGC CCAAGGAAGG TGAAAAA 637

INFORMATION FOR SEQ ID NO:8

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 874

(B) TYPE : Nucleic acid

(C) STRANDEDNESS : Single

(D) TOPOLOGY : Linear

(ii) MOLECULE TYPE : cDNA

(iv) ANTI-SENSE : No

(vi) ORIGINAL SOURCE :

(A) ORGANISM : Human

(F) TISSUE TYPE : Lung

(G) CELL TYPE : Fibroblast

(H) CELL LINE : MRC-5

(ix) FETURE :

(A) NAME KEY : CDS

(B) LOCATION : 279..752

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
GGATCCCCGG CCCGGGAAAA ATCGCAGCCC TCAGAGAGTC GCTGGCTGAA GTATCTAGAA   60
AAGGACTCCC AAGAACTGGA GCTGGAAGGA ACAGGAGTGT GTTTCAGCAA ACAGCCTTCA  120
TCCAAAATGG AGGAGCCAGG CCCCCGCTTC AGTCAAGACC TGCCTAGAAA AAGGAAGTGG  180
AGCGGGAGCA CCGTCCAGCC TCCGTGCAGC CGTGGCGTGC AGGACTCGGG TGGCTCTGAG  240
GTCGCCTGGG GACCCCAGAA GGGACAGGCT GGCCTGACAT GGAAGGTGAA ACAAGCAGCA  300
GCCCCTGCCT TCAGGAGAAC TCTGCAGACT GCAGTGCCGG GGAGCTGAGG GGTCCTGGGA  360
AGGAGCTATG GAGTCCCATC CAGCAGGTTA CAGCCACATC CTCTAAATGG GCGCGATTTG  420
TCCTGCCACC TAGAAAAAGT TCACATGTGG ACAGTGAGCA GCCAAGGTCT CTTCAGAGGG  480
ACCCCAGGCC AGCTGGTCCA GCACAGGCTA AGCAAGGGAC CCCCAGAGCA CAGGCCTCAA  540
GAGAAGGCCT CAGCAGGCCC ACTGCCGCTG TCCAGCTTCC TCGGGCCACA CACCCCGTCA  600
CATCTGGGTC TGAGAGGCCT TGCGGGAAGA CCTCATGGGA CGCAAGGACT CCCTGGGCAG  660
AGGGTGGGCC CCTGGTCCTG GAGGCACAGA ATCCTCGACC CACACGACTA TGTGACCTCT  720
TTATAACTGG GGAAGACTTC GATGATGATG TGTGATCTGG GACTGGCAGG TTATTAATCG  780
AGATACACTT GTTAGGAGGG ACAGGGTTCC CCTAAGGCAC TTTTAAAGAT ACTCTGTAAG  840
AACCATTAAC AATAAACTTA CTGTCAATCA AAAA                               874
```

INFORMATION FOR SEQ ID NO:9

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 640

(B) TYPE : Nucleic acid

(C) STRANDEDNESS : Single

(D) TOPOLOGY : Linear

(ii) MOLECULE TYPE : cDNA

(iv) ANTI-SENSE : No

(vi) ORIGINAL SOURCE :

(A) ORGANISM : Human
(F) TISSUE TYPE : Lung
(G) CELL TYPE : Fibroblast
(H) CELL LINE : MRC-5

(ix) FETURE :
(A) NAME KEY : CDS
(B) LOCATION : 201..377

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTGGAAGGAC CTGCTGCTTT GCAGACCCAT GTATATATCC AGAAACCAAT CGGAACTCAG 60
GGTTACACTG ATTCCCTTTT GAGTATAATC TGTGCCATGA AGAAGGGGAT TTATTTGAGG 120
GAGGGACTTT TCTTCACCTG CACTCCTTTT ATTTTATTTT CCTATGTTTA GTTTTCTTTG 180
GAGTTGAACA GCTAGGCTGA ATGAGATTAA AGTTTTCCAA ACACACATGG CAGTATGGAG 240
GTTTTATGAA AAGTGATGGT GAAGAGTTGG GAGAGATGGA GGAAAAAAAA TGCAGTCAGA 300
AGTTTCAGAA CAAATACACA AAATCCTATG TTAGTTTGAA TCTTTATTTT TCTGGCACAC 360
TTTTAAAAGG GCTGTATTAA AATAGTGATT TTTTTTTTTT TTGCCTCAGG GAACCTCAGT 420
CAACAGGAAT ACCTCTGTTT CTAACCTAGA GAATAATATT GTGAAAATTG CTTTGTTAAT 480
TTTTTTTCCT CAGGAATAAT TTTCTCTTTT GGAAAGCACT TTCCCCGTCT CAGTAGAAAA 540
GTCTAGCAGT TGTAACTTCT TGTTTCTTAT TTGCTTTGGG GGAAATCAAA GAAAACAGAC 600
GGTGAAGGAA AGGGTGGGAA AAATTAAGTC TCATGAAAAA 640

INFORMATION FOR SEQ ID NO:10

(i) SEQUENCE CHARACTERISTICS :
(A) LENGTH : 1560
(B) TYPE : Nucleic acid
(C) STRANDEDNESS : Single
(D) TOPOLOGY : Linear

(ii) MOLECULE TYPE : cDNA

(iv) ANTI-SENSE : No

(vi) ORIGINAL SOURCE :
(A) ORGANISM : Human
(F) TISSUE TYPE : Lung
(G) CELL TYPE : Fibroblast
(H) CELL LINE : MRC-5

(ix) FETURE :
(A) NAME KEY : CDS
(B) LOCATION : 296..1000

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGATCCCCGC GGACGAGGTG GCCGCGGCGG GGCAGCTGGG CCGCCAGCTT GGTGCCTCGG 60
GGACCGTCTC CCGCTGCTTT GGTCACCAGC CCCTGCCCGC CCGACCCGCT CCGTTCTCCG 120
GCCTGCGAGC CCTGCCGGCC GGACTTTGCG CCGCGTCCGG GCTGCTGCTG CGCTCGGGGC 180
CCCGCTCGGC GCCGGCGGTG ACCGGGAAGC CCGCGTTAAA GGGGCAACCG GGACCCTGGC 240
CCGGTATGGC TGAAGTCAGC ATCGACCAGT CCAAGCTGCC TGGAGTCAAG GAAGTATGCC 300
GAGATTTTGC TGTCCTGGAG GACCACACCC TGCTCACAGC CTGCAGGAAC AAGAGATTGA 360
GCATCATTTG GCATCGAACG TTCAGCGGAA CCGTTTGGTC CAGCATGATC TCCAGGTGGC 420
TAAGCAGCTC CAAGAGGAAG ATCTGAAAGC GCAGGCCCAG CTCCAGAAGC GTTACAAAGA 480
CCTTGAACAA CAAGACTGTG AAATTGCTCA GGAAATTCAG GAGAAGCTGG CTATTGAGGC 540
AGAGAGACGA CGCATTCAGG AGAAGAAGGA TGAGGACATA GCTCGCCTTT TGCAAGAAAA 600
GGAGTTACAG GAAGAGAAAA AGAGAAAGAA ACACTTTCCA GAGTTCCCTG CAACCCGTGC 660
TTATGCAGAT AGTTACTATT ATGAAGATGG AGGAATGAAG CCAAGAGTGA CGAAAGAAGC 720
TGTATCTACT CCATCACGAA TGGCCCACAG GGATCAGGAA TGGTATGATG CTGAAATTGC 780
CAGAAAACTG CAAGAAGAAG AACTTTTGGC TACCCAGGTG GACATGAGAG CCGCTCAAGT 840
AGCTCAAGAT GAAGAAATCG CTCGACTTCT AATGGCTGAA GAAAAGAAAG CTTACAAAAA 900
AGCCAAGGAG CGGGAGAAAT CATCTTTGGA CAAAAGAAAG CAAGACCCCG AGTGGAAGCC 960
AAAAACAGCT AAAGCAGCAA ATCAAAGTCA AAAGAGAGTA TGAACCTCAC CATTCTAAGA 1020
ATGAAAGGCC AGCACGGCCA CCACCACCTA TCATGACAGA TGGTGCAAGA TGCGGTACAC 1080
TCATTTTACA AACCAGCAGA GTTCCACACG GCATTTCTCA AAATCAGAGT CCTCTCATAA 1140
AGGTTTCATC ACAAACATTA AAAACCTAGG AATCTGCCTT GAAAATGGAC TCACTATAGC 1200
AAATATTACT GGGTGATACA GAATGAATTC TACACTTACT TTTTTTCTCC TGTGTTTGCA 1260
TGGCCTGGGA TTTACTCCTC AAGTGTCATT TCTGAACCAT AAGTAATTTT AATTCATTTC 1320
AAATGTTTTG GTTATTCATG ATCACTTGGG CAGTATAAGA AAATGTAGCT TCTGAATATT 1380
GGCCACCTCT ATGCTGCATA TACTTCTTGG GATATAGTAT CTAAGCCTTG TAAACTGCCA 1440
TTTGTTAGGT ATGGAGTTTG GTATCTAGGG AGTAGGCCTT ATTTAGCAAT TCAAATTTTA 1500
TGGAGATGAA TGATCAAAGT GAAACAATGT TTGGATGCAA CGCAGAATAA AAGAATATAA 1560

INFORMATION FOR SEQ ID NO:11

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 67

(B) TYPE : Amino acid

(ii) MOLECULE TYPE : Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Met Met Val Asp Ser Ala Glu Asp Gln Lys Ile His Arg Pro Met Ala
                5                  10                  15
Pro Lys Glu Ala Pro Lys Lys Leu Ile Arg Tyr Ile Asp Asn Gln Val
            20                  25                  30
Val Ser Thr Lys Gly Glu Arg Phe Lys Asp Val Arg Asn Pro Glu Ala
        35                  40                  45
Glu Glu Met Lys Ala Thr Tyr Ile Asn Leu Lys Pro Ala Arg Lys Tyr
    50                  55                  60
Arg Phe His
```

