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(54) Titre : DERIVE DE LA STROMALE HUMAINE FACTEUR 1 APLHA ET 1 BETA ET ADN ENCODANT CEUX-CI
(54) Title: HUMAN STROMAL DERIVED FACTOR 1 ALPHA AND 1 BETA AND DNAS ENCODING THE SAME

(57) Abrégé/Abstract:
The polypeptides of the present invention are produced and secreted in pro-B cells and may be used for diseases relating to undergrown or abnormal proliferation of hematopoietic cells, neuronal enhancement or depression, immunological enhancement and depression, for example, inflammatory diseases (rheumatoid arthritis, ulcerative colitis, etc.), hematopoietic stemcytopenia after bone marrow transplantation, leukocytopenia, thrombocytopenia, B lymphopenia and T lymphopenia after chemotherapy, anemia, infectious diseases, cancer, leukocytosis, AIDS, neurodegenerative diseases (Alzheimer, multiple sclerosis, etc.), prevention or treatment of neuronal injury, prevention or treatment of disorder of bone metabolism (osteoporosis, etc.) or tissue repair. The DNA of the present invention may be utilized as an important and essential template in preparing the polypeptides of the present invention which are expected to be useful for diagnosis and treatment of gene diseases.

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ABSTRACT OF THE DISCLOSURE

The polypeptides of the present invention are produced and secreted in pro-B cells and may be used for diseases relating to undergrown or abnormal proliferation of hematopoietic cells, neuronal enhancement or depression, immunological enhancement and depression, for example, inflammatory diseases (rheumatoid arthritis, ulcerative colitis, etc.), hematopoietic stemcytopenia after bone marrow transplantation, leukocytopenia, thrombocytopenia, B lymphopenia and T lymphopenia after chemotherapy, anemia, infectious diseases, cancer, leukocytosis, AIDS, neurodegenerative diseases (Alzheimer, multiple sclerosis, etc.), prevention or treatment of neuronal injury, prevention or treatment of disorder of bone metabolism (osteoporosis, etc.) or tissue repair. The DNA of the present invention may be utilized as an important and essential template in preparing the polypeptides of the present invention which are expected to be useful for diagnosis and treatment of gene diseases.

Human Stromal Derived Factor 1α and 1β and DNAs Encoding the Same

Field of the Invention

The present invention relates to novel polypeptides produced by the human pro-B cell line and DNA encoding these polypeptides.

Purpose of the Invention

The present invention relates to novel polypeptides produced by hematopoietic cells and DNA encoding them. Many kinds of growth and differentiation factors such as interleukin (IL) are known to be secreted from hematopoietic cells. This suggests that factors having similar or novel functions might be secreted therefrom in addition to presently known factors.

The present inventors have paid attention to this point and attempted to find novel factors (polypeptides) produced from hematopoietic cells. The factors of the present invention were screened by cross hybridization using mouse SDF-1 (Stromal Derived Factor 1; described in Japanese Patent Application No. 5-22098) cDNA as a probe and thus obtained human SDF-1 (2 kinds, α and β) produced from human pro-B cells to complete the present invention.

Polypeptides having sequences identical or highly homologous with that of the polypeptides of the present invention and the DNA encoding them could not be found by searching with a computer. Therefore, the polypeptide of the present invention and the DNA coding for the same are novel.

The present invention provides:

(1) a polypeptide having an amino acid sequence shown in SEQ. ID NO. 1,

(2) a DNA encoding the polypeptide described above (1),

(3) a DNA having a nucleotide sequence shown in SEQ. ID NO. 2,

(4) a DNA having a nucleotide sequence shown in SEQ. ID NO. 3,

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(5) a polypeptide having an amino acid sequence shown in SEQ. ID NO. 5,

(6) a DNA encoding the polypeptide described above (5),

(7) a DNA having a nucleotide sequence shown in SEQ. ID NO. 6, and

(8) a DNA having a nucleotide sequence shown in SEQ. ID NO. 7.

In another aspect, the present invention provides a stromal derived factor-1α (SDF-1α) polypeptide having the amino acid sequence as shown in SEQ. ID NO:1.

In another aspect, the present invention provides a stromal derived factor-1β (SDF-1β) polypeptide having the amino acid sequence as shown in SEQ. ID NO:5

In another aspect, the present invention provides a mature peptide of stromal derived factor-1α (SDF-1α) having an amino acid sequence as follows:

Lys Pro Val Ser Leu Ser Tyr Arg Cys Pro Cys Arg Phe Phe Glu
1 5 10 15
Ser His Val Ala Arg Ala Asn Val Lys His Leu Lys Ile Leu Asn
20 25 30
Thr Pro Asn Cys Ala Leu Gln Ile Val Ala Arg Leu Lys Asn Asn
35 40 45
Asn Arg Gln Val Cys Ile Asp Pro Lys Leu Lys Trp Ile Gln Glu
50 55 60
Tyr Leu Glu Lys Ala Leu Asn Lys.
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In another aspect, the present invention provides a mature peptide of stromal derived factor-1β (SDF-1β) having an amino acid sequence as follows:

Lys Pro Val Ser Leu Ser Tyr Arg Cys Pro Cys Arg Phe Phe Glu
1 5 10 15
Ser His Val Ala Arg Ala Asn Val Lys His Leu Lys Ile Leu Asn
20 25 30
Thr Pro Asn Cys Ala Leu Gln Ile Val Ala Arg Leu Lys Asn Asn
35 40 45
Asn Arg Gln Val Cys Ile Asp Pro Lys Leu Lys Trp Ile Gln Glu
50 55 60

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Tyr Leu Glu Lys Ala Leu Asn Lys Arg Phe Lys Met.
65 70

The present invention is concerned with polypeptides having the amino acid sequences shown in SEQ. ID NOS. 1 or 5, in substantially purified form, a homologue thereof, a fragment of the sequence or homologue of a fragment, and DNA encoding such a polypeptide. More particularly, the present invention relates to DNA having the nucleotide sequence shown in SEQ. ID NOS. 2 or 3, and 6 or 7 and DNA having a fragment which is selectively hybridizing to the nucleotide sequence shown in SEQ. ID NOS. 2 or 3, and 6 or 7.

A polypeptide of SEQ. ID NOS. 1 or 5 in substantially purified form will generally comprise the polypeptide in a preparation in which more than 90%, e.g. 95%, 98% or 99% of the polypeptide in the preparation is that of SEQ. ID NOS. 1 or 5.

