US005958748A

United States Patent [19]

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[11] **Patent Number: 5,958,748**

[45] **Date of Patent: Sep. 28, 1999**

[54] **DNA CODING FOR SERINE/THREONINE KINASE**

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[21] Appl. No.: **09/159,385**

[22] Filed: **Sep. 23, 1998**

[30] **Foreign Application Priority Data**

Sep. 26, 1997 [JP] Japan 9-261589

[51] **Int. Cl.**[6] **C12N 9/12**; C12N 1/12; C12N 15/00; C12N 5/00

[52] **U.S. Cl.** **435/194**; 435/320.1; 435/325; 435/252.1

[58] **Field of Search** 435/194, 320.1, 435/252.1, 325

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[57] **ABSTRACT**

There is provided a DNA coding for a serine/threonine kinase.

Thus, the present invention provides a DNA coding for a protein (a) or (b):

(a) a protein comprising the amino acid sequence as shown in SEQ ID NO: 1;

(b) a protein comprising an amino acid sequence having one or several amino acids deleted, substituted or added in the amino acid sequence as shown in SEQ ID NO: 1, and exhibiting a serine/threonine kinase activity.

2 Claims, 10 Drawing Sheets

FIG. 1

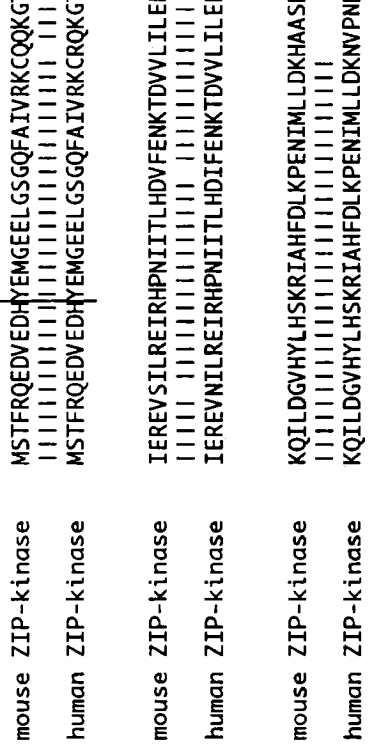

Protein kinase domain
mouse ZIP-kinase
human ZIP-kinase
Protein kinase domain
Leucine zipper domain

FIG. 2

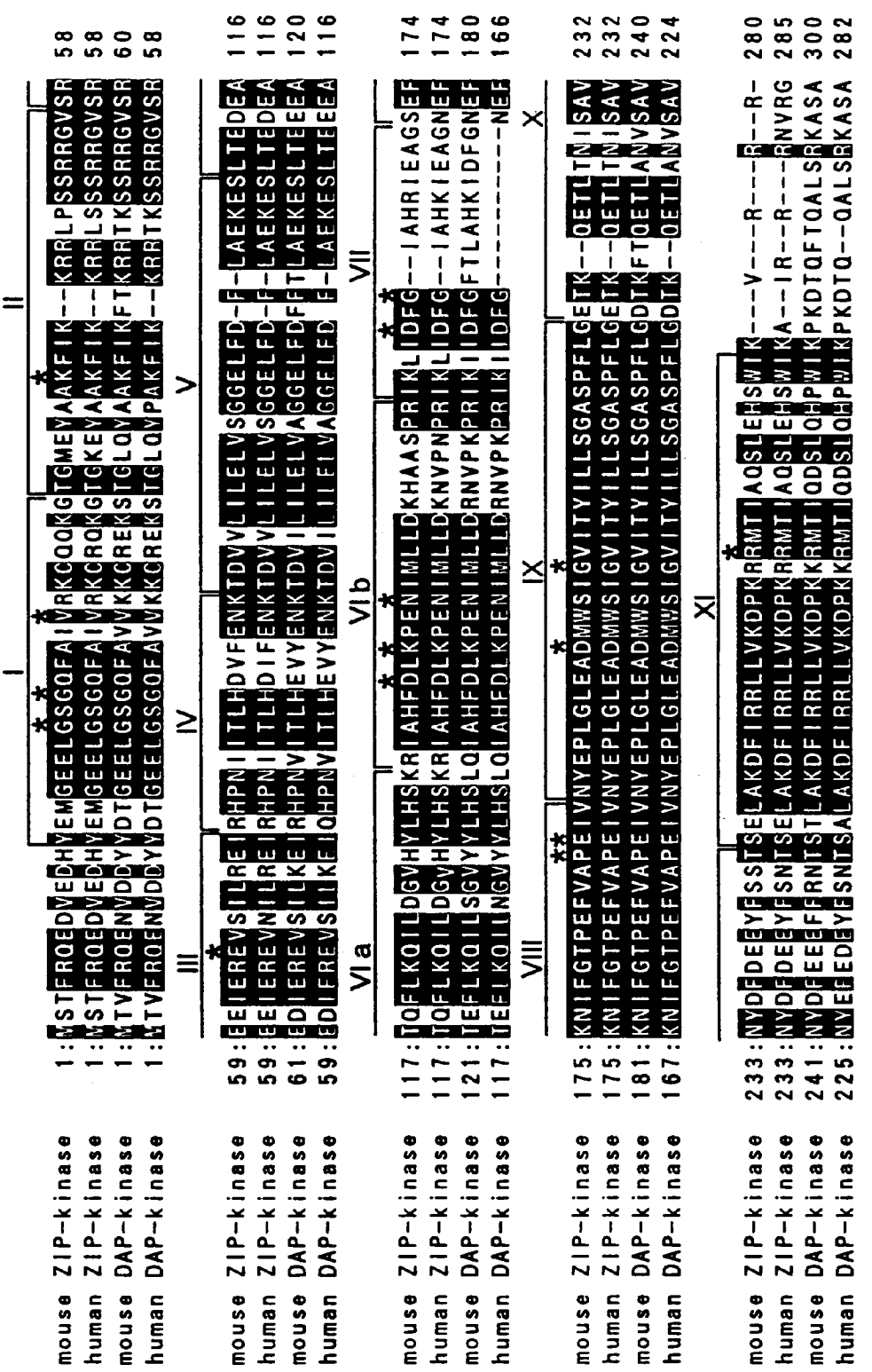

I
II
III
IV
V
VIa
VIb
VII
VIII
IX
X
XI
mouse ZIP-kinase 1:MSTFRQEDVEDHYEMGEELGSGQFAIVRKCQQKGTGMEYAAKFIK--KRRLPSSRRGVSR 58
human ZIP-kinase 1:MSTFRQEDVEDHYEMGEELGSGQFAIVRKCRQKGTGKEYAAKFIK--KRRLSSSRRGVSR 58
mouse DAP-kinase 1:MTVFRQENVDDYYDTGEELGSGQFAVVKKCREKSTGLQYAAKFIKFTKRRTKSSRRGVSR 60
human DAP-kinase 1:MTVFRQENVDDYYDTGEELGSGQFAVVKKCREKSTGLQVPAKFIK--KRRTKSSRRGVSR 58
mouse ZIP-kinase 59:EEIEREVSILREIRHPNIITLHDVFENKTDVVLILELVSGGELFD-FLAEKESLTEDEA 116
human ZIP-kinase 59:EEIEREVNILREIRHPNIITLHDIFENKTDVVLILELVSGGELFD-FLAEKESLTEDEA 116
mouse DAP-kinase 61:EDIEREVSILKEIRHPNVITLHEVYENKTDVILLELVAGGELFDFFTLAEKESLTEEEA 120
human DAP-kinase 59:EDIFREVSILKEIQHPNVITLHEVYENKTDVILIIFIVAGGFLFD FLAEKESLTEEEA 116
mouse ZIP-kinase 117:TQFLKQILDGVHYLHSKRIAHFDLKPENIMLLDKHAASPRIKLIDFG--IAHRIEAGSEF 174
human ZIP-kinase 117:TQFLKQILDGVHYLHSKRIAHFDLKPENIMLLDKNVPNPRIKLIDFG--IAHKIEAGNEF 174
mouse DAP-kinase 121:TEFLKQILSGVYYLHSLQIAHFDLKPENIMLLDRNVPKPRIKIIDFGFTLAHKIDFGNEF 180
human DAP-kinase 117:TEFIKQILNGVYYLHSLQIAHFDLKPENIMLLDRNVPKPRIKIIDFG----------NEF 166
mouse ZIP-kinase 175:KNIFGTPEFVAPEIVNYEPLGLEADMWSIGVITYILLSGASPFLGETKQETLTNISAV 232
human ZIP-kinase 175:KNIFGTPEFVAPEIVNYEPLGLEADMWSIGVITYILLSGASPFLGETKQETLTNISAV 232
mouse DAP-kinase 181:KNIFGTPEFVAPEIVNYEPLGLEADMWSIGVITYILLSGASPFLGDTKFTQETLANVSAV 240
human DAP-kinase 167:KNIFGTPEFVAPEIVNYEPLGLEADMWSIGVITYILLSGASPFLGDTKQETLANVSAV 224
mouse ZIP-kinase 233:NYDFDEEYFSNTSELAKDFIRRLLVKDPKRRMTIAQSLEHSWIK---V---R---R--R- 280
human ZIP-kinase 233:NYDFDEEYFSNTSELAKDFIRRLLVKDPKRRMTIAQSLEHSWIKA--IR--R---RNVRG 285
mouse DAP-kinase 241:NYDFEEEFFRNTSTLAKDFIRRLLVKDPKKRMTIQDSLQHPWIKPKDTQFTQALSRKASA 300
human DAP-kinase 225:NYEFEDEYFSNTSALAKDFIRRLLVKDPKKRMTIQDSLQHPWIKPKDTQ--QALSRKASA 282