INFORMATION FOR SEQ ID NO:12

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 158

(B) TYPE : Amino acid

(ii) MOLECULE TYPE : Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Met Glu Gly Glu Thr Ser Ser Ser Pro Cys Leu Gln Glu Asn Ser Ala
                5                  10                  15
Asp Cys Ser Ala Gly Glu Leu Arg Gly Pro Gly Lys Glu Leu Trp Ser
            20                  25                  30
Pro Ile Gln Gln Val Thr Ala Thr Ser Ser Lys Trp Ala Arg Phe Val
        35                  40                  45
Leu Pro Pro Arg Lys Ser Ser His Val Asp Ser Glu Gln Pro Arg Ser
    50                  55                  60
Leu Gln Arg Asp Pro Arg Pro Ala Gly Pro Ala Gln Ala Lys Gln Gly
65                  70                  75                  80
Thr Pro Arg Ala Gln Ala Ser Arg Glu Gly Leu Ser Arg Pro Thr Ala
                85                  90                  95
Ala Val Gln Leu Pro Arg Ala Thr His Pro Val Thr Ser Gly Ser Glu
            100                 105                 110
Arg Pro Cys Gly Lys Thr Ser Trp Asp Ala Arg Thr Pro Trp Ala Glu
        115                 120                 125
Gly Gly Pro Leu Val Leu Glu Ala Gln Asn Pro Arg Pro Thr Arg Leu
    130                 135                 140
Cys Asp Leu Phe Ile Thr Gly Glu Asp Phe Asp Asp Asp Val
145                 150                 155
```

INFORMATION FOR SEQ ID NO:13

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 59

(B) TYPE : Amino acid

(ii) MOLECULE TYPE : Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Met Arg Leu Lys Phe Ser Lys His Thr Trp Gln Tyr Gly Gly Phe Met
                5                  10                  15
Lys Ser Asp Gly Glu Glu Leu Gly Glu Met Glu Glu Lys Lys Cys Ser
            20                  25                  30
Gln Lys Phe Gln Asn Lys Tyr Thr Lys Ser Tyr Val Ser Leu Asn Leu
        35                  40                  45
Tyr Phe Ser Gly Thr Leu Leu Lys Gly Leu Tyr
    50                  55
```

INFORMATION FOR SEQ ID NO:14

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 235

(B) TYPE : Amino acid

(ii) MOLECULE TYPE : Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Met Pro Arg Phe Cys Cys Pro Gly Gly Pro His Pro Ala His Ser Leu
                5                  10                  15
Gln Glu Gln Glu Ile Glu His His Leu Ala Ser Asn Val Gln Arg Asn
            20                  25                  30
Arg Leu Val Gln His Asp Leu Gln Val Ala Lys Gln Leu Gln Glu Glu
        35                  40                  45
Asp Leu Lys Ala Gln Ala Gln Leu Gln Lys Arg Tyr Lys Asp Leu Glu
    50                  55                  60
Gln Gln Asp Cys Glu Ile Ala Gln Glu Ile Gln Glu Lys Leu Ala Ile
65                  70                  75                  80
Glu Ala Glu Arg Arg Arg Ile Gln Glu Lys Lys Asp Glu Asp Ile Ala
                85                  90                  95
```

```
Arg Leu Leu Gln Glu Lys Glu Leu Gln Glu Glu Lys Lys Arg Lys Lys
            100                 105                 110
His Phe Pro Glu Phe Pro Ala Thr Arg Ala Tyr Ala Asp Ser Tyr Tyr
        115                 120                 125
Tyr Glu Asp Gly Gly Met Lys Pro Arg Val Thr Lys Glu Ala Val Ser
    130                 135                 140
Thr Pro Ser Arg Met Ala His Arg Asp Gln Glu Trp Tyr Asp Ala Glu
145                 150                 155                 160
Ile Ala Arg Lys Leu Gln Glu Glu Glu Leu Leu Ala Thr Gln Val Asp
                165                 170                 175
Met Arg Ala Ala Gln Val Ala Gln Asp Glu Glu Ile Ala Arg Leu Leu
            180                 185                 190
Met Ala Glu Glu Lys Lys Ala Tyr Lys Lys Ala Lys Glu Arg Glu Lys
        195                 200                 205
Ser Ser Leu Asp Lys Arg Lys Gln Asp Pro Glu Trp Lys Pro Lys Thr
    210                 215                 220
Ala Lys Ala Ala Asn Gln Ser Gln Lys Arg Val
225                 230                 235
```

INFORMATION FOR SEQ ID NO:15

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 230

(B) TYPE : Amino acid

(ii) MOLECULE TYPE : Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
Met Val Lys Leu Phe Ile Gly Asn Leu Pro Arg Glu Ala Thr Glu Gln
                5                   10                  15
Glu Ile Arg Ser Leu Phe Glu Gln Tyr Gly Lys Val Leu Glu Ser His
            20                  25                  30
Ile Ile Lys Asn Tyr Arg Phe Val His Ile Glu Asp Lys Thr Ala Ala
        35                  40                  45
Glu Asp Ala Ile Arg Asn Leu His His Tyr Lys Leu His Gly Val Asn
    50                  55                  60
Ile Asn Val Glu Ala Ser Lys Asn Lys Ser Lys Thr Ser Thr Lys Leu
65                  70                  75                  80
```

```
His Val Gly Asn Ile Ser Pro Thr Cys Thr Asn Lys Glu Leu Arg Ala
                85                  90                  95
Lys Phe Glu Glu Tyr Gly Pro Val Ile Glu Cys Asp Ile Val Lys Asp
            100                 105                 110
Tyr Ala Phe Val His Met Glu Arg Ala Glu Asp Ala Val Glu Ala Ile
        115                 120                 125
Arg Gly Leu Asp Asn Thr Glu Phe Gln Gly Lys Arg Met His Val Gln
    130                 135                 140
Leu Ser Thr Ser Arg Leu Arg Thr Ala Pro Gly Met Gly Asp Gln Ser
145                 150                 155                 160
Gly Cys Tyr Arg Cys Gly Lys Glu Gly His Trp Ser Lys Glu Cys Pro
                165                 170                 175
Ile Asp Arg Ser Gly Arg Val Ala Asp Leu Thr Glu Gln Tyr Met Ser
            180                 185                 190
Asn Thr Glu Gln Cys Val Pro Leu His His Glu Leu Trp Gly Phe Ile
        195                 200                 205
Val Leu Gln Gln Arg Val Arg Ser Ala Arg Cys Leu Leu Gln Ala Leu
    210                 215                 220
Pro Cys Gly Ala Val Leu
225             230
```