A polypeptide homologue of the SEQ. ID NOS. 1 or 5 will be generally at least 70%, preferably at least 80 or 90% and more preferably at least 95% homologous to the polypeptide of SEQ. ID NO. 1 over a region of at least 20, preferably at least 30, for instance 40, 60 or 80 or more contiguous amino acids. Such polypeptide homologues will be referred to below as a polypeptide according to the invention.

Generally, fragments of SEQ. ID NOS. 1 or 5 or their homologues will be at least 10, preferably at least 15, for example 20, 25, 30, 40, 50 or 60 amino acids in length, and are also encompassed by the term "a polypeptide according to the invention" as used herein.

A DNA capable of selectively hybridizing to the DNA of SEQ. ID NOS. 2 or 3, and 6 or 7 will be generally at least 70%, preferably at least 80% or 90%

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and more preferably at least 95% homologous to the DNA of SEQ. ID NOS. 2 or 3 over a region of at least 20, preferably at least 30, for instance 40, 60 or 100 or more contiguous nucleotides. Such DNA will be encompassed by the term "DNA according to the invention".

Fragments of the DNA of SEQ. ID NOS. 2 or 3, 6 or 7 will be at least 15, preferably at least 20, for example 25, 30 or 40 nucleotides in length, and are also encompassed by the term "DNA according to the invention" as used herein.

A further embodiment of the invention provides replication and expression vectors comprising DNA according to the invention. The vectors may be, for example, plasmid, virus or phage vectors provided with an origin of replication, optionally a promoter for the expression of said DNA and optionally a regulator of the promoter. The vector may contain one or more selectable marker genes, for example an ampicillin resistance gene. The vector may be used in vitro, for example in the production of RNA corresponding to the DNA, or used to transfect or transform a host cell.

A further embodiment of the invention provides host cells transformed or transfected with vectors for the replication and expression of DNA according to the invention, including DNA SEQ. ID NOS. 2 or 3, and 6 or 7, or the open reading frame thereof. The cells will be chosen to be compatible with the vector and may for example be bacterial, yeast, insect or mammalian.

A further embodiment of the invention provides a method of producing a polypeptide which comprises culturing host cells of the present invention under conditions effective to express a polypeptide of the invention. Preferably, in addition, such a method is carried out under conditions in which the polypeptide of the invention is expressed and then produced from the host cells.

DNA according to the invention may also be inserted into the vectors described above in an antisense orientation in order to produce antisense RNA. Antisense RNA may also be produced by synthetic means. Such antisense

RNA may be used in a method of controlling the levels of a polypeptide of the invention in a cell.

The invention also provides monoclonal or polyclonal antibodies to a polypeptide according to the invention. The invention further provides a process for the production of monoclonal or polyclonal antibodies to the polypeptides of the invention. Monoclonal antibodies may be prepared by conventional hybridoma technology using a polypeptide of the invention, or a fragment thereof, as an immunogen. Polyclonal antibodies may also be prepared by conventional means which comprise inoculating a host animal, for example a rat or a rabbit, with a polypeptide of the invention and recovering immune serum.

The present invention also provides pharmaceutical compositions containing a polypeptide of the invention, or an antibody thereof, in association with a pharmaceutically acceptable diluent and/or carrier.

The polypeptides of the present invention include those lacking part of their amino acid sequence (e.g., a polypeptide comprised only of the essential sequence for revealing biological activity in an amino acid sequence shown in SEQ. ID NOS. 1 or 5), those in which a part of the amino acid sequence is replaced by other amino acids (e.g., those replaced by an amino acid having a similar property), and those in which other amino acids are added or inserted into a part of the amino acid sequence, as well as those having the amino acid sequence shown in SEQ. ID NOS. 1 or 5.

It is well known that there are from one to six different codons encoding each amino acid (for example, one codon is known to encode Methionine (Met), and six different codons are known to encode leucine (Leu)). Therefore, different nucleotide sequences of DNA can encode a polypeptide having the same amino acid sequence.

The DNA of the present invention specified in (2) and (6) includes every nucleotide sequence encoding the polypeptides shown in SEQ. ID NOS. 1 and

5. There is a probability of improving the yield of polypeptide production by changing the nucleotide sequence.

The DNA specified in (3) and (7) are embodiments of the DNA specified in (2) and (6), and are sequences in the natural form.

The DNA specified in (4) and (8) indicate the sequence of the DNA specified in (3) and (7) with an untranslated region.

A signal peptide is a hydrophobic region located immediately downstream of the translation initiation amino acid Met. It is assumed that the signal peptide in the polypeptide of the present invention resides in a region ranging from Met at the 1-position to Gly at the 21-position in the amino acid sequence represented by SEQ. ID NOS. 1 or 5. The region essentially responsible for the expression of the biological activity corresponds to the part of the amino acid sequences of the SEQ. ID. NO. 1 and 5 lacking the signal peptides, i.e. the mature protein part. Thus the signal peptides never relate to the activity.

DNA having the nucleotide sequences shown in SEQ. ID NOS. 3 or 7 may be prepared according to the following method:

(i) isolating mRNA from a cell which produces the polypeptide of the present invention (e.g., human pro-B cell line),

(ii) preparing a first strand (single strand cDNA) from mRNA thus obtained, followed by preparation of a second strand (double strand cDNA) (synthesis of cDNA),

(iii) inserting cDNA thus obtained into a proper phage vector,

(iv) transfecting recombinant phage into host cells (construction of cDNA library),

(v) screening with plaque hybridization a cDNA library using mouse SDF-1 cDNA as a probe,

(vi) preparing phage DNA containing the positive clone obtained, followed by subcloning cDNA which is cut out into a plasmid vector, and followed by

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preparing the restriction enzyme map, and

(vii) determining the nucleotide sequence of each fragment cut with the restriction enzyme, followed by assembling the full length sequence.

Explained in detail, step (i) may be carried out in accordance with the method of Okayama, H et al (described in Enzymology, vol. 154, p3, 1987) after a human pro-B cell line is stimulated by a proper stimulant (e.g. IL-1 etc.) or without stimulation.

The cells which secrete the polypeptides of the present invention are preferably of human pro-B cell line FLEB14. This human cell line FLEB14 may be supplied according to the first lecture, medicinal chemistry, School of Medicine, Kyoto University.

Steps (ii), (iii) and (iv) are a series of steps for preparing a cDNA library, and may be carried out in accordance with the method of Glubler & Hoffman (Gene, vol. 25, p. 263, 1983) with a slight modification.

As examples of the vector used in step (iii), many plasmid vectors (e.g. pB322™, pBluescript™, etc.) and phage vectors (e.g. λgt10™, λDASH II™ etc.) are known, with phage vector λgt10™ (43.3 kbp, Stratagene) being preferred.