FIG. 3

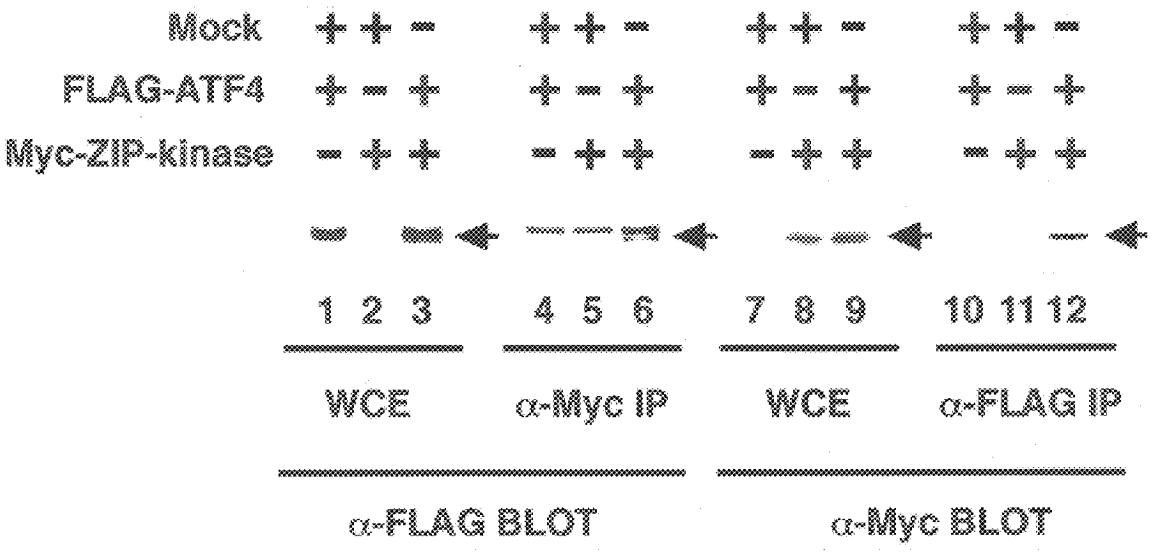

Mock
FLAG-ATF4
Myc-ZIP-kinase
1 2 3
4 5 6
7 8 9
10 11 12
WCE
α-Myc IP
WCE
α-FLAG IP
α-FLAG BLOT
α-Myc BLOT

FIG. 4

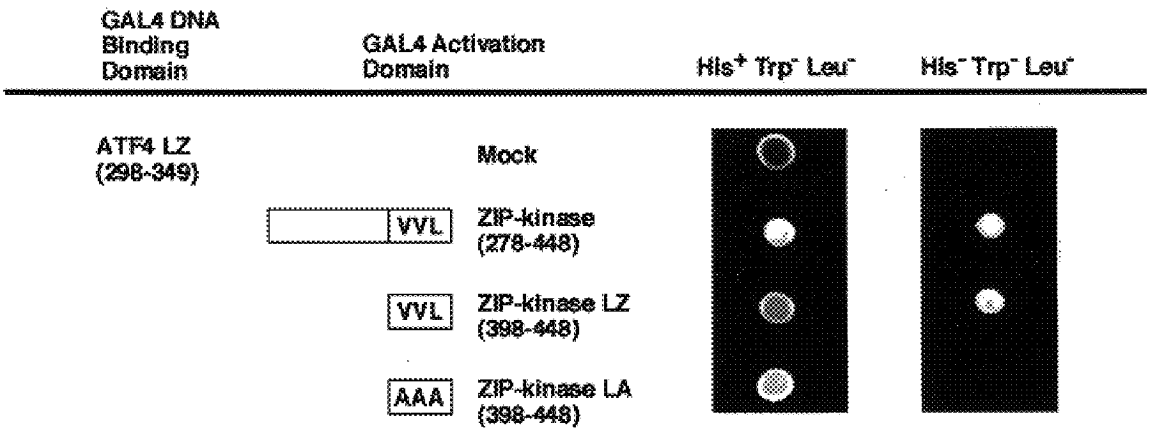

GAL4 DNA Binding Domain
GAL4 Activation Domain
His+ Trp- Leu-
His- Trp- Leu-
ATF4 LZ (298-349)
Mock
VVL
ZIP-kinase (278-448)
VVL
ZIP-kinase LZ (398-448)
AAA
ZIP-kinase LA (398-448)

FIG. 5

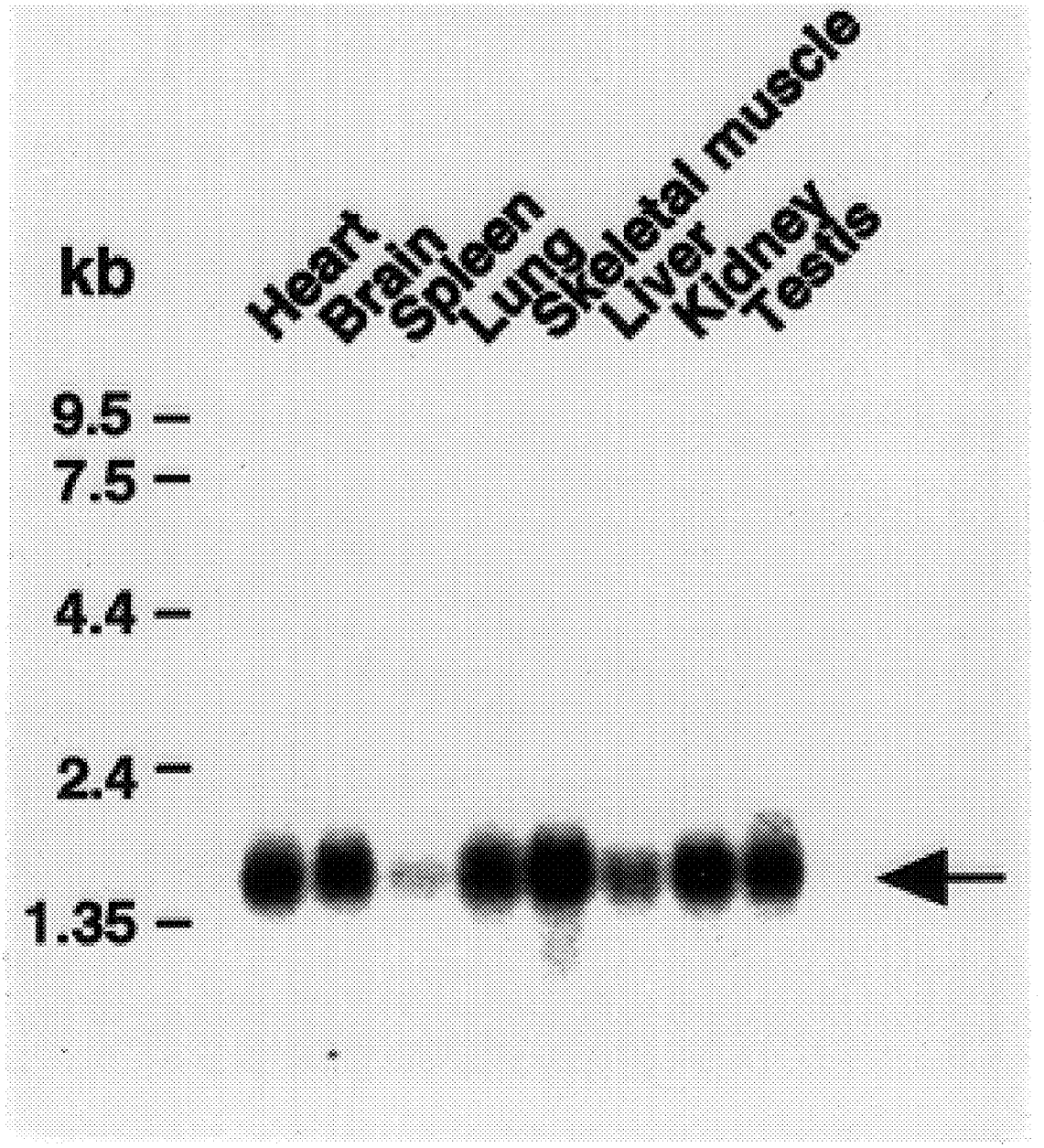

kb
Heart
Brain
Spleen
Lung
Skeletal muscle
Liver
Kidney
Testis
9.5
7.5
4.4
2.4
1.35

FIG. 6

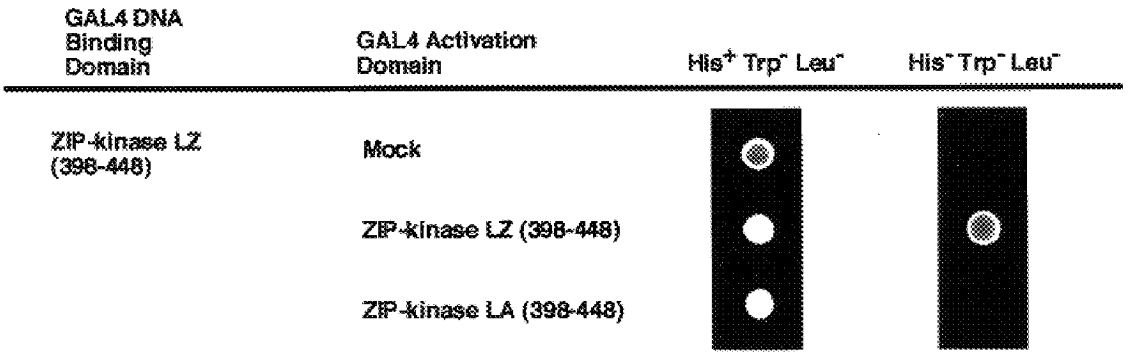

GAL4 DNA Binding Domain
GAL4 Activation Domain
His+ Trp- Leu-
His- Trp- Leu-
ZIP-kinase LZ (398-448)
Mock
ZIP-kinase LZ (398-448)
ZIP-kinase LA (398-448)