INFORMATION FOR SEQ ID NO:16

(i) SEQUENCE CHARACTERISTICS :

- (A) LENGTH : 1585
- (B) TYPE : Nucleic acid
- (C) STRANDEDNESS : Single
- (D) TOPOLOGY : Linear

(ii) MOLECULE TYPE : cDNA

(iv) ANTI-SENSE : No

(vi) ORIGINAL SOURCE :

- (A) ORGANISM : Human
- (F) TISSUE TYPE : Lung
- (G) CELL TYPE : Fibroblast
- (H) CELL LINE : MRC-5

(ix) FETURE :

- (A) NAME KEY : CDS
- (B) LOCATION : 51..740

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGATCCCCGG GGAGGAGGCC CTGCTGGTTT CTGTGCGGGC TCTTGTCAGG ATGGTGAAGC 60
TGTTCATCGG AAACCTGCCC CGGGAGGCTA CAGAGCAGGA GATTCGCTCA CTCTTCGAGC 120
AGTATGGGAA GGTGCTGGAA TCTCACATCA TTAAGAATTA CCGCTTTGTG CACATAGAAG 180
ACAAGACGGC AGCTGAGGAT GCCATACGCA ACCTGCACCA TTACAAGCTT CATGGGGTGA 240
ACATCAACGT GGAAGCCAGC AAGAATAAGA GCAAAACCTC AACAAAGTTG CATGTGGGCA 300
ACATCAGTCC CACCTGCACG AATAAGGAGC TTCGAGCCAA GTTTGAGGAG TATGGTCCGG 360
TCATCGAATG TGACATCGTG AAAGATTATG CCTTCGTACA CATGGAGCGG GCAGAGGATG 420
CAGTGGAGGC CATCAGGGGC CTTGATAACA CAGAGTTTCA AGGCAAACGA ATGCACGTGC 480
AGTTGTCCAC CAGCCGGCTT AGGACTGCGC CCGGGATGGG AGACCAGAGC GGCTGCTATC 540
GGTGCGGGAA AGAGGGGCAC TGGTCCAAAG AGTGTCCGAT AGATCGTTCA GGCCGCGTGG 600
CAGACTTGAC CGAGCAATAT ATGAGCAATA CGGAGCAGTG CGTACCCTTA CACCATGAGC 660
TATGGGGATT CATTGTATTA CAACAACGCG TACGGAGCGC TCGATGCCTA CTACAAGCGC 720
TGCCGTGCGG CGCGGTTCTA TGAGGCAGTG GCAGCTGCAG CCTCCGTGTA TAATTACGCA 780
GAGCAGACCC TGTCCCAGCT GCCACAAGTC CAGAATACAG CCATGGCCAG TCACCTCACC 840
TCCACCTCTC TCGATCCCTA CGATAGACAC CTGTTGCCGA CCTCAGGAGC TGCTGCCACA 900
CTGCGTGCTC GGACGAGCCG CTGCTGCTGT TACTGCAGCT TCCACTTCAT ATACGGGCGG 960
GATCGGAGCC CCCTCGTCCC TACAGCCCCA GTCCCCACTG TTGGAGAGGG CTACGGTTAC 1020
GGGCATGAGA GTGAGTTGTC CCAAGCTTCA GCAGCCGCGC GGAATTCTCT GTACGACATG 1080
GCCCGGTATG AGCGGGAGCA GTATGCCGAT CGGGCGCGGT ACTCAGCCTT TTAAAGCTTG 1140
AGGTGGGATG TGTGTGGGCT GAAATTCCGA GCTGCGGTTG TGCATGAGAA TCACCCTTCG 1200
TGGTACCCCA TCTTCGGGAC GTTCTCGGCT CTGTGCGTTC AGTCCCTCAG GAACCGTGGA 1260
CCTTAATTTA CCTTGCTAAG TTCAGACCTT CTCTTCCTTT CCTTTCCTTT CCTCTCCTGC 1320
CCATTTTCCT GTTCTTCTGT CCTCCAATAC TTCTGTAGCT ACCCATTCAT GTTCTCTTCT 1380
CCCAGAAGGC CTCATTGTGT GCAGAAACTG TGGTGGGGGC TGTGCTGTCT CCTCCCTGCC 1440
TCCTGCCTCT GCGGCTGTTG GATTTGGGAA TGACCTTGGT GAGAGTCTCA CTGCTCCAGG 1500
GTCTCTTTTT GGTCCAAAGG CTAGACCTAT AGAGTTGGAT CACTCTTTTT CTTTCCGGTG 1560
AGATAAATGG TTTTTCAACT TAAAA 1585

INFORMATION FOR SEQ ID NO:17

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 2500

(B) TYPE : Nucleic acid

(C) STRANDEDNESS : Single

(D) TOPOLOGY : Linear

(ii) MOLECULE TYPE : cDNA

(iv) ANTI-SENSE : No

(vi) ORIGINAL SOURCE :

(A) ORGANISM : Human

(F) TISSUE TYPE : Lung

(G) CELL TYPE : Fibroblast

(H) CELL LINE : MRC-5

(ix) FETURE :

(A) NAME KEY : CDS

(B) LOCATION : 1062..1736

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GGATCCCCGG GAACAGCGGC TCCGGGCCGC GCCGTCGCTG CTGCTGCTGC TGCTGTGGCT 60
GCTCGCGGTT CCCGGCGCTA ACGGGCCCCG GGTCGGCGCT CTATTCGCCT TCCGACCCGC 120
TGACGCTGCT GCAGGCGGAC ACGGTGCGCG GCGGGTGCTG GGCTCCCGCA GCGCCTGGGC 180
CGTGGAGTTC TTCCCCTCCT GGTGCGGCCA CTGCATCGCC TTCGCCCCGA CGTGGAAGGC 240
GCTGGCCGAA GACGTCAAAG CCTGGAGGCC GGCCCTGTAT CTCGCCGCCC TGGACTGTGC 300
TGAGGAGACC AACAGTGCAG TCTGCAGAGA CTTCAACATC CCTGGCTTCC CGACTGTGAG 360
GTTCTTCAAG GCCTTTACCA AGAACGGCTC GGGAGCAGTA TTTCCAGTGG CTGGTGCTGA 420
CGTGCAGACG CTGCGGGAGA GGCTCATTGA CGCCCTGGAG TCCATCATGA CACGTGGCCC 480
CCAGCCTGTC CCCCACTGGA GCCTGCCAAG CTGGAGGAGA TTGATGGATT CTTTGCGAGA 540
AATAACGAAG AGTACCTGGC TCTGATCTTT GAAAAGGGAG GCTCCTACCT GGGTAGAGAG 600
GTGGCTCTGG ACCTGTCCCA GCACAAAGGC GTGGCGGTGC GCAGGGTGCT GAACACAGAG 660
GCCCAATGTG GTGAGAAAGT TTGGTGTCAC CGACTTCCCC TCTTGCTACC TGCTGTTCCG 720
GAATGGCTCT GTCTCCCGAG TCCCCGTGCT CATGGAATCC AGGTCCTTCT ATACCGCTTA 780
CCTGCAGAGA CTCTCTGGGC TCACCAGGGA GGCTGCCCAG ACCACAGTTG CACCAACCAC 840
TGCTAACAAG ATAGCTCCCA CTGTTTGGAA ATTGGCAGAT CGCTCCAAGA TCTACATGGC 900
TGACCTGGAA TCTGCACTGC ACTACATCTG CGGATAGAAG TGGGCAGGTT CCCGGTCCTG 960
GAAGGGCAGC GCCTGGTGGC CCTGAAAAAG TTTGTGGCAG TGCTGGCCAA GTATTTCCCT 1020
GGCCGGCCCT TAGTCCAGAA CTTCCTGCAC TCCGTGAATG AATGGCTCAG AGGCAGAAGA 1080
GAAATAAAAT TCCCTACAGT TTCTTTAAAA CTGCCCTGGA CGACAGGAAA GAGGGTGCCG 1140
TTCTTGCCAA GAAGGTGAAC TGGATTGGCT GCCAGGGGAG TGAGCCGCAT TTCCGGGGCT 1200
TTCCCTGCTC CCTGGGCCTC CTCTTCCACT TCTTGACTGT GCAGGCAGCT CGGCAAAATG 1260
TAGACCACTC ACAGAACACC AAGGCCAAGG AGGTCCTCCC AGCCATCCGA GGCTACGTGC 1320
ACTACTTCTT CGGCTGCCGA GACTGCGCTA GCCACTTCGA GCAGATGGCT GCTGCCTCCA 1380
TGCACCGGGT GGGGAGTCCC AACGCCGCTG TCCTCTGGCT CTGGTCTAGC CACAACAGGG 1440
TCAATGCTCG CTTGCAGGTG CCCCCAGCGA GGACCCCCAG TTCCCCAAGG TGCAGTGGCC 1500
ACCCCGTGAA CTTTGTTCTG CCTGCCACAA TGAACGCCTG GATGTGCCCG TGTGGGACGT 1560