The host cell used in step (iv) is preferably E. coli NM514 (Stratagene).

Steps (v) and (vi) may be carried out in accordance with the method described in Molecular Cloning (written by J. Sambrook, E.F. Fritsh and T. Maniatis, published by Cold Spring Harbor Laboratory Press in 1989).

The sequencing in step (vii) may be carried out in accordance with the method of Maxam-Gilbert or the dideoxy termination method.

It is necessary to confirm that the cDNA obtained covers the complete or almost the complete length of the intact mRNA. This confirmation may be carried out by Northern analysis using the cDNA as a probe (see Molecular Cloning).

If the size of the mRNA obtained from the hybridized band and the size of

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the cDNA are almost the same, it will be confirmed that the cDNA is almost full length.

Once the nucleotide sequences shown in SEQ. ID NOS. 2, 3, 6 and 7 are determined, DNA of the present invention may be obtained by chemical synthesis, by the PCR method or by hybridization making use of a fragment of DNA of the present invention as a probe. Furthermore, DNA of the present invention may be obtained in a desired amount by transforming, with a vector having inserted therein a DNA of the present invention, a proper host, followed by culturing the transformant.

The polypeptides of the present invention (shown in SEQ. ID NOS. 1 or 5) may be prepared by:

(1) isolation and purification from an organism or a cultured cell,

(2) chemical synthesis, or

(3) using biotechnological techniques,

preferably, by method (3), biotechnological techniques.

Examples of expression systems which may be used to prepare a polypeptide by biotechnological techniques are, for example, the expression systems of bacteria, yeast, insect cells and mammalian cells.

For example, expression in E. coli may be carried out by adding the initiation codon (ATG) to the 5′ end of a DNA encoding the mature protein, connecting the DNA thus obtained downstream of a proper promoter (e.g., trp promoter, lac promoter, IPL promoter, T7 promoter, etc.), and then inserting it into a vector (e.g., pBR322™, pUC18™, pUC19™, etc.) which functions in an E. coli strain to prepare an expression vector.

When a signal peptide of bacteria (e.g., signal peptide of pel B) is utilized, the desired polypeptide may be also produced in periplasm. Furthermore, a fusion protein with other polypeptides may be also produced easily.

Expression in a mammalian cell may be carried out, for example, by inserting the DNA shown in SEQ. ID NOS. 3 or 6 downstream of a proper promoter (e.g., SV40 promoter, LTR promoter, metallothionein promoter, etc.) in a proper vector (e.g., retrovirus vector, papilloma virus vector, vaccinia virus vector, SV40 vector, etc.) to obtain an expression vector, and transfecting a proper mammalian cell (e.g., monkey COS-7 cell, Chinese hamster CHO cell, mouse L cell, etc.) with the expression vector thus obtained, and then culturing the transformant in a proper medium to obtain the desired polypeptide in the culture medium. The polypeptide thus obtained may be isolated and purified by conventional biochemical methods.

Brief Description of the Drawings

Fig. 1 shows the restriction enzyme map of human SDF-1α.

Fig. 2 shows the restriction enzyme map of human SDF-1β.

Fig. 3 shows the plasmid vector pUCSRαML2.

Effects of the Invention

The polypeptide of the present invention is produced and secreted in pro-B cells, so it may be used for diseases relating to undergrown or abnormal proliferation of hematopoietic cells, neuronal enhancement or depression, immunological enhancement and depression, for example, inflammatory diseases (rheumatoid arthritis, ulcerative colitis, etc.), hematopoietic stemcytopenia after bone marrow transplantation, leukocytopenia, thrombocytopenia, B lymphopenia and T lymphopenia after chemotherapy, anemia, infectious diseases, cancer, leukocytosis, AIDS, neurodegenerative diseases (Alzheimer, multiple sclerosis, etc.), prevention or treatment of neuronal injury, prevention or treatment of disorder of bone metabolism (osteoporosis, etc.) or tissue repair.

In the above activities, it was confirmed that mouse SDF-1α stimulated the proliferation of mouse myeloid progenitor cell line DA1G in the laboratory test. It was suggested that the human SDF-1α also have the same activity.

Further, polyclonal or monoclonal antibodies against the polypeptide of the present invention can be used in the determination of the amount of said polypeptide in an organism, and thereby, may be utilized for the purpose of investigating the relationship between said polypeptide and diseases, or for the purpose of diagnosing diseases, and the like. Polyclonal and monoclonal antibodies against the polypeptide of the present invention may be prepared by conventional methods by using said polypeptide or a fragment thereof as an antigen.

The DNA of the present invention may be utilized as an important and essential template in preparing polypeptides of the present invention, which are expected to be useful for diagnosis and treatment of gene diseases (the treatment of gene defect disease and treatment by inhibiting expression of the polypeptide by antisense DNA (RNA), and the like). Further, genomic DNA may be isolated by using the DNA of the present invention as a probe. Similarly, it is possible to isolate genes having high homology to the DNA of the present invention in human or other species.

Pharmaceutical Applications

The polypeptides of the present invention may be normally administered systemically or partially, usually by oral or parenteral administration, preferably orally, intravenously or intraventricularly.

The doses to be administered are determined depending upon age, body weight, symptom, the desired therapeutic effect, the route of administration, and the duration of the treatment, etc. In the human adult, the doses per person per day are generally between 100 μg and 100 mg, by oral administration, up to several times per day, and between 10 μg and 100 mg, by parenteral administration up to several times per day.

As mentioned above, the doses to be used depend upon various conditions. Therefore, there are cases in which doses lower than or greater than the ranges specified above may be used.

The compounds of the present invention may be administered as solid compositions, liquid compositions or other compositions for oral administration, as injections, liniments or suppositories etc. for parenteral administration.

Solid compositions for oral administration include compressed tablets, pills, capsules, dispersible powders and granules. Capsules include soft capsules and hard capsules.

In such compositions, one or more of the active compound(s) is or are admixed with at least one inert diluent (such as lactose, mannitol, glucose, hydroxypropyl cellulose, microcrystalline cellulose, starch, polyvinylpyrrolidone, magnesium metasilicate aluminate, etc.) As is normal practice, the compositions may also comprise additional substances other than inert diluents: e.g. lubricating agents (such as magnesium stearate, etc.), disintegrating agents (such as cellulose calcium glycolate, etc.), stabilizing agents (such as human serum albumin, lactose, etc.), and assisting agents for dissolving (such as arginine, asparaginic acid, etc.).