FIG. 7

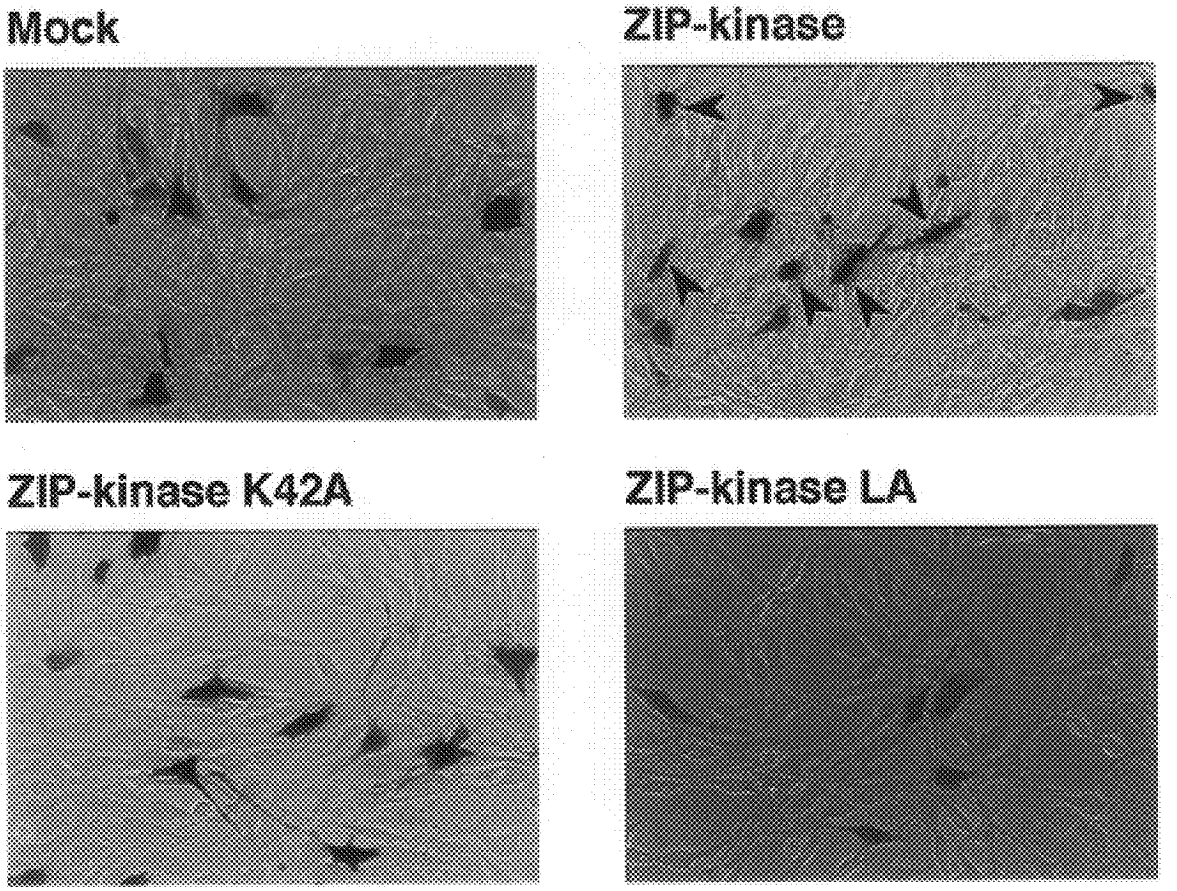


FIG. 8

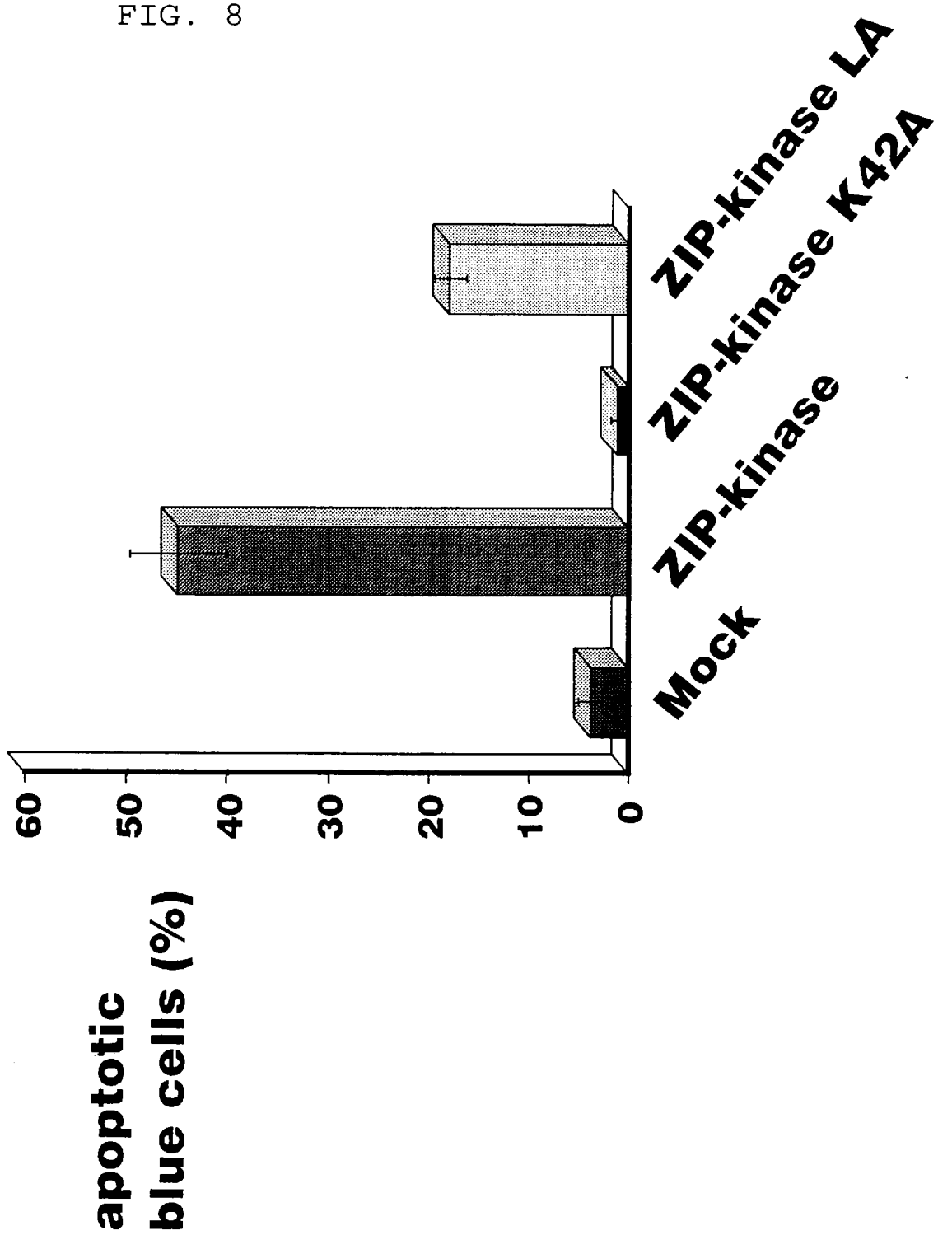

ZIP-kinase LA
ZIP-kinase K42A
ZIP-kinase
Mock
60
50
40
30
20
10
0
apoptotic blue cells (%)

FIG. 9A

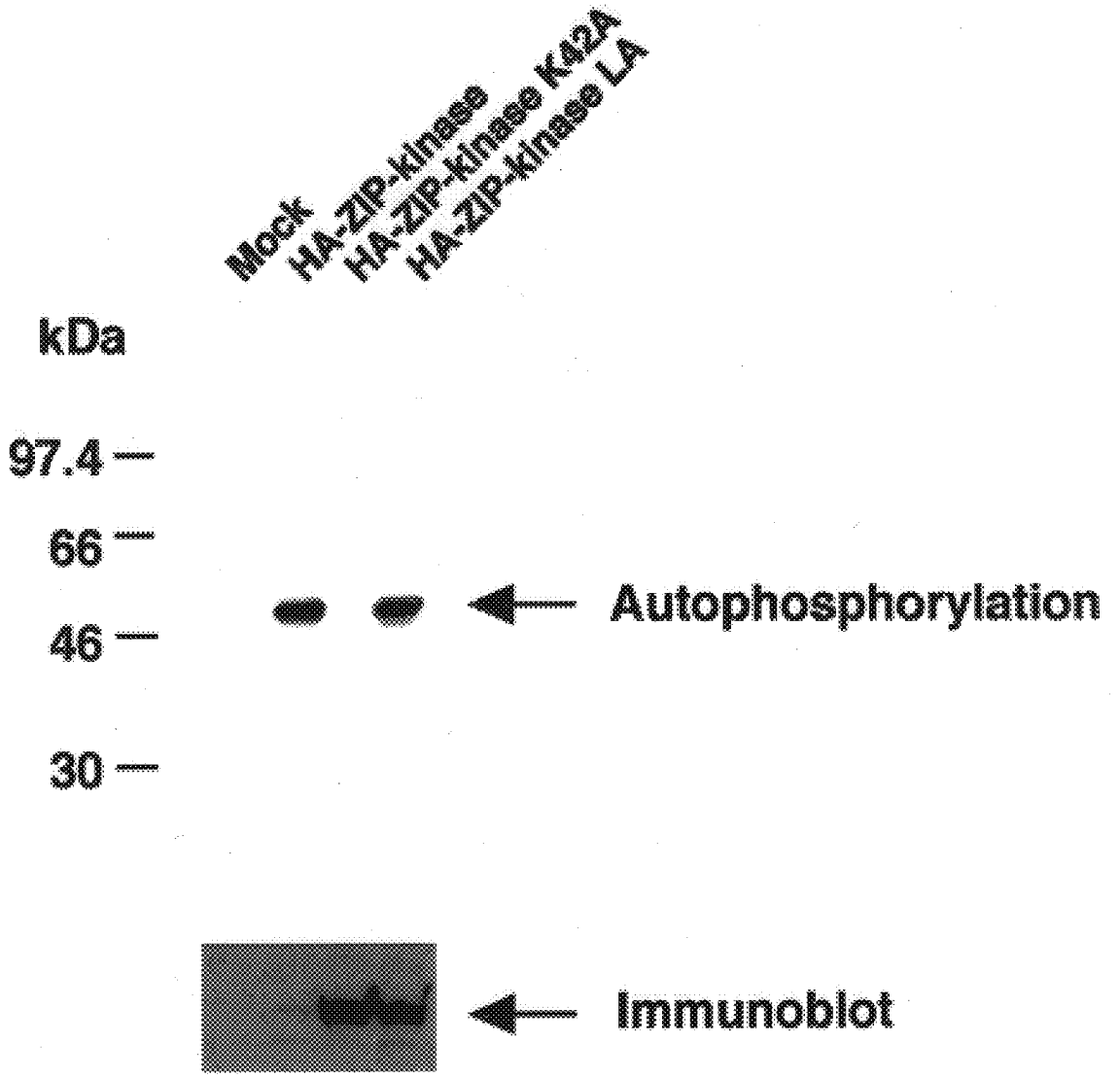
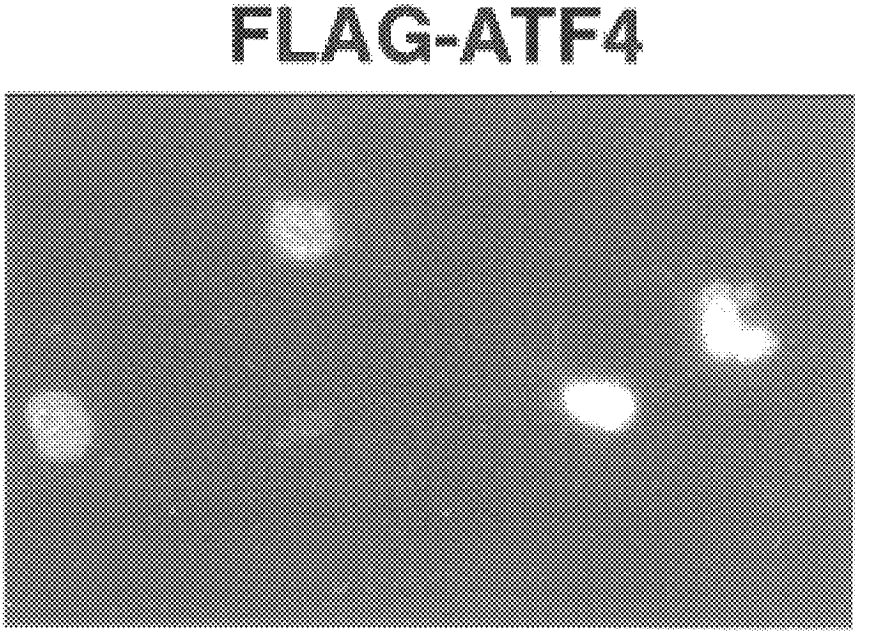