GGAAGCCACC CTCAACTTCC TCAAGGCCCA CTTCTCCCCA AGCAACATCA TCCTGGACTT 1620
CCCTCAGCTG GGTCAGCTGC CCGGAGGGAT GTGCAGAATG TGGCAGCCGC CCCAGAGCTG 1680
GCGATGGGAG CCCTGGAGCT GGGAAGCCGG AATTCAACTC TGGACCCTGG GAAGCCTGAG 1740
ATGATGAAGT CCCCCACAAA CACCACCCCA CATGTGCCGG CTGAGGGACC TGAGCTTATT 1800
TGAAGTCCTG CCTCATTCTC ACTGGAGCCT CAGTCTCTCC TGCTTGGTCT TGGCCCTCAA 1860
CTGGGGCAAG TGAAGCCAGA GGAGGGTCCC CCAGCTGGGT GGGCTGGAAT GGAACTCCTC 1920
ACTAGCTGCT GGCTCCGCCC ACCCTGCTCC CTTCCGGACA ATGAAGAAGC CTTTGCACCC 1980
TGGGAGGAAG GACCACCCCG GGCCCTCTAT GCCTGGCCAG CCTCCAGCTC CTCAGACCTC 2040
CTGGGTGGGG TTTGGCTTCA GGGTGGGGTT TGGAAGCTTC TGGAAGTCGT GCTGGTCTCC 2100
CAGGTGAGGC AAGCCATGGT TGCTGGGCTG TAGGGTGAGG TGGCTTCCTT GGTGGGACCT 2160
GACGAGTTGG TGGCATGGGA AGGATGTGGG TCTCTAGTGC CTTGCCCTGG CTTAGCGGCA 2220
GGAGAAGATG GCGGCTTTCA CTTCCCCCCA ATTGAGCTCT GCTCCCTCTG AGCCTGGGTC 2280
TTTTGTCCTT TTTTATTTTG GTCTCCAAGA TGAATGCTCA TCTTTGGAGG GTCCCAGGTA 2340
GAAGCTAGGG AGGGGAGTGT CTTCTCTCTC CCAGGTTTCA CCTTCCAGTG TGCAGAAGTT 2400
AGAAGGGTCT GGCGGGGGCA GTGCCTTACA CATGCTTGAT TCCCACGCTA CCCCCTGCCT 2460
TGGGAGGTGT GTGGAATAAA TTATTTTTGT TAAGGCAAAA 2500

INFORMATION FOR SEQ ID NO:18

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 1246

(B) TYPE : Nucleic acid

(C) STRANDEDNESS : Single

(D) TOPOLOGY : Linear

(ii) MOLECULE TYPE : cDNA

(iv) ANTI-SENSE : No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GGATCCCCGG GCCCGGGAGA AGCAGAGCTC AGAGGAAGAA GAAAAGGAAA CAAGAGGGGT 60
TCAGAAGAGG CGAGGAGGGA GCACAGTACC CAAAGATGGG CCAGTGAGAC CTCAGAACGC 120
TGAAGAAGAA AAAAGAGGCT TAGACCTGCG TGTGTCGGGG TACCTGAATC TGGCTGCTGA 180
CTTGGCACAC AACTTCACTG ATGGTCTGGC CATTGGGGCT TCCTTTCGAG GGGGCCGGGG 240
ACTAGGGATC CTGACCACAA TGACTGTCCT GCTACATGAA GTGCCCCACG AGGTCGGAGA 300
CTTTGCCATC TTGGTCCAGT CTGGCTGCAG CAAAAAGCAG GCGATGCGTC TGCAACTACT 360
GACAGCAGTA GGGGCACTGG CAGGCACAGC CTGTGCCCTT CTCACTGAAG GAGGAGCAGT 420
GGGCAGTGAA ATTGCAGGTG GTGCAGGTCC TGACTGGCTC CTACCATTTA CTGCAGGTGG 480
CATTATCCTA CGTAACAAAT AGTGTGTGTG TTCCCCGAGC TGCTGAGGGA GGCATCACCA 540
TTGCAATCAC TTCTGGAGGG TGCTGGGGCT GCTGGGGGGA ATTATCATGA TGGTGCTGAT 600

TCCCCACCTT GAGTGAGGGG TGGATAAACT ACCCCTCCCC AAACCTCTAC CCCTAACTCC 660
AGGTCAGGGG TGCGTAGAGG TTGGGGGCCC TGGCCAGGGA CATCTGCCAA AGGAAGGAAC 720
TGTAGCCTGG GAGAATGGTT ACTTTGGCAT TAGGGCCTTC AAGGGCTGGC AGTCTTACAG 780
AGGCTGGAGC GGTGAGAATG AGAGGCCAGA GGGACCATAG TGTTGGGCAC TGTCTGACCA 840
TGTTGCATTT GGAAGGCTAA ATGGGGCCAT GAAGAAGGCT GGAAGGGACA GGGGGTGATG 900
GCAGCCTACC TGGTGTCCCC TACCCCACCT GTTCTCGGAG AACCAAGTTG CTACACAGGA 960
AGTTCTCCAA GGTCCAGTTT CCTTTCTCCC ACCAGTTGGT GGAGGCTTCA GGGAAGACCA 1020
GAGTCCTGGA CAGAGAGGGT AACAGGAGGA GTCGGGGATA AACATCAAAC ATCAATCGTG 1080
TGTCCTGATT TGGGAGTGAT TGGGGGGATG GGGTGGGAGA GGGTTAGTTG GTATTCTCAT 1140
GGCCTGATTT TTTTTGTTTC TATTCCTTTT ATATCACTGT GTTTGAATCG AGGGGGAGGG 1200
GTGGTAACCG GAAATAAAGA CCTCCGATCT TCCGCCCCAC CAAAAA 1246

INFORMATION FOR SEQ ID NO:19

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 661

(B) TYPE : Nucleic acid

(C) STRANDEDNESS : Single

(D) TOPOLOGY : Linear

(ii) MOLECULE TYPE : cDNA

(iv) ANTI-SENSE : No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGATCCCCGG GCTCCTGTCC ATGAACTGGG CCACGTGGCC GACATTCTGC TGTACGTGGT 60
GATCCCTACC CGACGCTCCA CCGCCGAGGC CTTCCAGATC GTGCTGTCCC ACCTGCTGGG 120
TGATGCTGGG AGCCCCTACC TCATTGGCCT GATCTCTGAC CGCCTGCGCC GGAACTGGCC 180
CCCCTCCTTC TTGTCCGAGT TCCGGGCTCT GCAGTTCTCG CTCATGCTCT GCGCGTTTGT 240
TGGGGCACTG GCGCGCGCAG CCTTCCTGGG CACCGCCATC TTCATTGAGG CCGACCGCCG 300
GCGGGACAGC TGCACGTGCA GGGCCTGCTG CACGAAGCAG GGTCCACAGA CGACCGGATT 360
GTGGTCCGCA GCGGGGCCGC TCCACCCGCG TGCCCGTGGG CAGGGTGCTC ATCTGAGAGG 420
CTGCCGCTCA CCTACCAGCC TGACATCTCC ACAGCTGCCC TGGCCCACCC ACAAGGGGCC 480
TGGCCTAACC CCTTGGCCTG GCCCAGCTTC CAGAGGGACC CTGGGCCGTG TGCCAGCTCC 540
CAGACACTAC ATGGGTAGCT CAGGGGAGGA GGTGGGGGTC CAGGAGGGGG ATCCCTCTCC 600
ACAGGGGCAG CCCCAAGGGC TCGGTGCTAT TTGTAACGGA ATAAAATTTG TAGCCAGAAA 660
A 661