The tablets or pills may, if desired, be coated with a film of gastric or enteric material (such as sugar, gelatin, hydroxypropyl cellulose or hydroxypropylmethyl cellulose phthalate, etc.), or be coated with more than two films. Furthermore, the term "coating" includes containment within capsules of absorbable materials such as gelatin.

Other compositions for oral administration include spray compositions which may be prepared by known methods and which comprise one or more active compounds. Spray compositions may comprise additional substances in addition to inert diluents: e.g. stabilizing agents (sodium sulfite, etc.), isotonic

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buffer (sodium chloride, sodium citrate, citric acid, etc.). For preparation of such spray compositions, for example, the method described in United States Patent Nos. 2,868,691 or 3,095,355 may be used.

Injectable compositions may comprise additional ingredients other than inert diluents: e.g. preserving agents, wetting agents, emulsifying agents, dispersing agents, stabilizing agents (such as human serum albumin, lactose, etc.), and assisting agents such as assisting agents for dissolving (arginine, asparaginic acid, etc.).

The injectable compositions may be sterilized for example, by filtration through a bacteria-retaining filter, by incorporation of sterilizing agents in the compositions or by irradiation. They may also be manufactured in the form of sterile solid compositions, for example, by freeze-drying, which can be dissolved in sterile water or some other sterile diluent for injection immediately before use.

Other compositions for parenteral administration include liquids for external use, and endermic liniments (ointment, etc.), suppositories for rectal administration and pessaries which comprise one or more of the active compounds and may be prepared by known methods.

Examples

The following examples illustrate, but do not limit, the scope of the present invention.

Example 1: Northern Analysis of Human Cell Line FLEB14

Human pro-B cell line FLEB14 cells (see Katamine, S., et al., Nature, 309, 369 (1984)) were homogenated. The homogenate was incubated with oligo-dT cellulose. Poly(A) RNA was eluted after washing out (Vennstorm, B., et al., Cell, 28, 135 (1982)). 1 μg of poly(A) RNA was electrophoresed in a 1.0% agarose

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gel and then blotted onto a nitrocellulose membrane.

The membrane was hybridized with ^{32}P-labelled mouse SDF-1 (described as SEQ. ID NO. 3 in Japanese Patent Application No. 5-22098 and shown in SEQ. ID NO. 9; the factor is now called "SDF-1α", as another SDF-1 was found from mouse). cDNA with 50% formamide, 5 X SSC, 0.1% SDC, 0.1% SDS, 5 X Denhaldt's, 0.1 mg/ml Salmon sperm DNA at 39° C and washed with 0.3 M NaCl, 30 mM Na citrate, 0.1% SDS at 50° C and then autoradiogrammed. 3.5 kb and 1.9 kb mRNA were hybridized.

Example 2: Preparation of cDNA from mRNA of Human pro-B Cell Line

A cDNA library was constructed from human pro-B cell line FLEB14 cells by a conventional method (see Molecular Cloning; J. Sambrook, E.F. Fritsh, and T. Maniatis, Cold Spring Harbor Laboratory Press (1989)). cDNA was synthesized using the Time Saver™ cDNA synthesis kit (Pharmacia).

The first stand was synthesized from FLEB14 poly(A)-RNA (5 μg) using a reverse transcriptase and an oligo-dT primer. Double strand cDNA was synthesized using a DNA polymerase I.

The cDNA was ligated to an EcoRI-NotI adapter:

AATTCGCGGCCGCT (SEQ. ID NO. 10)
GCGCCGGCGAp (SEQ. ID NO. 11)

and then phosphorylated. cDNA which are larger than 800 bp were recovered from a 0.8% agarose gel with a glass powder (Geneclean II™ DNA purification kit, available from Bio101™).

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Example 3: Preparation of cDNA Library and Cross Hybridization

The cDNA obtained was ligated to a λgt10™ phage vector (available from Stratagene) which have the EcoRI arm treated with phosphatase.

In vitro packaging was followed by the protocol of the in vitro packaging kit LAMDA INN™ (available from Nihon gene). The recombinant phages were transfected to host E. Coli NM514 (available from Stratagene). A cDNA library containing 1 X 10^6 plaques was obtained. 1 X 10^6 λgt10™ phage plaques of the cDNA library were transfected to nitrocellulose membranes. The membranes were hybridized with the ^{32}P-labelled mouse SDF-1α cDNA (shown in SEQ. ID NO. 9, the same cDNA used in Example 1) in 50% formamide, 5 X SSD, 0.1% SDS, 5 X Denhaldt's 0.1 mg/ml Salmon sperm DNA, at 39° C and washed in 0.3 M NaCl, 30 mM Na citrate, 0.1% SDS at 50° C and autoradiogrammed. 40 positive clones were obtained.

Example 4: Isolation of Positive Clones

Phage DNA was prepared from 9 positive clones by the conventional method (see New Cell Technology Experimental Protocol, pp. 88, edited by the Department of Oncology, The Institute of Medical Science, The University of Tokyo, published by Shujunsha Co. Ltd., Tokyo, 1991, 1993.). Phage DNA was digested at Not I. The length of insert cDNA was measured by agarose gel electrophoresis. The length of 8 clones was 1.9 kb, and the length of one clone was 3.5 kb. It was thought that these two types of clones are almost the full length of human SDF-1α and SDF-β cDNA from the result of Northern analysis.

The cDNA from one clone was picked up from 8 clones of 1.9 kb length, and one clone of 3.5 kb length digested at Not I was subjected to agarose electrophoresis, and the fragments were cut out and subcloned at Not I site of plasmid pBluescript™.

Example 5: Preparation of Restriction Enzyme Map and Sequencing

A restriction enzyme map of human SDF-1 (1.9 kb) was prepared (shown in Fig. 1). Each fragment of restriction enzyme was cDNA subcloned into pBluescript™, followed by determination of about 300 bp nucleotide sequences at both ends of each insert. Assembling these sequences, nucleotide sequences of full length were determined (shown in SEQ. ID NO. 3).

An open reading frame and an amino acid sequence were determined from the nucleotide sequence of full length cDNA, with the results shown in SEQ. ID NO. 1. 30-40 amino acids at the N-termini were compared with known signal peptide and the signal peptide of the polypeptides of the present invention was thus deduced to have the sequence shown in SEQ. ID NO. 4 (see Von Heuane, G., Nucleic Acids Res. 14, 4683 (1986)).