Mock
HA-ZIP-kinase
HA-ZIP-kinase K42A
HA-ZIP-kinase LA
kDa
97.4
66
46
30
Autophosphorylation
Immunoblot

FIG. 9B

FIG. 10A



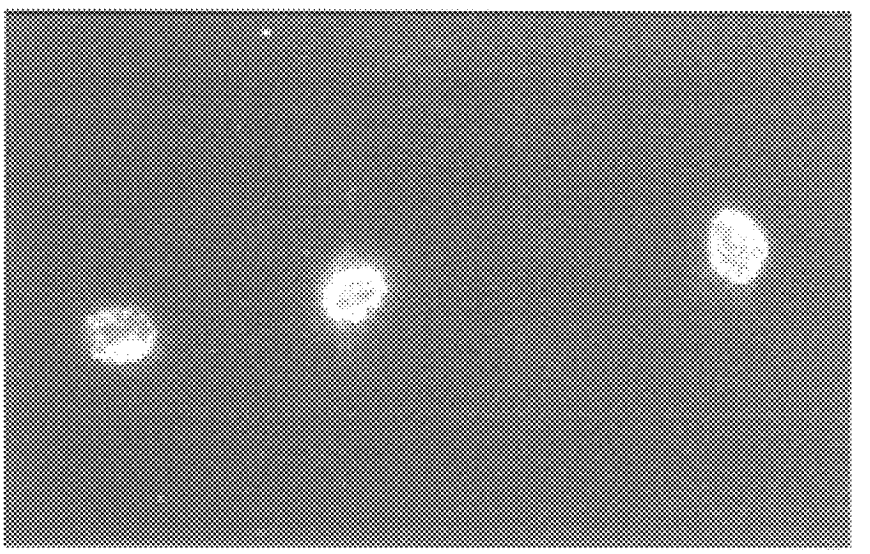


FIG. 10B

DNA CODING FOR SERINE/THREONINE KINASE

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a serine/threonine kinase, a DNA coding for said kinase, a recombinant vector comprising said DNA, a transformant transformed with said vector, and a process for preparing the serine/threonine kinase.

2. Prior Art

Various signals from the exterior of a cell are transmitted through receptors on the cell surface into the cell and ultimately into the nucleus. The signals transmitted into the nucleus activate transcription factors and, as a result, expression of a group of genes is induced or repressed to produce phenotypes such as cell proliferation, differentiation and cell death. Many transcription factors have been cloned and the structure of functional domains have been elucidated: MOLECULAR BIOLOGY OF THE CELL THIRD EDITION, pp. 401–469. These functional domains are known to include leucine zipper, helix-loop-helix and zinc finger structures. Among them, the leucine zipper structure is a motif commonly found in such transcription factors as Jun/Fos, ATF/CREB and C/EBP families and these transcription factors form homo- or hetero-dimers through their leucine zipper structures to control the transcription of specific genes: Hai, T. et al., Proc. Natl. Acad. Sci., USA, 88:3720–3724 (1991).

Recently, it is reported that the leucine zipper structure is also found other functional molecules than the transcription factors (Holzman, L. B. et al., J. Biol. Chem., 269:30808–30817, 1994), suggesting that the leucine zipper structure not only facilitates the binding between transcription factors but also acts generally as a protein-protein interactional domain in cells.

Therefore, identification of molecules interacting with the leucine zipper domain is considered to be useful in analyzing not only new functions of transcription factors but also functions of the leucine zipper structure in other molecules than the transcription factors.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a serine/threonine kinase, a DNA coding for said kinase, a recombinant vector comprising said DNA, a transformant transformed with said vector, and a process for preparing the serine/threonine kinase.

As a result of their eager studies based on the above described problems, the present inventors have succeeded in isolating a DNA coding for a serine/threonine kinase from cDNA libraries prepared from human placenta and mouse brain and thus completed the present invention.

Accordingly, the present invention is the following recombinant protein (a) or (b):

(a) a protein comprising the amino acid sequence as shown in SEQ ID NO: 1;

(b) a protein comprising an amino acid sequence having one or several amino acids deleted, substituted or added in the amino acid sequence as shown in SEQ ID NO: 1, and exhibiting a serine/threonine kinase activity.

Also, the present invention is the following recombinant protein (c) or (d):

(c) a protein comprising the amino acid sequence as shown in SEQ ID NO: 2;

(d) a protein comprising an amino acid sequence having one or several amino acids deleted, substituted or added in the amino acid sequence as shown in SEQ ID NO: 2, and exhibiting a serine/threonine kinase activity.

Further, the present invention is a DNA coding for said protein. The DNA include, for example, those comprising the base sequence as shown in SEQ ID NO: 3 or 4.

The present invention is also a recombinant vector comprising said DNA.

Still further, the present invention is a transformant transformed with the recombinant vector.

Finally, the present invention is a process for producing a serine/threonine kinase comprising cultivating the transformant in a culture medium and collecting the serine/threonine kinase from the resulting culture.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will hereinbelow be described in detail with reference to the drawings attached in which:

FIG. **1** shows the results of homology search in amino acid sequence between human ZIP kinase (SEQ ID NO:1) and mouse ZIP kinase (SEQ ID NO:2);

FIG. **2** shows the results of homology search in amino acid sequence between human ZIP kinase (SEQ ID NO:1), mouse ZIP kinase (SEQ ID NO:2), human DAP kinase, and mouse DAP kinase;

FIG. **3** is an electrophoretic photograph showing the results of western blot;

FIG. **4** is a photograph (the form of an organism) showing the results of colony formation in a selective medium;

FIG. **5** is an electrophoretic photograph showing the results of northern blot;

FIG. **6** is a photograph (the form of an organism) showing the results of colony formation in a selective medium;

FIG. **7** is a photograph of NIH3T3 (the form of an organism) showing the form of apoptosis;

FIG. **8** shows the fraction of LacZ expression cells showing the form of apoptosis;

FIGS. **9**A & **9**B are electrophoretic photographs showing the kinase activity of ZIP-kinase; and

FIGS. **10**A & **10**B are photographs (the form of an organism) showing the intracellular localization of ZIP-kinase.

DESCRIPTION OF THE INVENTION

The recombinant protein according to the present invention, hereinafter also referred to as "ZIP-kinase", is a protein molecule binding to the leucine zipper domain of a transcription factor called ATF4, and has a serine/threonine kinase activity. The ZIP-kinase is a novel nuclear serine/threonine kinase having the leucine zipper structure and has an activity to induce apoptosis. ATF4 is a leucine zipper type transcription factor which binds to cAMP response element (CRE) and belongs to the ATF/CREB family.

On the other hand, the DNA according to the present invention is obtained from cDNA libraries prepared from human placenta and mouse brain by screening them using so-called yeast two-hybrid system, and codes for ZIP-kinase. Hereinafter, the DNA will also be referred to as "ZIP-kinase DNA".

The DNA according to the present invention may be cloned in the following manner:

1. Cloning of ZIP-kinase DNA

(1) Preparation of CDNA Libraries From Human Placenta and Mouse Brain

Sources of mRNA may include tissues such as human placenta and mouse brain. Established cell lines from these tissues may also be used as the source.

The mRNA may be prepared by conventional procedures. For instance, total RNA may be obtained by treating the tissue or cell with a guanidine reagent, and poly (A+) RNA (mRNA) may be obtained by an affinity column method using oligo dT-cellulose or poly U-Sepharose on Sepharose 2B as a carrier, or by a batch method. Also, the poly (A+) RNA may further be fractionated by sucrose density-gradient centrifugation.

The resulting mRNA is used as a template to synthesize a single-stranded cDNA which is in turn used to synthesize a double-stranded cDNA. A recombinant plasmid is prepared from a suitable vector DNA and used to transform *Escherichia coli* or the like to yield a cDNA library.

Alternatively, the cDNA library may be commercially available (CLONETECH).

(2) Construction of Plasmid pAS2-1

From the cDNA library obtained in (1) above, a plasmid is prepared for screening for a desired clone.

Such a plasmid may be obtained by preparing a chimeric DNA by ligating a DNA coding for mouse ATF4 leucine zipper domain (amino acids 298 to 349 in the sequence of ATF4) to a DNA coding for GAL4 DNA binding domain, and ligating the chimeric DNA to bait plasmid pAS2-1.