INFORMATION FOR SEQ ID NO:20

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 1951
(B) TYPE : Nucleic acid
(C) STRANDEDNESS : Single
(D) TOPOLOGY : Linear
(ii) MOLECULE TYPE : cDNA
(iv) ANTI-SENSE : No
(vi) ORIGINAL SOURCE :
(A) ORGANISM : Human
(F) TISSUE TYPE : Lung
(G) CELL TYPE : Fibroblast
(H) CELL LINE : MRC-5
(ix) FETURE :
(A) NAME KEY : CDS
(B) LOCATION : 55..1488
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GTCGCGGCCG CTGTTCCTGG GACGTCCGGT TGACCGCGGT CTGCTGCAGA GACCATGTCT 60
GCCGACGGGG CAGAGGCTGA TGGCAGCACC CAGGTGACAG TGGAAGAACC GGTACAGCGG 120
CCCAGTGTGG TGGACCGTGT GGCCAGCATG CCTCTGATCA GCTCCACCTG CGACATGGTG 180
TCCGCAGCCT ATGCCTCCAC CAAGGAGAGC TACCCGCACG TCAAGACTGT CTGCGACGCC 240
GCAGAGAAGG GAGTGAGGAC CCTCACGGCG GCTGCTGTCA GCGGGGCTCA GCCGATCCTC 300
TCCAAGCTGG AGCCCCAGAT TGCATCAGCC AGCGAATACG CCCACAGGGG GCTGGACAAG 360
TTGGAGGAGA ACCTCCCCAT CCTGCAGCAG CCCACGGAGA AGTGCTGGCG GACACCAAGG 420
AGCTTGTGTC GTCTAAGGTG TCGGGGGCCC AAGAGTTGGT GTCTAGCGCC AAGGACACGT 480
TGGCCACCCA ATTGTCGGAG GCGGTGGACC GACCCGCGGT GCTGTGCAGA GCGGCGTGGA 540
CAAGACAAAG TCCGTAGTGA CCGGCGGCGT CCAATCAGTC ATGGGCTCCC GCTTGGGCCA 600
GATGGTGCTG AGTGGGGTCG ACACGGTGCT GGGGAATTCG GAGCCGTGCG CGCACAACCA 660
CCTGCCCTTA CGGATGCCGA ACTGGCCCGC ATCGCCACAT CCCTGGATGG CTTCGACGTC 720
GCGTCCGTGC AGCAGCAGCG GCAGGAACAG AGCTACTTCG TACGTCTGGG CTCCCTGTCG 780
GAGAGGCTGC GGCAGCACGC CTATGAGCAC TCGCTGGGCA AGCTTCGAGC CACCAAGCAG 840
AGGGCACAGG AGGCTCTGCT GCACGTGTCG CAGGCCCTAA GCCTGATGGA AACTGTCAAG 900
CAAGGCGTTG ATCAGAAGCT GGTGGAAGGC CAGGAGAAGC TGCACCAGAT GTGGCTCAGC 960
TGGAACCAGA AGCAGCTCCA GGGCCCCGAG AAGGAGCCGC CCAAGCCAGA GCAGGTCGAG 1020
TCCCGCGCGC TCACCATGTT CCGGGACATT GCCCAGCAAC TGCAGGCCAC CTGTACCTCC 1080
CTGGGGTCCA GCATTCAGGG CCTCCCCACC AATGTGAAGG ACCAGGTGCA GCAGGCCCGC 1140
CGCCAGGTGG AGGACCTCCA GGCCACGTTT TCCAGCATCC ACTCCTTCCA GGACCTGTCC 1200
AGCAGCATTC TGGCCCAGAG CCGTGAGCGT GTCGCCCGCG CCCGCGAGGC CCTGGACACA 1260

TGGTGGGATA TGTGGCCCAG CACACACCTG TCACGTGGCT CGTGGGACCC TTTGCCCCTG 1320
GAATCACTGA GAAAGCCCCG GAGGAGAAGA AGTAGGGGGA GAGGAGAGGA CTCAGCGGGC 1380
CCCGTCTCTA TAATGCAGCT GTGCTCTGGA GTCCTCAACC CGGGGCTCAT TTCAAACTTA 1440
TTTTCTAGCC ACTCCTCCCA GCTCTTCTGT GCTGTCCACT TGGGAAGCTA AGGCTCTCAA 1500
AACGGGCATC ACCCAGTTGA CCCATCTCTC AGCCTCTCTG AGCTTGGAAG AAGCCTGTTC 1560
TGAGCCTCAC CCTATCAGTC AGTAGAGAGA GATGTCCAGA AAAAATATCT TTCAGGAAAG 1620
TTCTCCCCGG CAGAATTTTT TTTCCTTGTT AGATATCAGG GATATAGGCC GGGTGCGGTG 1680
GCTCACACCT GTAATCCCAG CACTTTGGGA GGCTGAGGCG GGCGGACACC TGAGGTCAGG 1740
TGTTCGAGAC CAGCCAGGCC AACATGGTGA AACCCCGTCT CTACTAAAAA TACAAAAAAA 1800
AATGAGCCGC GCATGGTAGC AGGTGTCTGT TATCCCAGTT AGGAGGCTGA GGGAAGAGAA 1860
TCTCTTGAAC CTGAGAGGCG GAGGTTGCAG TGAGCCAAGA TCGCGCCTTG CACTCCAGCC 1920
TGGGGAACAA GAGTGAGACT TAGTCTCAAA A 1951

INFORMATION FOR SEQ ID NO:21

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 513

(B) TYPE : Nucleic acid

(C) STRANDEDNESS : Single

(D) TOPOLOGY : Linear

(ii) MOLECULE TYPE : cDNA

(iv) ANTI-SENSE : No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GGATCCCCGG GGCGCAGGAC AGCCTCTTCC AGCTATGGAA GAAGAAGCGC GGGGTGCTCA 60
CCTCCGACCG CCTGAGCCTG TTCCCCGCGA GCCCCCGGGC GCGCCCCAAG GAGCTGCGCT 120
TCCACTCCAT CCTAAGGTGG ACTGCGTGGG GGACGGCAAG TACGTGTACT CACCATCGTC 180
ACCACCGACC ACAAGGAGAT CGACTTCCGC TGCGGGCGAG AGCTGCTGGA ACCGGCCATC 240
GGCGGCGCTC ATCGATTTCC AGAACCGCCG CGCCCTGCAG GACTTTCGCA GCCGCCAGAA 300
CGCACCGCAC CCGCCGCACC CGCCGAGGAC GCCGTGGCTG CCGCGGCCGC CGACCCTCCG 360
AGCCCTCGGA GCCCTCCAGG CCATCCCCGC AGCCCAAACC CCGCACGCCA TGAGCCCGCC 420
GCGGGCCATA CGCTGGACGA GTCGGACCGA GGCTAGGACA TGGCCCGCGC TCTCCAGCCC 480
TGCAGCAGAA GAACTTCCCG TGCGCGCGGA TCC 513