By the same procedure as described above, a restriction enzyme map (shown in Fig. 2), full length nucleotide sequences (shown in SEQ. ID NO. 7), an open reading frame (shown in SEQ. ID NO. 6, an amino acid sequence (shown in SEQ. ID NO. 5) and a sequence shown with signal peptide (shown in SEQ. ID NO. 8) of 3.5 kb clone were obtained.

The deduced amino acid sequences of the 3.5 kb clone and the 1.9 kb clone were very similar to each other, so the 1.9 kb clone was named SDF-1α and the 3.5 kb clone was named SDF-1β.

The nucleotide sequences were determined by the cycle sequence method using a fluorescence determinator (supplied by Applied Biosystem Inc.). Reading of nucleotide sequences was carried out by a DNA sequencer (Model 373, supplied by Applied Biosystem Inc.).

The nucleotide sequences and deduced amino acid sequences of SDF-1α and 1β were homology searched by a computer in data bases GENBANK™

and EMBL™ for DNA, and NBRF™ and SWISSPROT™ for amino acid sequences. It was thereby confirmed that the cDNA's of the present invention encode novel peptides.

Example 6: Construction of Plasmid Vector for Use in the Preparation of Expression Vector

As an expression vector, a pUC-SRαML-1 (this vector and its preparation are disclosed in European Patent Publication No. 559428) derivative was used. This derivative was constructed to insert two kinds of fragments, as shown below:

Fragment T7

5' GTAATACGACTCACTATAGGGGAGAGCT 3' (SEQ. ID NO. 12)

3' ACGTCATTATGCTGAGTGATATCCCCTC 5' (SEQ. ID NO. 13)

between PstI and SacI and

Fragment SP6

5' CTAGTCTATAGTGTCACCTAAATCGTGGGTAC 3' (SEQ. ID NO. 14)

3' AGATATCACAGTGGATTTAGCAC 5' (SEQ. ID NO. 15)

between the SpeI and KpnI sites in the multi-cloning site, respectively.

The pUC-SRαML-1 vector was digested with PstI and SacI and the resulting digest was subjected to the agarose gel electrophoresis to prepare and recover a fragment of about 4.1 kbp, followed by removal of the 5′-end phosphoric acid group by BAP (bacterial alkaline phosphatase) treatment. The phosphorylated DNA fragment T7 was ligated with the about 4.1 kbp fragment from pUC-SRαML-1 to convert them into a circular form.

Alternatively, the pUC-SRαML-1 vector was digested with SpeI and KpnI and the resulting digest was subjected to agarose gel electrophoresis to prepare and recover an about 4.1 kbp fragment and thereafter removing the 5′-end phosphoric acid group by BAP (bacterial alkaline phosphatase) treatment. The phosphorylated DNA fragment SP6 was ligated with the thus prepared about 4.1 kbp fragment to convert them into a circular form. The plasmid vector constructed in this manner was named pUC-SRαML-2 (see Fig. 3).

Example 7: Construction of Expression Vector

Regarding hSDF-1α, primer X, Y and YH were synthesized. The sequences of primers X, Y and YH are as follows:

Primer X

5′- A ATA TAG TCG ACC ACC ATG AAC GCC AAG GTC GTG GTC GTG CTG G-3′

(SEQ. ID NO. 16)

Primer Y

5′-CGG CGG ACT AGT TTA CTT GTT TAA AGC TTT CTC CAG G-3′

(SEQ. ID NO. 17)

Primer YH

5′- GCC GCC ACT AGT TTA GTG GTG GTG GTG GTG GTG CTT GTT TAA AGC TTT CTC CAG G-3′

(SEQ. ID NO. 18)

The hSDF-1α plasmid was subjected to PCR using the thus synthesized oligonucleotides X and Y as primers. The thus obtained PCR fragment contains a sequence placed 5′-adjacent to the initiation codon, corresponding to the Kozac sequence which is known among those skilled in the art, and cDNA which encodes a protein molecule consisting of the hSDF-1α protein. The PCR fragment was digested with SalI and SpeI and the resulting digest was separated and purified and then inserted into the SalI - SpeI site of the pUC-SRαML2 prepared in Example 6 to obtain an expression vector pUC-SRαML2 - hSDF-1αA.

Alternatively, the hSDF-1α plasmid was subjected to PCR using the synthesized oligonucleotides X and YH as primers. The thus obtained PCR fragment contains a sequence placed 5′-adjacent to the initiation codon, corresponding to the Kozac sequence which is known among those skilled in the art, and cDNA which encodes a protein molecule consisting of the hSDF-1α protein and six additional histidine (His) residues attached to its C-terminal end. The PCR fragment was digested with SalI and SpeI and the resulting digest was separated and purified and then inserted into the SalI - SpeI site of the pUC-SRαML2 prepared in Example 6 to obtain an expression vector pUC-SRαML2 - hSDF-1αB.

As for hSDF-1β, primer Z and ZH were synthesized. Sequences of primer Z and ZH are as follows:

Primer Z

5′-CGG CGG ACT AGT TCA CAT CTT GAA CCT CTT GTT TAA AGC -3′

(SEQ. ID NO. 19)

Primer ZH

5'- GCC GCC ACT AGT TCA GTG GTG GTG GTG GTG GTG CAT CTT GAA CCT CTT GTT TAA AGC -3'

(SEQ. ID NO. 20)

The hSDF-1β plasmid was subjected to PCR using the thus synthesized oligonucleotides X and Z as primers. The thus obtained PCR fragment contains a sequence placed 5'-adjacent to the initiation codon, corresponding to the Kozac sequence which is known amount those skilled in the art, and cDNA which encodes a protein molecule consisting of the hSDF-1β protein. The PCR fragment was digested with SalI and SpeI and the resulting digest was separated and purified and then inserted into the SalI - SpeI site of the pUC-SRαML2 prepared in Example 6 to obtain an expression vector pUC-SRαML2 - hSDF-1βA.

Alternatively, the hSDF-1β plasmid was subjected to PCR using the synthesized oligonucleotides X and ZH as primers. The thus obtained PCR fragment contains a sequence placed 5'-adjacent to the initiation codon, corresponding to the Kozac sequence which is known among those skilled in the art, and cDNA which encodes a protein molecule consisting of the hSDF-1β protein and six additional histidine (His) residues attached to its C-terminal end. The PCR fragment was digested with SalI and SpeI and the resulting digest was separated and purified and then inserted into the SalI - SpeI site of the pUC-SRαML2 prepared in Example 6 to obtain an expression vector pUC-SRαML2 - hSDF-1βB.