(3) Screening

Then said plasmid is used to screen the CDNA library. In the screening, yeast two-hybrid system may be used. The yeast two-hybrid system is an experimental system capable of detecting interaction between proteins in yeast and is capable of screening the library for cDNA of a protein interacting with the desired protein (bait).

Positive clones may be selected using the growth in a selective medium free of hystidine, tryptophan or leucine and the activity of β-galactosidase.

(4) Determination of Base Sequence

The base sequence is determined for the resulting clone. The sequencing may be carried out by any known method such as Maxam-Gilbert method or the dideoxy method and is usually done using an automated base sequencer.

SEQ ID NOs: 1 and 2 exemplify the amino acid sequence of ZIP-kinase and the base sequence of ZIP-kinase DNA, respectively, according to the present invention. As far as a protein comprising said amino acid sequence has an activity as a serine/threonine kinase, there may be a mutation or variation of deletion, substitution and/or addition of one or several amino acids in said amino acid sequence as shown in SEQ ID NO: 1. For example, a protein having the amino acid sequence as shown in SEQ ID NO: 1 from which the first amino acid methionine has been deleted may also be included in the present invention.

Herein the serine/threonine kinase activity means an activity of transferring the terminal phosphate group of ATP to a certain amino acid (serine or threonine) of a protein. The introduction of mutation or variation may be carried out by any known method (Deng, W. P. et al., Anal. Biochem., 200: 81, 1992) or using a commercially available kit (Site-Directed Mutagenesis Kit of CLONETECH).

Once the base sequence of ZIP-kinase DNA according to the present invention is determined, ZIP-kinase DNA according to the present invention may then be obtained by chemical synthesis, or by PCR with various tissues-derived cDNA as a template, or hybridization of a DNA fragment having said base sequence as a probe.

2. Construction of Recombinant Vector and Transformant

(1) Construction of Recombinant Vector

The recombinant vector of the present invention may be obtained by ligating or inserting ZIP-kinase DNA of the present invention into an appropriate vector. The vector for inserting ZIP-kinase DNA of the present invention is not particularly limited as long as it can be replicated in a host, and may include plasmid DNA, phage DNA, etc. The plasmid DNA may be prepared from *E. coli* or Agrobacterium by the alkali extraction (Birnboim, H. C. & Doly, J., (1979) Nucleic acid Res., 7:1513) or modified method. Further, commercially available plasmids may also be used, for example, pUC18 (Takara Shuzo), pUC19 (Takara Shuzo), pBluescript SK+ (Stratagene), pGEM-T (Promega), pT7Blue (Novagen) and PBR322 (Takara Shuzo).

The phage DNA may include, for example, M13mp18, M13mp19, λ gt10, λgt11, etc.

When the DNA of the present invention is inserted into a vector, the purified DNA may first be cut with a suitable restriction enzyme and inserted into a restriction enzyme site or multi cloning site of a suitable vector DNA to ligate with the vector.

The DNA of the present invention should be incorporated into a vector such that the function of the DNA can be realized. In addition to a promoter and the DNA of the present invention, the vector of the present invention may comprise a terminator, a ribosome-binding sequence and the like. The terminator may be a stop codon such as TGA, TAG or TAA and the ribosome-binding sequence may be a leader sequence.

(3) Preparation of Transformant

The transformant of the present invention may be obtained by introducing the recombinant expression vector of the present invention into a host such that the desired gene can be expressed therein.

The host is not particularly limited so long as the DNA of the present invention can be expressed and may include, for example, bacteria belonging to the genus Escherichia or Bacillus, such as *Escherichia coli* and *Bacillus subtillus*; yeast, such as *Saccharomyces cerevisiae* and *Saccharomyces pombe*; animal cells, such as COS and CHO cells; and insect cells, such as Sf9.

When a bacterium, such as *E. coli*, is used as a host, it is preferred that the vector of the present invention is capable of autonomously replicating in said bacterium and comprises a promoter, a ribosome-binding sequence, the DNA of the present invention, and a transcription terminating sequence. The vector may also comprise a gene controlling the promoter.

For example, pET and pGEX (Pharmacia) may be used as the expression vector.

Any promoter may be used so long as the expression can be effected in the host such as *E. coli*. A promoter derived from *E. coli* or phage, such as trp, lac, PL or PR promoter, may be used. An artificially designed and modified promoter, such as T7 or T3, may also be used.

The method for introducing the recombinant vector into a bacterium is not particularly limited so long as a DNA can be introduced into a bacterium. For example, the method using calcium ion (Proc. Natl. Acad. Sci., USA, 69, 2110–2114 (1972)) and the electroporation method may be used.

When a yeast is used as a host, YEp13, YEp24 and YCp50 may be used as the expression vector. The promoter used is

not particularly limited so long as the expression in the yeast can be effected, and may include, for example, gal1, gal10, heat shock protein, MFα1 and SV40 promoters.

The method for introducing the recombinant vector into a yeast is not particularly limited so long as a DNA can be introduced into a yeast, and include, for example, the electroporation method (Methods Enzymol., 194, 182–187 (1990)), the spheroplast method (Proc. Natl. Acad. Sci., USA, 84, 1929–1933 (1978)), and the lithium acetate method (J. Bacteriol., 153, 163–168 (1983)).

When an animal cell is used as a host, an expression vector, such as pcDNAI/Amp or pcDNAI (Invitrogen) is used. The promoter used may also be the early gene promoter of human cytomegalovirus.

The method for introducing the recombinant vector into an animal cell may include, for example, the electroporation method, the calcium phosphate method and the lipofection method.

The recombinant vectors of the present invention (one vector containing ZIP-kinase DNA from human placenta and another vector containing ZIP-kinase DNA from mouse brain) have been introduced into *E. coli* DH5, *E. coli* (hZIP-kinase) DH5 and *E. coli* (mZIP-kinase) DH5, respectively, and deposited at National Institute of Bioscience and Human-Technology (NIBH), Agency of Industrial Science and Technology, 1–3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken 305, Japan on Sep. 25, 1997 under Accession Nos. FERM BP-6487 and FERM BP-6488, respectively.

3. Production of ZIP-kinase

ZIP-kinase of the present invention may be obtained by cultivating the transformant in a culture medium and collecting from the resulting culture.

The transformant of the present invention may be cultivated in a culture medium by any method conventionally used to cultivate a host.

The culture medium for cultivating a transformant obtained from a microorganism such as *E. coli* or yeast as a host may be either a natural or synthetic medium so long as it contains a carbon source, a nitrogen source and inorganic salts which can be utilized by the microorganism and the transformant can efficiently be cultivated.

The carbon source used may include carbohydrates, such as glucose, fructose, sucrose, starch, and dextrose; organic acids, such as acetic acid and propionic acid; and alcohols, such as ethanol and propanol.

The nitrogen source which may be used includes ammonia; ammonium salts of inorganic or organic acids, such as ammonium chloride, ammonium sulfate, ammonium acetate and ammonium phosphate; other nitrogen containing compounds; peptone, meat extract, corn steep liquor, and yeast extract.

The minerals which may be used include monobasic potassium phosphate, dibasic potassium phosphate, magnesium phosphate, magnesium sulfate, sodium chloride, ferrous sulfate, manganese sulfate, copper sulfate, calcium carbonate, calcium chloride, and disodium phosphate.

The cultivation is generally carried out at 37° C. for 12 to 18 hours under aerobic conditions, such as shaking culture and aerated spinner culture. During the cultivation, pH is kept at 7.0 to 7.5. The pH is adjusted with an inorganic or organic acid or alkaline solution, or carbonic acid gas.

During the cultivation, an antibiotic such as ampicillin or tetracycline may optionally be added to the medium.

When a microorganism transformed with an expression vector comprising an inducible promoter is cultivated, an inducer may be added to the medium, if necessary. For example, when a microorganism transformed with an expression vector comprising Lac promoter is cultivated, isopropyl-β-D-thiogalactopyranoside (IPTG) or the like may be added to the medium. When a microorganism transformed with an expression vector comprising trp promoter is cultivated, indole-acrylic acid (IAA) may be added to the medium.

When a transformant obtained from an animal cell as a host is cultivated, a conventional culture medium such as RPMI 1640 or DMEM medium or these media to which fetal bovine serum is added may be used.

The cultivation is generally carried out at 37° C. for 1 to 3 days in the presence of 5% CO_2.

During the cultivation, an antibiotic such as kanamycin or penicillin may be added to the medium.

After the cultivation, when ZIP-kinase of the present invention is produced in the host cell, the ZIP-kinase is extracted by disruption of the cell. When ZIP-kinase of the present invention is produced in the exterior of the cell, the culture may be directly used as it is, or the ZIP-kinase of the present invention may be isolated and purified from the culture, after removing the cell by centrifugation, using any conventional biochemical methods generally used in the isolation and purification of proteins, such as ammonium sulfate precipitation, gel chromatography, ion exchange chromatography and affinity chromatography, singly or in any combination thereof.

EXAMPLES

The present invention will be further illustrated by the following examples. However, the scope of the present invention is not limited to these examples.

Example 1

Cloning of ZIP-kinase DNA

(1) Preparation of cDNA Library

A commercially available cDNA (CLONETECH) was used in the present invention.

(2) Construction of Plasmid

DNA coding for leucine zipper domain of mouse ATF4 was obtained by PCR method.

The composition of the PCR reaction was 1.0 μg DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.5 μM primer, and 1 U Taq.

The following primers were used:

Sense primer: 5'-GGGAATTCGCGGAGCAGGAGGCT-3' (SEQ ID NO: 5)

Antisense primer: 5'-GGGGATCCCTAGGGGACCCTTTTCTA-3' (SEQ ID NO: 6).

PCR reaction was first carried out at 94° C. for 1 minute. Then, 25 cycles of reactions at 94° C. for 20 seconds, at 56° C. for 20 seconds and at 72° C. for 30 seconds were carried out. Finally, the reaction at 72° C. for 10 minutes was effected.