INFORMATION FOR SEQ ID NO:22

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 225

(B) TYPE : Amino acid

(ii) MOLECULE TYPE : Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Met Ala Gln Arg Gln Lys Arg Asn Lys Ile Pro Tyr Ser Phe Phe Lys
5 10 15
Thr Ala Leu Asp Asp Arg Lys Glu Gly Ala Val Leu Ala Lys Lys Val
20 25 30
Asn Trp Ile Gly Cys Gln Gly Ser Glu Pro His Phe Arg Gly Phe Pro
35 40 45
Cys Ser Leu Gly Leu Leu Phe His Phe Leu Thr Val Gln Ala Ala Arg
50 55 60
Gln Asn Val Asp His Ser Gln Asn Thr Lys Ala Lys Glu Val Leu Pro
65 70 75 80
Ala Ile Arg Gly Tyr Val His Tyr Phe Phe Gly Cys Arg Asp Cys Ala
85 90 95
Ser His Phe Glu Gln Met Ala Ala Ala Ser Met His Arg Val Gly Ser
100 105 110
Pro Asn Ala Ala Val Leu Trp Leu Trp Ser Ser His Asn Arg Val Asn
115 120 125
Ala Arg Leu Gln Val Pro Pro Ala Arg Thr Pro Ser Ser Pro Arg Cys
130 135 140
Ser Gly His Pro Val Asn Phe Val Leu Pro Ala Thr Met Asn Ala Trp
145 150 155 160
Met Cys Pro Cys Gly Thr Trp Lys Pro Pro Ser Thr Ser Ser Arg Pro
165 170 175
Thr Ser Pro Gln Ala Thr Ser Ser Trp Thr Ser Leu Ser Trp Val Ser
180 185 190
Cys Pro Glu Gly Cys Ala Glu Cys Gly Ser Arg Pro Arg Ala Gly Asp
195 200 205
Gly Ser Pro Gly Ala Gly Lys Pro Glu Phe Asn Ser Gly Pro Trp Glu
210 215 220
Ala
225

INFORMATION FOR SEQ ID NO:23

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 240

(B) TYPE : Amino acid

(ii) MOLECULE TYPE : Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
Met Ser Ala Asp Gly Ala Glu Ala Asp Gly Ser Thr Gln Val Thr Val
                5                  10                  15
Glu Glu Pro Val Gln Arg Pro Ser Val Val Asp Arg Val Ala Ser Met
            20                  25                  30
Pro Leu Ile Ser Ser Thr Cys Asp Met Val Ser Ala Ala Tyr Ala Ser
        35                  40                  45
Thr Lys Glu Ser Tyr Pro His Val Lys Thr Val Cys Asp Ala Ala Glu
    50                  55                  60
Lys Gly Val Arg Thr Leu Thr Ala Ala Ala Val Ser Gly Ala Gln Pro
65                  70                  75                  80
Ile Leu Ser Lys Leu Glu Pro Gln Ile Ala Ser Ala Ser Glu Tyr Ala
                85                  90                  95
His Arg Gly Leu Asp Lys Leu Glu Glu Asn Leu Pro Ile Leu Gln Gln
            100                 105                 110
Pro Thr Glu Lys Cys Trp Arg Thr Pro Arg Ser Leu Cys Arg Leu Arg
        115                 120                 125
Cys Arg Gly Pro Lys Ser Trp Cys Leu Ala Pro Arg Thr Arg Trp Pro
    130                 135                 140
Pro Asn Cys Arg Arg Arg Trp Thr Asp Pro Arg Cys Cys Ala Glu Arg
145                 150                 155                 160
Arg Gly Gln Asp Lys Val Arg Ser Asp Arg Arg Arg Pro Ile Ser His
                165                 170                 175
Gly Leu Pro Leu Gly Pro Asp Gly Ala Glu Trp Gly Arg His Gly Ala
            180                 185                 190
Gly Glu Phe Gly Ala Val Arg Ala Gln Pro Pro Ala Leu Thr Asp Ala
        195                 200                 205
Glu Leu Ala Arg Ile Ala Thr Ser Leu Asp Gly Phe Asp Val Ala Ser
    210                 215                 220
Val Gln Gln Gln Arg Gln Glu Gln Ser Tyr Phe Val Arg Leu Gly Ser
225                 230                 235                 240
```

INFORMATION FOR SEQ ID NO:24

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 448
(B) TYPE : Nucleic acid
(C) STRANDEDNESS : Single
(D) TOPOLOGY : Linear
(ii) MOLECULE TYPE : cDNA
(iv) ANTI-SENSE : No
(vi) ORIGINAL SOURCE :
(A) ORGANISM : Human
(F) TISSUE TYPE : Lung
(G) CELL TYPE : Fibroblast
(H) CELL LINE : MRC-5
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GGATCCCCGG GACCAAGAAC TTATCGGAAG TGTGCCTCTG TGTCTCCTTC CTCGGGGTAA 60
GGAGGGGACA GTGCTTCCCA AGTTCCAGCT GCAAGTCCAA CTTAACCAAC TTTCCTTCAA 120
AGTCAGTTAC TGCCAATTTT CTGAAAAAAG CATGTTCCAT ATACTAAGTC TCTCTTCTCA 180
CGGTAGGAAA TAATACAGCC AAGATATGCA GCATCCTTCT CATTGATGTA GAAAATTCTG 240
AAAATTCTGC GATAGACCAG AAAAATCCTG GCAGCTTTTC TCCAGGCATC TGGGTCACTA 300
AAAACTGATT TTCTAAAATT ATTGGATTTG TATTTTGTTA TTAAGGGGGG AAATGTGATT 360
TGTGCCTGAT CTTTCATCTG TGATTCTAAT AAGAGCTTTG TCTTCAGAGA AACTAAAAAT 420
AAAAGGCATT GACTTAAACA GCTGAAAA 448

INFORMATION FOR SEQ ID NO:25
(i) SEQUENCE CHARACTERISTICS :
(A) LENGTH : 1352
(B) TYPE : Nucleic acid
(C) STRANDEDNESS : Single
(D) TOPOLOGY : Linear
(ii) MOLECULE TYPE : cDNA
(iv) ANTI-SENSE : No
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GGATCCCCGG GCTGCCGCGG CTCCCCGAGC TGTTCGAAAC TGGTAGACAG TTACTGGACG 60
AAGTAGAAGT GGTGACTGAA CCCGCCGGTT CCCGGATAGT CCAGGAGAAG GTGTTCAAGG 120
GCTTGGACCT CCTTGAGAAG GCTGCCGAAA TGTTATCGCA GCTCGACTTG TTCAGCCGAA 180
ATGAAGATTT GGAAGAGATT GCTTCCACCG ACCTGAAGTA CCTTTTGGTG CCAGCGTTTC 240
AAGGAGCCCT CACCATGAAA CAAGTCAACC CCAGCAAGCG TCTAGATCAT TTGCAGCGGG 300

CTCGAGAACA CTTTATAAAC TACTTAACTC AGTGCCATTG CTATCATGTC GCAGAGTTTG 360
GGCTATCCCA AACCATGAAC AACTCTGCTG AAAATCACAC TGCCAATTCC TCCATGGCTT 420
ATCCTAGTCT CGTTGCTATG GCATCTCAAA GACAGGCTAA AATACAGAGA TACAAGCAGA 480
AGAAGGAGTT GGAGCATAGG TTGTCTGCAA TGAAATCTGC TGTGGAAAGT GGTCAAGCAG 540
ATGATGAGCG TGTTCGTGAA TATTATCTTC TTCACCTTCA GAGGTGGATT GATATCAGCT 600
TAGAAGAGAT TGAGAGCATT GACCAGGAAA TAAAGATCCT GAGAGAAAGA GACTCTTCAA 660
GAGAGGCATC AACTTCTAAC TCATCTCGCC AGGAGAGGCC TCCAGTGAAA CCCTTCATTC 720
TCACTCGGAA CATGGCTCAA GCCAAAGTAT TTGGAACTGG TTATCCAAGT CTGCCAACTA 780
TGACGGTGAG TGACTGGTAT GAGCAACATC GGAAATATGG AGCATTACCG GATCAGGGAA 840
TAGCCAAGGC AGCACCAGAG GAATTCAGAA AAGCAGCTCA GCAACAGGAA GAACAAGAAG 900
AAAAGGAGGA AGAGGATGAT GAACAAACAC TCCACAGAGC CCGGGAGTGG GATGACTGGA 960
AGGACACCCA TCCTAGGGGC TATGGGAACC GACAGAACAT GGGCTGATCT TCCCACAACA 1020
CCACAGGACT GCAGGGTGCA CAACTCCCCT GCCAAGGAAA ACCATGCAGT CCTCCCCTCC 1080
CTGGTCTCCT GCTTCAGCTC TGTACAACGA GGGCAAAGAT GCTAAATCTT GCTTTGCATT 1140
CAGTAAAGTG TCAAGTGATT AAGTGTGTAT TTGTACCCTA GATGATATGA ACCAGCAGTC 1200
TTGTTTTGGC ATCATCCTCA TCATGTTGTA TTCCAGCTTC TTAAGTGGAA GGAAAAGAGT 1260
GCTGAGAAAT GGCTCTGTAT AATCTATGGC TATCCCGAAT TCTCTGAAAA AATAATAAAA 1320
GTCCCCTCTA TTATATGAGC CTGTACAGAA AA 1352

INFORMATION FOR SEQ ID NO:26

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 632

(B) TYPE : Nucleic acid

(C) STRANDEDNESS : Single

(D) TOPOLOGY : Linear

(ii) MOLECULE TYPE : cDNA

(iv) ANTI-SENSE : No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GGATCCCCCC CGGGCCGATT TTCTCCTGCT GCTGTGGCCC GGACATGGCG ACTCCCGGCC 60
CTGTGATTCC GGAGGTCCCC TTTGAACCAT CGAAGCCTCC AGTCATTGAG GGGCTGAGCC 120
CCACTGTTTA CAGGAATCCA GAGAGTTTCA AGGAAAAGTT CGTTCGCAAG ACCCGCGAGA 180
ACCCGGTGGT ACCCATAGGT TGCCTGGCCA CGGTGGGCGN CCTCANCTAC GGTCTCTACT 240
CCTTCCACCG GGGGAACAGC CAGCGCTCTC AGCTCATGAT GCGCACCCGG ATCGCCGCCC 300
AGGGTTTCAC GGTCGCAGCC ATCTTGCTGG GTCTGGCTGT CACTGCTATG AAGTCTCGAC 360
CCTAAGCCCA GGGTCTGGCC TTGAAAGCTC CGCAGAAATG ATTCCAAAAC CCAGGGAGCA 420
ACCACTGGCC CTAACCGTGG GACTTACTCC CTCCTCTCCT TTGAGAGGCC CATGTGTCGC 480

TGGGGAGGAA GTGACCCTTT GTGTAACTGT AACCGAAAGT TTTTTCAAAA ATCCTAGATG 540
CTGTTGTTTG AATGTTACAT ACTTCTATTT GTGCCACATC TCCCCTCCAC TCCCCTGCTT 600
AATAAACTCT AAAAATCCAC TTGTATTTAA AA 632

INFORMATION FOR SEQ ID NO:27

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 285

(B) TYPE : Amino acid

(ii) MOLECULE TYPE : Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Met Leu Ser Gln Leu Asp Leu Phe Ser Arg Asn Glu Asp Leu Glu Glu
5 10 15

Ile Ala Ser Thr Asp Leu Lys Tyr Leu Leu Val Pro Ala Phe Gln Gly
20 25 30

Ala Leu Thr Met Lys Gln Val Asn Pro Ser Lys Arg Leu Asp His Leu
35 40 45

Gln Arg Ala Arg Glu His Phe Ile Asn Tyr Leu Thr Gln Cys His Cys
50 55 60

Tyr His Val Ala Glu Phe Gly Leu Ser Gln Thr Met Asn Asn Ser Ala
65 70 75 80

Glu Asn His Thr Ala Asn Ser Ser Met Ala Tyr Pro Ser Leu Val Ala
85 90 95

Met Ala Ser Gln Arg Gln Ala Lys Ile Gln Arg Tyr Lys Gln Lys Lys
100 105 110

Glu Leu Glu His Arg Leu Ser Ala Met Lys Ser Ala Val Glu Ser Gly
115 120 125