Each of the thus constructed pUC-SRαML2-hSDF-1αA, pUC-SRαML2-hSDF-1αB, pUC-SRαML2-hSDF-1βA and pUC-SRαML2-hSDF-1β plasmids were transfected into an E. coli strain DH5, recovered from a 100 ml culture of the resulting transformant and then purified twice by CsCl density gradient

B

centrifugation.

Example 8: Expression in COS Cells

Each of the plasmid DNA preparations pUC-SRαML2, pUC-SRαML2-hSDF-1αA, pUC-SRαML2-hSDF-1αB, pUC-SRαML2-hSDF-1βA and pUC-SRαML2-hSDF-1βB were introduced into COS-7 cells (Cell, vol. 23, p. 175, 1981) by means of the diethylaminoethyl (DEAE) dextran method (J. Immunology, vol. 136, p. 4291, 1986).

That is, about 1.8 X 10^6 COS-7 cells were inoculated into a 225 cm^2 capacity flask (manufactured by Corning) together with 50 ml of a liquid culture medium (Dulbecco's modified MEM medium supplemented with 10% decomplemented fetal bovine serum). After overnight incubation in a carbon dioxide incubator (37° C, 5% CO_2) and subsequent removal of the culture supernatant, 12 ml of a DNA cocktail (Dulbecco's modified MEM medium supplemented with 15 μg of each plasmid DNA, 50 mM Tris-HCl buffer (pH 7.4) and 400 μg/ml of DEAE-dextran) was added to each flask and culturing was carried out for 3 hours at 37° C in an atmosphere of 5% CO_2. Thereafter, the DNA cocktail was replaced by 15 ml of a chloroquine solution (Dulbecco's modified MEM medium supplemented with 150 μM chloroquine and 7% decomplemented fetal bovine serum), followed by 3 additional hours of culturing.

After removing the chloroquine solution, the aforementioned liquid culture medium (50 ml) was added to each of the resulting flasks which were then incubated at 37° C in an atmosphere of 5% CO_2 for 72 hours to produce growth of the cells in each flask of an almost monolayer form. After removing the culture supernatant, the cells in each flask were washed with a serum-free liquid culture medium (trade name, SFM-101™; available from Nissui Pharmaceutical Co., Ltd.) and then supplied with 75 ml of the same serum-free liquid culture medium, and the culturing was continued for another 72 hours. Thereafter, the resulting culture supernatant was recovered and filtered through a membrane filter (trade name, STERIVEX-GS™; available from Millipore Corp.) to remove the cells and cell debris. The thus obtained culture supernatant samples were stored at 4° C for future use. The culture supernatant of COS cells transformed with plasmid containing the hSDF-1α and β cDNA inserts are expected to contain expressed and secreted mature protein moieties of polypeptides which correspond to hSDF-1α and β.

Example 9: Confirmation of Expression

A 2 ml portion of each of the culture supernatants of the transformed COS cells obtained in Example 8 was concentrated to a volume of 100 ml using a centrifugal concentration filter (trade name, Centricon-10™; available from Millipore Corp.). A 1 μl portion of each of the thus concentrated samples was mixed with the same volume of a loading buffer (0.125 M Tris-HCl buffer (pH 6.8), 4% sodium dodecyl sulfate and 30% glycerol) for SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) use, and the mixture was treated at 90° C for 3 minutes and then subjected to SDS-PAGE.

For the hSDF-1αB and βB proteins having His hexamer introduced to the C-terminus of the proteins, the COS cell culture supernatant as well as the purified products were subjected to the SDS-PAGE analysis.

Purification of the protein was carried out by means of metal chelate affinity chromatography (Biotechnology, vol. 9, p. 273, 1991), making use of the function of His to form complex compounds with various transition metal ions. That is, a culture supernatant (350 ml) obtained from COS cells was mixed with a sodium chloride aqueous solution in such an amount that the final concentration of the salt became 1 M, and the resulting mixture was applied to a column packed with 4 ml of a zinc-linked chelating Sepharose (trade name, Chelating Sepharose Fast-Flow™; available from Pharmacia) to adsorb the protein to the resin. The column was washed with 50 mM phosphate buffer (pH 7.0) containing 1 M sodium chloride aqueous solution (40 ml), and the protein retained in the column was eluted with 50 mM phosphate buffer (pH 7.0) containing 1 M sodium chloride aqueous solution and 0.4 M imidazole. Thereafter, the resulting elute was concentrated to a volume of 100 μl, and a portion of the concentrated sample was subjected to SDS-PAGE analysis. The SDS-PAGE analysis was carried out using a SDS 10/20 gradient gel and a product which corresponds to a molecular weight of hSDF-1α and SDF-1β was detected respectively.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Ono Pharmaceutical Co., Ltd.
(B) STREET: 1-5, Doshomachi 2-chome
(C) CITY: Chuo-ku, Osaka-shi
(D) STATE: Osaka
(E) COUNTRY: Japan
(F) POSTAL CODE (ZIP): 541

(ii) TITLE OF INVENTION: Novel Polypeptides and DNAs encoding them

(iii) NUMBER OF SEQUENCES: 20

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 89 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

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Cys Leu Ser Asp Gly Lys Pro Val Ser Leu Ser Tyr Arg Cys Pro Cys
            20                  25                  30

Arg Phe Phe Glu Ser His Val Ala Arg Ala Asn Val Lys His Leu Lys
        35                  40                  45

Ile Leu Asn Thr Pro Asn Cys Ala Leu Gln Ile Val Ala Arg Leu Lys
    50                  55                  60

Asn Asn Asn Arg Gln Val Cys Ile Asp Pro Lys Leu Lys Trp Ile Gln
65                  70                  75                  80

Glu Tyr Leu Glu Lys Ala Leu Asn Lys
                85
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 267 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

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

ATGAACGCCA AGGTCGTGGT CGTGCTGGTC CTCGTGCTGA CCGCGCTCTG CCTCAGCGAC 60
GGGAAGCCCG TCAGCCTGAG CTACAGATGC CCATGCCGAT TCTTCGAAAG CCATGTTGCC 120
AGAGCCAACG TCAAGCATCT CAAAATTCTC AACACTCCAA ACTGTGCCCT TCAGATTGTA 180
GCCCGGCTGA AGAACAACAA CAGACAAGTG TGCATTGACC CGAAGCTAAA GTGGATTCAG 240
GAGTACCTGG AGAAAGCTTT AAACAAG 267

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1856 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