The PCR products were cut with EcoRI/BamHI and inserted into EcoRI/BamHI site of pAS2-1 vector. The plasmids were used to transform *E. coli* DH5α and purified by means of a commercially available kit (Wizard miniprep: Promega) based on alkali-SDS method. This plasmid capable of expressing a fused protein of GAL4 DNA binding domain in yeast was used as a bait.

(3) Screening

Yeast strain Y190 was transformed with the plasmid as a bait using MATCHMAKER Two-Hybrid System kit of CLONETECH. Transformants were selected by growth in

tryptophan(−) medium as an index. Further, cDNA libraries capable of expressing a fusion protein with GAL4 transcription activating domain (CLONETECH, mouse brain and human placenta MATCHMAKER cDNA a libraries) were transformed. Transformants can grow in tryptophan(−), leucine(−) medium. Further, since reporter genes, HIS3 and LacZ genes, were transcribed if the bait bound to the DNA coding for the protein from the library, positive clones can grow in tryptophan(−), leucine(−), histidine(−) medium and provides blue color in the presence of X-gal because of their β-galactosidase activity. Plasmids were purified from the positive clones using MATCHMAKER Two-Hybrid System kit of CLONETECH, and used to transform *E. coli*. Plasmids were purified from the resulting transformants and the base sequences thereof were determined (ABI model 377). The resulting base sequences were searched for homology using GenBank, EMBL, DDBJ data base.

As a result, those having high homology (20% or higher) with the previously reported C/EBP family, AP-1 family and genes having leucine zipper structure were identified as novel genes. Seven (7) and 2 clones of such genes were obtained from mouse brain and human placenta CDNA libraries, respectively. All these genes were derived from an identical gene.

(4) Determination of Base Sequence

The thus obtained gene was considered to code for a kinase and the DNA coding for this novel kinase was designated as ZIP-kinase DNA (Zipper Interacting Protein Kinase DNA). The base sequence of the full length ZIP-kinase DNA was determined.

The base sequences of ZIP-kinase DNA obtained from human placenta and mouse brain are shown in SEQ ID NOs: 3 and 4, respectively. The amino acid sequences encoded by the base sequences of SEQ ID NOs: 3 and 4 are shown in SEQ ID NOs: 1 and 2, respectively.

The amino acid sequence encoded by ZIP-kinase DNA obtained from human placenta (human ZIP-kinase) and the amino acid sequence encoded by ZIP-kinase DNA obtained from mouse brain (mouse ZIP-kinase) were searched for homology therebetween and the leucine zipper domain and serine/threonine kinase domain were found in the C- and N-terminal of the respective amino acid sequences, respectively (FIG. **1**). Further, mouse and human ZIP-kinases consisted of 448 and 454 amino acids, respectively, and the homology between mouse and human was 84.9% at amino acid level.

Moreover, the kinase domains of the ZIP-kinases showed high homology with DAP-kinases positively controlling apoptosis caused by IFN-γ, suggesting that these kinases form a new family (FIG. **2**).

Example 2

Construction of Recombinant Vector and Preparation of Transformant

To construct a recombinant vector of ZIP-kinase DNA, cDNA coding for ZIP-kinase was synthesized by PCR method.

The PCR reaction mixture and primers used were as follows.

The composition of the PCR reaction was 1.0 μg DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.5 μM primer, and 1 U Taq.

Sense primer: 5'-GGGTCGACCAC CATGGCTTAC CCATACGATG TTCCAGATTA CGCTATGTCC ACATTCAGGC AA-3' (SEQ ID NO: 7)

Antisense primer: 5'-GGGTCGACTA GCGCACGCCG CACTCAGCCT GC-3' (SEQ ID NO: 8)

PCR reaction was first carried out at 96° C. for 1 minute. Then, 30 cycles of reactions at 96° C. for 30 seconds, at 56° C. for 30 seconds and at 72° C. for 1 minute were carried out. Finally, the reaction at 72° C. for 10 minutes was effected.

The resulting PCR products were cut with SalI, inserted into expression vector pEF-BOS (Takara, Ligation kit), and used to transform *E. coli* DH5 (TOYOBO). Plasmids were purified from the *E. coli* (Promega, Wizard miniprep) and confirmed by DNA sequence (ABI, model 377).

A DNA coding for a variant of ZIP-kinase in which the 42nd amino acid lysine in the amino acid sequence as shown in SEQ ID NO: 2 was changed to alanine, hereinafter referred to as "ZIP-kinase K42A", was constructed by using Site-Directed Mutagenesis Kit of CLONETECH. Also, a DNA coding for another variant in which the 422nd and 429th amino acids valine and the 436th amino acid leucine in the amino acid sequence as shown in SEQ ID NO: 2 were changed to alanines, hereinafter referred to as "ZIP-kinase LA", was similarly constructed.

Example 3

Function of DNA of the Present Invention

(1) Binding of ZIP-kinase to ATF4 in Cells

Whether ZIP-kinase binds to ATF4 in cells as well or not was investigated.

First, DNA coding for mouse ZIP-kinase (309–448 amino acids in the amino acid sequence as shown in SEQ ID NO: 2) was inserted into expression vector pEF-BOS. Thus, a tag of the transcription factor Myc was provided at the N-terminal end of ZIP-kinase, whereby a DNA coding for Myc-ZIP-kinase complex was designated to have the tag as an epitope to construct the vector (pEF-BOS-Myc-ZIP-kinase). Also, an expression vector (pEF-BOS-FLAG-ATF4) was constructed comprising a DNA coding for human ATF4 (full length)-FLAG complex in which FLAG epitope had been added to the N-terminal end of human ATF4.

These vectors were transiently introduced into COS-7 cell line by the lipofection method and expressed (FIG. **3** in which lanes 1, 4, 7 and 10 represent FLAG-ATF4; lanes 2, 5, 8 and 11 Myc-ZIP-kinase; lanes 3, 6, 9 and 12 FLAG-ATF4 and Myc-ZIP-kinase). 36 hours after the introduction, the cells were collected and solubilized with 0.5% Nonidet P-40 lysis buffer. The resulting solubilized cell (WCE: whole cell extract) was developed in SDS-PAGE, and transferred to nitrocellulose membrane. Western blot analysis was done using anti-FLAG monoclonal antibody (FIG. **3**, lanes 1, 2 and 3) and anti-Myc monoclonal antibody (lanes 7, 8 and 9) to confirm the expression of Myc-ZIP-kinase and FLAG-ATF4.

Subsequently, the WCE was immunoprecipitated with anti-Myc monoclonal antibody and the precipitate was subjected to the western blot analysis using anti-FLAG monoclonal antibody, attempting to detect co-immunoprecipitation of Myc-ZIP-kinase and FLAG-ATF4 (FIG. **3**, lanes 4 to 6).

As a result, a band of FLAG-ATF4 was detected in lane 6 (FIG. **3**). For further confirmation, the WCE was then immunoprecipitated with anti-FLAG monoclonal antibody and the precipitate was subjected to the western blot analysis using anti-Myc monoclonal antibody (lanes 10, 11 and 12). A band of Myc-ZIP-kinase immunoprecipitated with FLAG-ATF4 was found only in lane 12.

Thus, it was shown that ZIP-kinase and ATF4 bind to each other in cells as well.

From this result that ZIP-kinase and ATF4 binds to each other, it may be considered that ATF4 may possibly control the activity of ZIP-kinase.

(2) Determination of Domain Necessary for Binding of ZIP-kinase to ATF4

The site to which ZIP-kinase and ATF4 bind was determined using yeast two-hybrid system. First, variants of mouse ZIP-kinase were prepared: 1) amino acids 278 to 448 of ZIP-kinase (ZIP-kinase 278–448); 2) leucine zipper domain of ZIP-kinase (amino acids 398 to 448) (ZIP-kinase LZ); and 3) a variant of ZIP-kinase in which valine and leucine in the leucine zipper domain were substituted with alanine (ZIP-kinase LA). Each of these variants was designed to produce a chimeric protein with GAL4 trans activating domain, and DNA coding for said chimeric protein was inserted into pACT2 and introduced into yeast strain Y190 together with pAS2-1-ATF4 LZ. The strain was cultivated on histidine+, tryptophan−, leucine−, and histidine−, tryptophan−, leucine− selective media.

Yeast containing DNA coding for ZIP-kinase 278–448 and yeast containing DNA coding for ZIP-kinase LZ could form a colony on the histidine−, tryptophan−, leucine− medium, indicating that ZIP-kinase bound to ATF4 through leucine zipper domain present at the C-terminal (FIG. **4**). Further, when valine and leucine in the leucine zipper domain structure were substituted with alanine, the binding to ATF4 was no longer found.

Accordingly, it has been elucidated that ZIP-kinase and ATF4 bind to each other through their respective leucine zipper domain.

(3) Expression of ZIP-kinase in Each Tissue

Northern blot analysis was carried out to investigate the expression of ZIP-kinase in each tissue.

As shown in FIG. **5**, mRNA of ZIP-kinase (about 1.4 kb) was distributed almost all tissues investigated. However, only low expression was observed in the spleen.

(4) Confirmation of Formation of Homodimer of ZIP-kinase

The leucine zipper domain present at the C-terminal of ZIP-kinase is considered to be a domain through which proteins bind to each other. Whether ZIP-kinase forms a homodimer or not was investigated. Plasmid pAS2-1 into which DNA coding for leucine zipper domain of ZIP-kinase was inserted, and plasmid pACT2 into which DNA coding for the leucine zipper domain of ZIP-kinase and a variant in which valine and leucine in said domain were substituted with alanine, were co-introduced into yeast and colony formation was observed in a selective medium.

As shown in FIG. **6**, only yeast co-expressing the ZIP-kinase leucine zipper domain could grow in histidine−, tryptophan−, leucine− medium. Thus, it has been elucidated that ZIP-kinase forms a homodimer through its leucine zipper structure.

(5) Induction of Apoptosis by ZIP-kinase

It has been shown that DAP-kinase, which has high homology with kinase domain of ZIP-kinase, induces apoptosis in HeLa cell. Whether ZIP-kinase has an apoptosis activity was investigated.