```
Gln Ala Asp Asp Glu Arg Val Arg Glu Tyr Tyr Leu Leu His Leu Gln
    130                 135                 140

Arg Trp Ile Asp Ile Ser Leu Glu Glu Ile Glu Ser Ile Asp Gln Glu
145                 150                 155                 160

Ile Lys Ile Leu Arg Glu Arg Asp Ser Ser Arg Glu Ala Ser Thr Ser
                165                 170                 175

Asn Ser Ser Arg Gln Glu Arg Pro Pro Val Lys Pro Phe Ile Leu Thr
            180                 185                 190

Arg Asn Met Ala Gln Ala Lys Val Phe Gly Thr Gly Tyr Pro Ser Leu
        195                 200                 205

Pro Thr Met Thr Val Ser Asp Trp Tyr Glu Gln His Arg Lys Tyr Gly
    210                 215                 220

Ala Leu Pro Asp Gln Gly Ile Ala Lys Ala Ala Pro Glu Glu Phe Arg
225                 230                 235                 240

Lys Ala Ala Gln Gln Gln Glu Glu Gln Glu Glu Lys Glu Glu Glu Asp
                245                 250                 255

Asp Glu Gln Thr Leu His Arg Ala Arg Glu Trp Asp Asp Trp Lys Asp
            260                 265                 270