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

TCTCCGTCAG CCGCATTGCC CGCTCGGCGT CCGGCCCCCG ACCCGTGCTC GTCCGCCCGC 60
CCGCCCGCCC GCCCGCGCCA TGAACGCCAA GGTCGTGGTC GTGCTGGTCC TCGTGCTGAC 120
CGCGCTCTGC CTCAGCGACG GGAAGCCCGT CAGCCTGAGC TACAGATGCC CATGCCGATT 180
CTTCGAAAGC CATGTTGCCA GAGCCAACGT CAAGCATCTC AAAATTCTCA ACACTCCAAA 240
CTGTGCCCTT CAGATTGTAG CCCGGCTGAA GAACAACAAC AGACAAGTGT GCATTGACCC 300
GAAGCTAAAG TGGATTCAGG AGTACCTGGA GAAAGCTTTA AACAAGTAAG CACAACAGCC 360
AAAAAGGACT TTCCGCTAGA CCCACTCGAG GAAAACTAAA ACCTTGTGAG AGATGAAAGG 420
GCAAAGACGT GGGGGAGGGG GCCTTAACCA TGAGGACCAG GTGTGTGTGT GGGGTGGGCA 480
CATTGATCTG GGATCGGGCC TGAGGTTTGC AGCATTTAGA CCCTGCATTT ATAGCATACG 540
GTATGATATT GCAGCTTATA TTCATCCATG CCCTGTACCT GTGCACGTTG GAACTTTTAT 600
TACTGGGGTT TTTCTAAGAA AGAAATTGTA TTATCAACAG CATTTTCAAG CAGTTAGTTC 660
CTTCATGATC ATCACAATCA TCATCATTCT CATTCTCATT TTTTAAATCA ACGAGTACTT 720

CAAGATCTGA ATTTGGCTTG TTTGGAGCAT CTCCTCTGCT CCCCTGGGGA GTCTGGGCAC 780

AGTCAGGTGG TGGCTTAACA GGGAGCTGGA AAAAGTGTCC TTTCTTCAGA CACTGAGGCT 840

CCCGCAGCAG CGCCCCTCCC AAGAGGAAGG CCTCTGTGGC ACTCAGATAC CGACTGGGGC 900

TGGGGCGCCG CCACTGCCTT CACCTCCTCT TTCAAACCTC AGTGATTGGC TCTGTGGGCT 960

CCATGTAGAA GCCACTATTA CTGGGACTGT CTCAGAGACC CCTCTCCCAG CTATTCCTAC 1020

TCTCTCCCCG ACTCCGAGAG CATGCTTAAT CTTGCTTCTG CTTCTCATTT CTGTAGCCTG 1080

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CCTCCCAGGG CCTGCCCCAC AGCTCGGGCC CTCTGTGAGA TCCGTCTTTG GCCTCCTCCA 1200

GAATGGAGCT GGCCCTCTCC TGGGGATGTG TAATGGTCCC CCTGCTTACC CGCAAAAGAC 1260

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CACAGCAGGG GTTCAGGTTC CAATCAGAAC TGTTGGCAAG GTGACATTTC CATGCATAGA 1500

TGCGATCCAC AGAAGGTCCT GGTGGTATTT GTAACTTTTT GCAAGGCATT TTTTTATATA 1560

TATTTTTGTG CACATTTTTT TTTACGATTC TTTAGAAAAC AAATGTATTT CAAAATATAT 1620

TTATAGTCGA ACAAGTCATA TATATGAATG AGAGCCATAT GAATGTCAGT AGTTTATACT 1680

TCTCTATTAT CTCAAACTAC TGGCAATTTG TAAAGAAATA TATATGATAT ATAAATGTGA 1740

TTGCAGCTTT TCAATGTTAG CCACAGTGTA TTTTTTCACT TGTACTAAAA TTGTATCAAA 1800

TGTGACATTA TATGCACTAG CAATAAAATG CTAATTGTTT CATGGTAAAA AAAAAA 1856

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1856 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
(H) CELL LINE: FLEB14

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 80..349
(C) IDENTIFICATION METHOD: by similarity to some other pattern

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 80..142
(C) IDENTIFICATION METHOD: by similarity with known sequence or to an established consensus

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 143..346
(C) IDENTIFICATION METHOD: by similarity with known sequence or to an established consensus

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

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                     -21 -20                 -15

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Val Leu Thr Ala Leu Cys Leu Ser Asp Gly Lys Pro Val Ser Leu Ser
-10                 -5                  1               5

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Tyr Arg Cys Pro Cys Arg Phe Phe Glu Ser His Val Ala Arg Ala Asn
             10                  15                  20

GTC AAG CAT CTC AAA ATT CTC AAC ACT CCA AAC TGT GCC CTT CAG ATT      256
Val Lys His Leu Lys Ile Leu Asn Thr Pro Asn Cys Ala Leu Gln Ile
         25                  30                  35

GTA GCC CGG CTG AAG AAC AAC AAC AGA CAA GTG TGC ATT GAC CCG AAG      304
Val Ala Arg Leu Lys Asn Asn Asn Arg Gln Val Cys Ile Asp Pro Lys
     40                  45                  50

CTA AAG TGG ATT CAG GAG TAC CTG GAG AAA GCT TTA AAC AAG TAAGCACAAC   356
Leu Lys Trp Ile Gln Glu Tyr Leu Glu Lys Ala Leu Asn Lys
 55                 60                  65

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GGCACATTGA TCTGGGATCG GGCCTGAGGT TTGCAGCATT TAGACCCTGC ATTTATAGCA       536

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TTATTACTGG GGTTTTTCTA AGAAAGAAAT TGTATTATCA ACAGCATTTT CAAGCAGTTA       656

GTTCCTTCAT GATCATCACA ATCATCATCA TTCTCATTCT CATTTTTTAA ATCAACGAGT       716

ACTTCAAGAT CTGAATTTGG CTTGTTTGGA GCATCTCCTC TGCTCCCCTG GGGAGTCTGG       776

GCACAGTCAG GTGGTGGCTT AACAGGGAGC TGGAAAAAGT GTCCTTTCTT CAGACACTGA       836
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GGCTCCATGT AGAAGCCACT ATTACTGGGA CTGTCTCAGA GACCCCTCTC CCAGCTATTC 1016

CTACTCTCTC CCCGACTCCG AGAGCATGCT TAATCTTGCT TCTGCTTCTC ATTTCTGTAG 1076

CCTGATCAGC GCCGCACCAG CCGGGAAGAG GGTGATTGCT GGGGCTCGTG CCCTGCATCC 1136

CTCTCCTCCC AGGGCCTGCC CCACAGCTCG GGCCCTCTGT GAGATCCGTC TTTGGCCTCC 1196

TCCAGAATGG AGCTGGCCCT CTCCTGGGGA TGTGTAATGG TCCCCCTGCT TACCCGCAAA 1256

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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 93 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