RNA wild type ZIP-kinase tagged with HA (pEF-BOS-HA-ZIP-kinase), a variant thereof in which lysine (42nd amino acid), which is present in ZIP-kinase subdomain II and conserved in other kinases, was substituted with alanine (pEF-BOS-HA-ZIP-kinase K42A), and a variant in which valine and leucine in the leucine zipper domain were substituted with alanine (pEF-BOS-HA-ZIP-kinase LA) were prepared, and DNA coding for each of these proteins was transiently introduced into NIH 3T3 cell together with LacZ expression vector (pEF-BOS-LacZ). After 36 hours from the introduction, X-gal staining was effected.

As a result, a form of cells stained blue was observed under a microscope (FIG. **7**). As compared with a control pEF-BOS-mock (FIG. **7**, left, upper), the cell into which the wild type ZIP-kinase was introduced (FIG. **7**, right, upper) exhibited a typical form of apoptosis associated with agglomeration of nucleus. The fraction of LacZ expression cells showing the apoptosis form was measured to be 44.9% (FIG. **8**).

On the other hand, such change of form was not observed in the ZIP-kinase-K42A variant (FIG. **7**, left, lower) and there was no significant difference in the fraction of apoptosis between the vaiant and control. Further, in the ZIP-kinase-LA (FIG. **7**, right, lower), some cells caused apoptosis but the fraction thereof was significantly reduced as compared with the wild type.

From the above results, the kinase activity of ZIP-kinase is considered to be essential for the induction of apoptosis by the expression of ZIP-kinase. Further, since apoptosis was suppressed in vatiants in which a homodimer between ZIP-kinases was inhibited, it is suggested that ZIP-kinases form a homodimer to become an activated form.

(6) Kinase Activity of ZIP-kinase

Whether ZIP-kinase indeed has an activity as a kinase was investigated.

Each of pEF-BOS-HA-ZIP-kinase, pEF-BOS-HA-ZIP-kinase K42A, and pEF-BOS-HA-ZIP-kinase LA was transiently introduced into COS-7 cell, and 36 hours later, the cell was collected and solubilized with 0.5% Nonidet P-40 lysis buffer. The solubilized cell was immunoprecipitated with anti-HA monoclonal antibody and the kinase activity in the precipitate was detected by in vitro kinase assay (FIG. **9**A).

As a result, a band of phosphorylation by ZIP-kinase was observed at about 50 kDa in the wild type ZIP-kinase (FIG. **9**A, lane of HA-ZIP-kinase), while no band corresponding thereto was observed in ZIP-kinase K42A. On the other hand, a phosphorylation band was observed in ZIP-kinase LA. However, when the expression of HA-ZIP-kinase and its variant in the solubilized cell was checked by the western blotting using anti-HA monoclonal antibody, the amount of HA-ZIP-kinase expressed was markedly reduced as compared with the other two variants (FIG. **9**B). From this result, it may be considered that the kinase activity observed in ZIP-kinase LA would be very weak as compared with the wild type.

Further, the amount of wild type ZIP-kinase expressed was low in COS-7 cells; this is considered to be resulted from some lethal effect, such as apoptosis, of ZIP-kinase on COS-7 cells.

(7) Localization of ZIP-kinase in Cells

Knowledge of intracellular localization of ZIP-kinase would be considered to be very effective in analyzing the functions of ZIP-kinase. The present inventors have investigated the localization of ZIP-kinase using a confocal laser microscope.

An expression vector (pEF-BOS-FLAG-ATF4) comprising DNA coding for ATF4 tagged with FLAG or another vector (pEF-BOS-FLAG-ZIP-kinase K42A) coding for ZIP-kinase K42A tagged with FLAG was transiently introduced into COS-7 cells. After 36 hours, the cells were fixed, reacted with anti-FLAG monoclonal antibody, and stained using FITC-labelled anti-mouse immunoglobulin antibody as a secondary antibody.

When observed under the confocal laser microscope, the cytoplasm was not stained and the nucleus was stained in the FLAG-ATF4 introduced cells (FIG. **10**A). When the localization of FLAG-ZIP-kinase was similarly investigated, the

same staining pattern as in ATF4 was observed, confirming that it was localized in the nucleus (FIG. **10**B).

Accordingly, it could be concluded that the ZIP-kinase is a novel nuclear serine/threonine kinase.

Advantages of the Invention

According to the present invention, there are provided a serine/threonine kinase, a DNA coding for said kinase, a recombinant vector comprising said DNA, and a transformant transformed with said vector, and a process for the preparation of the serine/threonine kinase.

Since the ZIP-kinase has a function of inducing apoptosis, the ZIP-kinase and DNA coding for said kinase are useful in being utilizable as a gene therapeutical agent against a cancer and as an anti-cancer agent.

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Lys Gly Thr Gly Lys Glu Tyr Ala Ala Lys Phe Ile Lys Lys Arg Arg
         35                  40                  45

Leu Ser Ser Ser Arg Arg Gly Val Ser Arg Glu Glu Ile Glu Arg Glu
     50                  55                  60

Val Asn Ile Leu Arg Glu Ile Arg His Pro Asn Ile Ile Thr Leu His
 65                  70                  75                  80

Asp Ile Phe Glu Asn Lys Thr Asp Val Val Leu Ile Leu Glu Leu Val
                 85                  90                  95

Ser Gly Gly Glu Leu Phe Asp Phe Leu Ala Glu Lys Glu Ser Leu Thr
            100                 105                 110

Glu Asp Glu Ala Thr Gln Phe Leu Lys Gln Ile Leu Asp Gly Val His
        115                 120                 125

Tyr Leu His Ser Lys Arg Ile Ala His Phe Asp Leu Lys Pro Glu Asn
    130                 135                 140

Ile Met Leu Leu Asp Lys Asn Val Pro Asn Pro Arg Ile Lys Leu Ile
145                 150                 155                 160

Asp Phe Gly Ile Ala His Lys Ile Glu Ala Gly Asn Glu Phe Lys Asn
                165                 170                 175

Ile Phe Gly Thr Pro Glu Phe Val Ala Pro Glu Ile Val Asn Tyr Glu
            180                 185                 190

Pro Leu Gly Leu Glu Ala Asp Met Trp Ser Ile Gly Val Ile Thr Tyr
        195                 200                 205

Ile Leu Leu Ser Gly Ala Ser Pro Phe Leu Gly Glu Thr Lys Gln Glu
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Thr Leu Thr Asn Ile Ser Ala Val Asn Tyr Asp Phe Asp Glu Glu Tyr
225                 230                 235                 240

Phe Ser Asn Thr Ser Glu Leu Ala Lys Asp Phe Ile Arg Arg Leu Leu
                245                 250                 255

Val Lys Asp Pro Lys Arg Arg Met Thr Ile Ala Gln Ser Leu Glu His
            260                 265                 270

Ser Trp Ile Lys Ala Ile Arg Arg Arg Asn Val Arg Gly Glu Asp Ser
        275                 280                 285

Gly Arg Lys Pro Glu Arg Arg Arg Leu Lys Thr Thr Arg Leu Lys Glu
    290                 295                 300

Tyr Thr Ile Lys Ser His Ser Ser Leu Pro Pro Asn Asn Ser Tyr Ala
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Glu Gly Leu Arg Glu Leu Gln Arg Ser Arg Arg Leu Cys His Glu Asp
            340                 345                 350

Val Glu Ala Leu Ala Ala Ile Tyr Glu Glu Lys Glu Ala Trp Tyr Arg
        355                 360                 365

Glu Glu Ser Asp Ser Leu Gly Gln Asp Leu Arg Arg Leu Arg Gln Glu
    370                 375                 380

Leu Leu Lys Thr Glu Ala Leu Lys Arg Gln Ala Gln Glu Glu Ala Lys
385                 390                 395                 400

Gly Ala Leu Leu Gly Thr Ser Gly Leu Lys Arg Arg Phe Ser Arg Leu
                405                 410                 415

Glu Asn Arg Tyr Glu Ala Leu Ala Lys Gln Val Ala Ser Glu Met Arg
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Lys Gly Thr Gly Met Glu Tyr Ala Ala Lys Phe Ile Lys Lys Arg Arg
         35                  40                  45

Leu Pro Ser Ser Arg Arg Gly Val Ser Arg Glu Glu Ile Glu Arg Glu
     50                  55                  60

Val Ser Ile Leu Arg Glu Ile Arg His Pro Asn Ile Ile Thr Leu His
 65                  70                  75                  80

Asp Val Phe Glu Asn Lys Thr Asp Val Val Leu Ile Leu Glu Leu Val
                 85                  90                  95

Ser Gly Gly Glu Leu Phe Asp Phe Leu Ala Glu Lys Glu Ser Leu Thr
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Glu Asp Glu Ala Thr Gln Phe Leu Lys Gln Ile Leu Asp Gly Val His
        115                 120                 125

Tyr Leu His Ser Lys Arg Ile Ala His Phe Asp Leu Lys Pro Glu Asn
    130                 135                 140

Ile Met Leu Leu Asp Lys His Ala Ala Ser Pro Arg Ile Lys Leu Ile
145                 150                 155                 160

Asp Phe Gly Ile Ala His Arg Ile Glu Ala Gly Ser Glu Phe Lys Asn
                165                 170                 175

Ile Phe Gly Thr Pro Glu Phe Val Ala Pro Glu Ile Val Asn Tyr Glu
            180                 185                 190

Pro Leu Gly Leu Glu Ala Asp Met Trp Ser Ile Gly Val Ile Thr Tyr
        195                 200                 205

Ile Leu Leu Ser Gly Ala Ser Pro Phe Leu Gly Glu Thr Lys Gln Glu
    210                 215                 220

Thr Leu Thr Asn Ile Ser Ala Val Asn Tyr Asp Phe Asp Glu Glu Tyr
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225                 230                 235                 240

Phe Ser Ser Thr Ser Glu Leu Ala Lys Asp Phe Ile Arg Arg Leu Leu
                245                 250                 255

Val Lys Asp Pro Lys Arg Arg Met Thr Ile Ala Gln Ser Leu Glu His
            260                 265                 270

Ser Trp Ile Lys Val Arg Arg Arg Glu Asp Gly Ala Arg Lys Pro Glu
        275                 280                 285

Arg Arg Arg Leu Arg Ala Ala Arg Leu Arg Glu Tyr Ser Leu Lys Ser
    290                 295                 300