Thr His Pro Arg Gly Tyr Gly Asn Arg Gln Asn Met Gly
        275                 280                 285
```

CLAIMS

What is claimed is:

1. A method of screening for a DNA encoding a eukaryotic cell growth inhibiting factor comprising :

(a) introducing a human DNA operably linked to an inducible promoter into a eukaryotic host cell ;

(b) testing said host cell for the presence of said DNA by measuring host cell growth rate under conditions in which the promoter is induced and not induced ; and

(c) determing the differential growth of these two groups at selected times whereby a host cell showing at least about 25% growth rate inhibition under the inducible condition as compared with the cell growth rate under the non-inducible condition is identified as containing the DNA encoding a eukaryotic cell growth inhibiting factor.

2. The method as claimed in claim 1, wherein a host cell showing at least about 50% growth rate inhibition is identified as containing the DNA.

3. The method as claimed in claim 1, wherein a host cell that has at least about 75% growth rate inhibition is identified as containing the DNA.

4. The method as claimed in claim 1, further comprising the step of isolating the DNA encoding the eukaryotic cell growth inhibiting factor from the host identified as containing the DNA.

5. The method as claimed in claim 1, the inducible promoter is, PHO5 promoter, nmt1 promoter or hsp promoter.

6. The method as claimed in claim 1, the selected times is about 12 to 144 hours after culture.

7. A method of screening for a DNA encoding a eukaryotic cell growth inhibiting factor, which comprises introducing a human DNA to be tested into a eukaryotic cell host so as to be controlled by an inducible promoter and selecting the host cell which does not grow under the inducible condition of said promoter but grows under the non-inducible condition.

8. The method as claimed in claim 1 or 7, wherein said eukaryotic cell host is a yeast.

9. The method as claimed in claim 8, wherein said yeast is a fission yeast.

10. The method as claimed in claim 9, wherein said fission yeast is a Shizosaccharomyces pombe.

11. An isolated DNA encoding a eukaryotic cell growth inhibiting factor, which is screened by the method as claimed in claim 1.

12. A DNA which codes for a eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 11.

13. The DNA as claimed in claim 12, wherein said DNA comprises a nucleotide sequence at least from the 248th to the 448th residues of the nucleotide sequence represented by SEQ ID NO. 7.

14. A DNA which codes for a eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 12.

15. The DNA as claimed in claim 14, wherein said DNA comprises a nucleotide sequence at least from the 279th to the 752nd residues of the nucleotide sequence

represented by SEQ ID NO. 8.

16. A DNA which codes for a eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 13.

17. The DNA as claimed in claim 16, wherein said DNA comprises a nucleotide sequence at least from the 201st to the 377th residues of the nucleotide sequence represented by SEQ ID NO. 9.

18. A DNA which codes for a eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 14.

19. The DNA as claimed in claim 18, wherein said DNA comprises a nucleotide sequence at least from the 296th to the 1000th residues of the nucleotide sequence represented by SEQ ID NO. 10.

20. A DNA which codes for a eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 15.

21. The DNA as claimed in claim 20, wherein said DNA comprises a nucleotide sequence at least from the 51st to the 740th residues of the nucleotide sequence represented by SEQ ID NO. 16.

22. A DNA which codes for a eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 22.

23. The DNA as claimed in claim 22, wherein said DNA comprises a nucleotide sequence at least from the 1062nd to the 1736th residues of the nucleotide sequence represented by SEQ ID NO. 17.

24. A DNA which codes for a eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 23.

25. The DNA as claimed in claim 24, wherein said DNA comprises a nucleotide sequence at least from the 55th to the 1488th residues of the nucleotide sequence represented by SEQ ID NO. 20.

26. A DNA coding for a eukaryotic cell growth inhibiting factor which comprises the nucleotide sequence of SEQ ID NO. 18.

27. A DNA coding for a eukaryotic cell growth inhibiting factor which comprises the nucleotide sequence of SEQ ID NO. 19.

28. A DNA coding for a eukaryotic cell growth inhibiting factor which comprises the nucleotide sequence of SEQ ID NO. 21.

29. A DNA coding for a eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 27.

30. The DNA as claimed in claim 29, wherein said DNA comprises a nucleotide sequence at least from the 150th to the 1004th residues of the nucleotide sequence represented by SEQ ID NO. 25.

31. A DNA coding for a eukaryotic cell growth inhibiting factor which comprises the nucleotide sequence of SEQ ID NO. 24.

32. A DNA coding for a eukaryotic cell growth inhibiting factor which comprises the nucleotide

sequence of SEQ ID NO. 26.

33. A vector comprising any one of DNAs as claimed in claim 11 to 32.

34. A transformant harboring the vector as claimed in claim 33.

35. A eukaryotic cell growth inhibiting factor which is coded by the DNA obtained by the method as claimed in claim 1.

36. A eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 11.

37. A eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 12.

38. A eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 13.

39. A eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 14.

40. A eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 15.

41. A eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 22.

42. A eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 23.

43. A eukaryotic cell growth inhibiting factor which comprises the amino acid sequence of SEQ ID NO. 27.

44. A eukaryotic cell growth inhibiting factor which is encoded by the DNA as claimed in claim 26.

45. A eukaryotic cell growth inhibiting factor which is encoded by the DNA as claimed in claim 27.

46. A eukaryotic cell growth inhibiting factor which is encoded by the DNA as claimed in claim 28.

47. A eukaryotic cell growth inhibiting factor which is encoded by the DNA as claimed in claim 31.

48. A eukaryotic cell growth inhibiting factor which is encoded by the DNA as claimed in claim 32.

49. A method for preparing the eukaryotic cell growth inhibiting factor as claimed in claim 35 which comprises cultivating a transformant containing a DNA encoding said factor under conditions suitable for expression of the said factor and recovering said factor.

50. A pharmaceutical composition which comprises an effective amount of any one of eukaryotic cell growth inhibiting factors as claimed in claim 35.

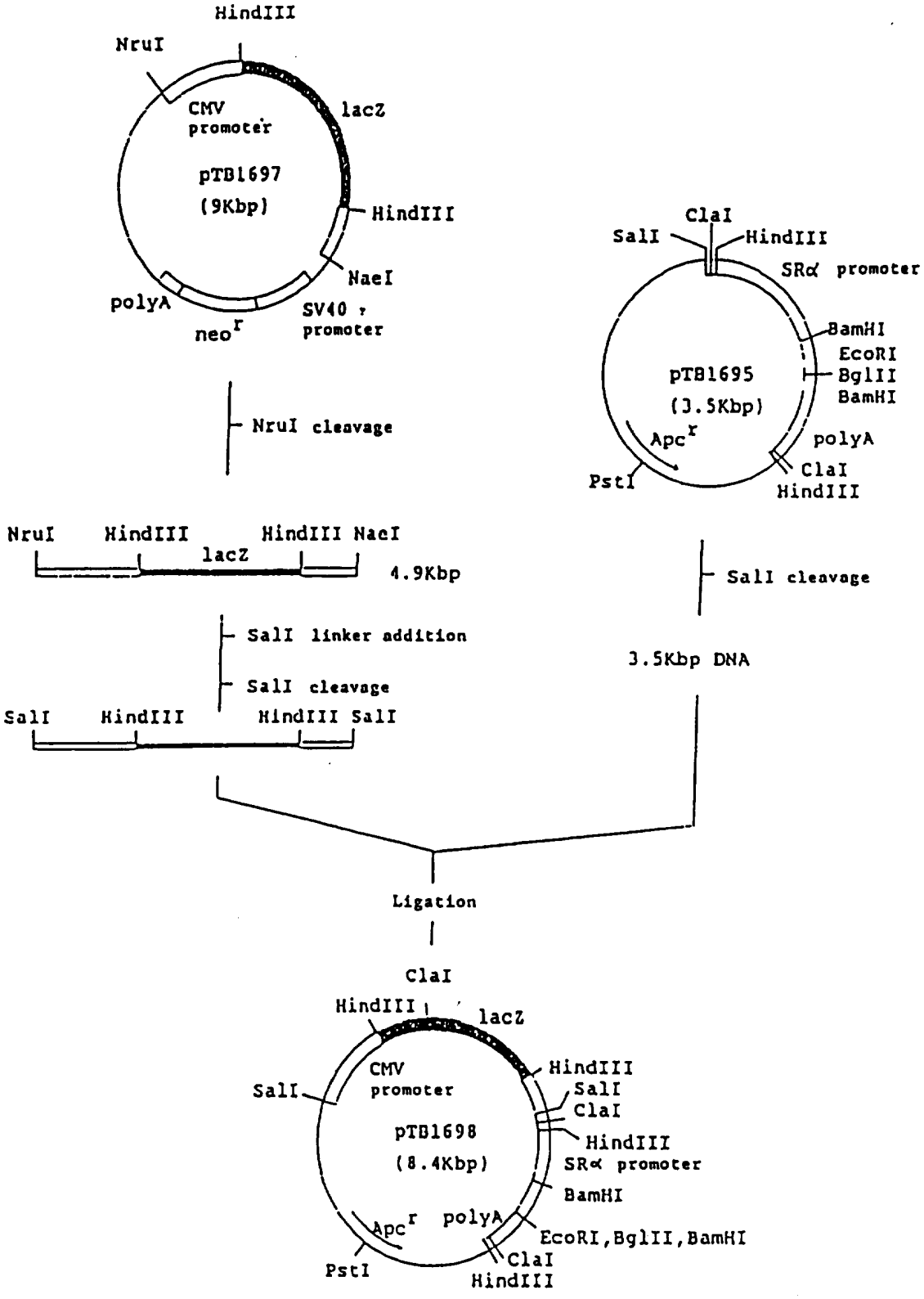
51. Use of the eukaryotic cell growth inhibiting factor as claimed in claim 35 for preparing an anticancer agent or infection remedy.

52. A method for treating for a patient suffering from cancer or infection which comprises administering to said said patient an effective effective amount of the eukaryotic cell growth inhibiting factor as claimed in claim 35 in the form of a pharmaceutical composition containing said factor as the effective component.

53. A method for inhibiting nucleic acid synthesis in target cell comprising containing said cell with an

effective amount of a eukaryotic cell growth inhibiting factor encoded by the DNA of claim 11.

Fig. 1


HindIII
NruI
CMV promoter
lacZ
pTB1697
(9Kbp)
HindIII
NaeI
polyA
neo^r
SV40 promoter
NruI cleavage
NruI HindIII HindIII NaeI
lacZ
4.9Kbp
SalI linker addition
SalI cleavage
SalI HindIII HindIII SalI
ClaI
SalI
HindIII
SRα promoter
BamHI
EcoRI
BglII
BamHI
pTB1695
(3.5Kbp)
Apc^r
polyA
PstI
ClaI
HindIII
SalI cleavage
3.5Kbp DNA
Ligation
ClaI
HindIII
lacZ
CMV promoter
SalI
HindIII
SalI
ClaI
HindIII
SRα promoter
BamHI
pTB1698
(8.4Kbp)
Apc^r
polyA
EcoRI,BglII,BamHI
PstI
ClaI
HindIII