His Ser Ser Met Pro Arg Asn Thr Ser Tyr Ala Ser Phe Glu Arg Phe
305                 310                 315                 320

Ser Arg Val Leu Glu Asp Val Ala Ala Ala Glu Gln Gly Leu Arg Glu
                325                 330                 335

Leu Gln Arg Gly Arg Arg Gln Cys Arg Glu Arg Val Cys Ala Leu Arg
            340                 345                 350

Ala Ala Ala Glu Gln Arg Glu Ala Arg Cys Arg Asp Gly Ser Ala Gly
        355                 360                 365

Leu Gly Arg Asp Leu Arg Arg Leu Arg Thr Glu Leu Gly Arg Thr Glu
    370                 375                 380

Ala Leu Arg Thr Arg Ala Gln Glu Glu Ala Arg Ala Ala Leu Leu Gly
385                 390                 395                 400

Ala Gly Gly Leu Lys Arg Arg Leu Cys Arg Leu Glu Asn Arg Tyr Asp
                405                 410                 415

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                                      Met Ser Thr Phe Arg Gln Glu
                                        1               5

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Asp Val Glu Asp His Tyr Glu Met Gly Glu Glu Leu Gly Ser Gly Gln
         10                  15                  20

ttt gcg atc gtg cgg aag tgc cgg cag aag ggc acg ggc aag gag tac      210
Phe Ala Ile Val Arg Lys Cys Arg Gln Lys Gly Thr Gly Lys Glu Tyr
     25                  30                  35

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Ala Ala Lys Phe Ile Lys Lys Arg Arg Leu Ser Ser Ser Arg Arg Gly
 40                  45                  50                  55

gtg agc cgg gag gag atc gag cgg gag gtg aac atc ctg cgg gag atc      306
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             75                  80                  85

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ctc aag cag atc ctg gac ggc gtt cac tac ctg cac tct aag cgc atc      498
Leu Lys Gln Ile Leu Asp Gly Val His Tyr Leu His Ser Lys Arg Ile
120                 125                 130                 135

gca cac ttt gac ctg aag ccg gaa aac atc atg ctg ctg gac aag aac      546
Ala His Phe Asp Leu Lys Pro Glu Asn Ile Met Leu Leu Asp Lys Asn
                140                 145                 150

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Val Pro Asn Pro Arg Ile Lys Leu Ile Asp Phe Gly Ile Ala His Lys
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Val Ala Pro Glu Ile Val Asn Tyr Glu Pro Leu Gly Leu Glu Ala Asp
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200                 205                 210                 215

ccg ttc ctg ggc gag acc aag cag gag acg ctc acc aac atc tca gcc      786
Pro Phe Leu Gly Glu Thr Lys Gln Glu Thr Leu Thr Asn Ile Ser Ala
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Val Asn Tyr Asp Phe Asp Glu Glu Tyr Phe Ser Asn Thr Ser Glu Leu
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        250                 255                 260

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    265                 270                 275

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280                 285                 290                 295

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Gln Gln Lys Gly Thr Gly Met Glu Tyr Ala Ala Lys Phe Ile Lys Lys
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    80                  85                  90

ctg gtg tcc ggt ggc gag ctt ttc gac ttc ctg gcc gag aag gag tca      339
Leu Val Ser Gly Gly Glu Leu Phe Asp Phe Leu Ala Glu Lys Glu Ser
 95                 100                 105                 110

ttg acg gag gat gag gcc acg cag ttc ctc aaa caa atc cta gac ggt      387
Leu Thr Glu Asp Glu Ala Thr Gln Phe Leu Lys Gln Ile Leu Asp Gly
                115                 120                 125

gtc cac tac ctg cac tcc aag cgc atc gca cac ttt gac ctg aag ccc      435
Val His Tyr Leu His Ser Lys Arg Ile Ala His Phe Asp Leu Lys Pro
            130                 135                 140
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gag aac atc atg ttg ctg gac aag cac gca gcc agc ccc cgc att aag      483
Glu Asn Ile Met Leu Leu Asp Lys His Ala Ala Ser Pro Arg Ile Lys
        145                 150                 155

ctc atc gac ttt ggc atc gcg cac agg atc gag gct ggc agc gag ttc      531
Leu Ile Asp Phe Gly Ile Ala His Arg Ile Glu Ala Gly Ser Glu Phe
    160                 165                 170

aag aac atc ttt ggc aca ccc gag ttt gtc gcc ccc gag atc gtg aac      579
Lys Asn Ile Phe Gly Thr Pro Glu Phe Val Ala Pro Glu Ile Val Asn
175                 180                 185                 190

tat gag cca ctt ggc ttg gag gct gac atg tgg agc att ggc gtc atc      627
Tyr Glu Pro Leu Gly Leu Glu Ala Asp Met Trp Ser Ile Gly Val Ile
                195                 200                 205

acc tac atc ctc ctg agc gga gcg tcc cca ttc ctg ggc gag acc aag      675
Thr Tyr Ile Leu Leu Ser Gly Ala Ser Pro Phe Leu Gly Glu Thr Lys
            210                 215                 220

cag gag acg ctg acg aac atc tca gca gtg aac tat gac ttt gat gag      723
Gln Glu Thr Leu Thr Asn Ile Ser Ala Val Asn Tyr Asp Phe Asp Glu
        225                 230                 235

gaa tac ttc agc agc acc agc gag ctg gcc aag gac ttc atc cgc agg      771
Glu Tyr Phe Ser Ser Thr Ser Glu Leu Ala Lys Asp Phe Ile Arg Arg
    240                 245                 250

ctg ctg gtc aaa gac ccc aag agg agg atg acc atc gca cag agc ctg      819
Leu Leu Val Lys Asp Pro Lys Arg Arg Met Thr Ile Ala Gln Ser Leu
255                 260                 265                 270

gag cat tcc tgg atc aag gtg cgc agg cgc gag gac ggc gcc cgg aag      867
Glu His Ser Trp Ile Lys Val Arg Arg Arg Glu Asp Gly Ala Arg Lys
                275                 280                 285

cca gag cga cgg cgg ctg cgc gcc gcg cgc ctg cgc gag tac agc ctc      915
Pro Glu Arg Arg Arg Leu Arg Ala Ala Arg Leu Arg Glu Tyr Ser Leu
            290                 295                 300

aag tcc cac tcg agc atg ccg cgc aac acg agc tac gcc agc ttc gag      963
Lys Ser His Ser Ser Met Pro Arg Asn Thr Ser Tyr Ala Ser Phe Glu
        305                 310                 315

cgc ttc tca cgc gtg ctg gag gac gtg gcg gcg gca gag cag ggg ctg     1011
Arg Phe Ser Arg Val Leu Glu Asp Val Ala Ala Ala Glu Gln Gly Leu
    320                 325                 330

cgc gag ctg cag cga ggc agg cgc cag tgc cgg gag cgc gtg tgt gcg     1059
Arg Glu Leu Gln Arg Gly Arg Arg Gln Cys Arg Glu Arg Val Cys Ala
335                 340                 345                 350

ctg cgc gcg gcc gcc gag cag cgg gag gcg cgc tgc cgc gac ggg agc     1107
Leu Arg Ala Ala Ala Glu Gln Arg Glu Ala Arg Cys Arg Asp Gly Ser
                355                 360                 365

gca ggg cta ggg cgc gac ctg cga cgc ctg cgc acg gag ctg ggg cgc     1155
Ala Gly Leu Gly Arg Asp Leu Arg Arg Leu Arg Thr Glu Leu Gly Arg
            370                 375                 380

acc gag gct ctg cgc acg cgc gcg cag gag gag gcg cgg gcg gcg ctg     1203
Thr Glu Ala Leu Arg Thr Arg Ala Gln Glu Glu Ala Arg Ala Ala Leu
        385                 390                 395

ttg ggt gcc ggg ggc ctg aag cgt cgc ctg tgt cgc ctg gag aac cgt     1251
Leu Gly Ala Gly Gly Leu Lys Arg Arg Leu Cys Arg Leu Glu Asn Arg
    400                 405                 410

tac gac gcg cta gcc gct cag gtg gcc gct gag gtg caa ttc gtg cgc     1299
Tyr Asp Ala Leu Ala Ala Gln Val Ala Ala Glu Val Gln Phe Val Arg
415                 420                 425                 430

gac ctg gtg cgt gcg ctg gag cag gaa cgg ctg cag gct gag tgc ggc     1347
Asp Leu Val Arg Ala Leu Glu Gln Glu Arg Leu Gln Ala Glu Cys Gly
                435                 440                 445

gtg cgc taggctgcgg caccccaga ccccgaccca cccccagaat aaagctgctt      1403
Val Arg
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tccacgtaaa aaaaaaaaaa aaaaaa 1429

<210> SEQ ID NO 5
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic oligonucleotides

<400> SEQUENCE: 5

gggaattcgc ggagcaggag gct 23

<210> SEQ ID NO 6
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic oligonucleotides

<400> SEQUENCE: 6

ggggatccct aggggaccct tttcta 26

<210> SEQ ID NO 7
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic oligonucleotides

<400> SEQUENCE: 7

gggtcgacca ccatggctta cccatacgat gttccagatt acgctatgtc cacattcagg 60

caa 63

<210> SEQ ID NO 8
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic oligonucleotides

<400> SEQUENCE: 8

gggtcgacta gcgcacgccg cactcagcct gc 32

What is claimed is:

1. A recombinant protein comprising the amino acid sequence selected from the group consisting of:

(a) a protein comprising the amino acid sequence as shown in SEQ ID NO:1; and

(b) a protein comprising an amino acid sequence having the amino acid sequence as shown in SEQ ID NO:1 in which the first amino acid is deleted.

2. A recombinant protein comprising the amino acid sequence selected from the group consisting of:

(a) a protein comprising the amino acid sequence as shown in SEQ ID NO:2; and

(b) a protein comprising an amino acid sequence having the amino acid sequence as shown in SEQ ID NO:2 in which the 42^{nd} amino acid residue is changed to alanine; and

(c) a protein comprising an amino acid sequence having the amino acid sequence as shown in SEQ ID NO:2 in which the 422^{nd}, 429^{th} and 436^{th} amino acid residue are changed to alanines.

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