Met Asn Ala Lys Val Val Val Val Leu Val Leu Val Leu Thr Ala Leu
1 5 10 15

Cys Leu Ser Asp Gly Lys Pro Val Ser Leu Ser Tyr Arg Cys Pro Cys
20 25 30

Arg Phe Phe Glu Ser His Val Ala Arg Ala Asn Val Lys His Leu Lys

35 40 45

Ile Leu Asn Thr Pro Asn Cys Ala Leu Gln Ile Val Ala Arg Leu Lys
50 55 60

Asn Asn Asn Arg Gln Val Cys Ile Asp Pro Lys Leu Lys Trp Ile Gln
65 70 75 80

Glu Tyr Leu Glu Lys Ala Leu Asn Lys Arg Phe Lys Met
85 90

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 279 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

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

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GGGAAGCCCG TCAGCCTGAG CTACAGATGC CCATGCCGAT TCTTCGAAAG CCATGTTGCC 120

AGAGCCAACG TCAAGCATCT CAAAATTCTC AACACTCCAA ACTGTGCCCT TCAGATTGTA 180

GCCCGGCTGA AGAACAACAA CAGACAAGTG TGCATTGACC CGAAGCTAAA GTGGATTCAG 240

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(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3526 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

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

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GTTTTGCACA CTTTGCCATA TTTTCACCAT TTGATTATGT AGCAAAATAC ATGACATTTA 540
TTTTTCATTT AGTTTGATTA TTCAGTGTCA CTGGCGACAC GTAGCAGCTT AGACTAAGGC 600
CATTATTGTA CTTGCCTTAT TAGAGTGTCT TTCCACGGAG CCACTCCTCT GACTCAGGGC 660
TCCTGGGTTT TGTATTCTCT GAGCTGTGCA GGTGGGGAGA CTGGGCTGAG GGAGCCTGGC 720
CCCATGGTCA GCCCTAGGGT GGAGAGCCAC CAAGAGGGAC GCCTGGGGGT GCCAGGACCA 780
GTCAACCTGG GCAAAGCCTA GTGAAGGCTT CTCTCTGTGG GATGGGATGG TGGAGGGCCA 840
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CCTCATCCCG AGCACCTCCA CATCCTCCAC GTTCTGCTCA TCATTCTCTG TCTCATCCAT 1020
CATCATGTGT GTCCACGACT GTCTCCATGG CCCCGCAAAA GGACTCTCAG GACCAAAGCT 1080
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AAATGTTCAC TTTTGACAAA GGCAAGCACT TGTGGGTTTT TGTTTTGTTT TTCATTCAGT 1860
CTTACGAATA CTTTTGCCCT TTGATTAAAG ACTCCAGTTA AAAAAAATTT TAATGAAGAA 1920

AGTGGAAAAC AAGGAAGTCA AAGCAAGGAA ACTATGTAAC ATGTAGGAAG TAGGAAGTAA 1980

ATTATAGTGA TGTAATCTTG AATTGTAACT GTTCGTGAAT TTAATAATCT GTAGGGTAAT 2040

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AAACCCATCA ACAAAAATTG TCCCTTAAAC AAAAATTAAA ATCCTCAATC CAGCTATGTT 2160

ATATTGAAAA AATAGAGCCT GAGGGATCTT TACTAGTTAT AAAGATACAG AACTCTTTCA 2220

AAACCTTTTG AAATTAACCT CTCACTATAC CAGTATAATT GAGTTTTCAG TGGGGCAGTC 2280

ATTATCCAGG TAATCCAAGA TATTTTAAAA TCTGTCACGT AGAACTTGGA TGTACCTGCC 2340

CCCAATCCAT GAACCAAGAC CATTGAATTC TTGGTTGAGG AAACAAACAT GACCCTAAAT 2400

CTTGACTACA GTCAGGAAAG GAATCATTTC TATTTCTCCT CCATGGGAGA AAATAGATAA 2460

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CAGTGTTAAA TGTGATGAAT ACTGTATTTT GTATTGTTTC AAGTGCATCT CCCAGATAAT 3360

GTGAAAATGG TCCAGGAGAA GGCCAATTCC TATACGCAGC GTGCTTTAAA AAATAAATAA 3420

GAAACAACTC TTTGAGAAAC AACAATTTCT ACTTTGAAGT CATACCAATG AAAAAATGTA 3480

TATGCACTTA TAATTTTCCT AATAAAGTTC TGTACTCAAA TGTAAA 3526

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3526 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
(H) CELL LINE: FLEB14

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 80..361
(C) IDENTIFICATION METHOD: by similarity to some other pattern

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 80..142
(C) IDENTIFICATION METHOD: by similarity with known sequence or to an established consensus

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 143..358
(C) IDENTIFICATION METHOD: by similarity with known sequence or to an established consensus

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

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TCTCCGTCAG CCGCATTGCC CGCTCGGCGT CCGGCCCCCG ACCCGTGCTC GTCCGCCCGC       60

CCGCCCGCCC GCCCGCGCC ATG AAC GCC AAG GTC GTG GTC GTG CTG GTC CTC       112
                     Met Asn Ala Lys Val Val Val Val Leu Val Leu
                     -21 -20                 -15

GTG CTG ACC GCG CTC TGC CTC AGC GAC GGG AAG CCC GTC AGC CTG AGC       160
Val Leu Thr Ala Leu Cys Leu Ser Asp Gly Lys Pro Val Ser Leu Ser
-10                 -5                  1                   5

TAC AGA TGC CCA TGC CGA TTC TTC GAA AGC CAT GTT GCC AGA GCC AAC       208
Tyr Arg Cys Pro Cys Arg Phe Phe Glu Ser His Val Ala Arg Ala Asn
            10                  15                  20

GTC AAG CAT CTC AAA ATT CTC AAC ACT CCA AAC TGT GCC CTT CAG ATT       256
Val Lys His Leu Lys Ile Leu Asn Thr Pro Asn Cys Ala Leu Gln Ile
        25                  30                  35

GTA GCC CGG CTG AAG AAC AAC AAC AGA CAA GTG TGC ATT GAC CCG AAG       304
Val Ala Arg Leu Lys Asn Asn Asn Arg Gln Val Cys Ile Asp Pro Lys
    40                  45                  50

CTA AAG TGG ATT CAG GAG TAC CTG GAG AAA GCT TTA AAC AAG AGG TTC       352
Leu Lys Trp Ile Gln Glu Tyr Leu Glu Lys Ala Leu Asn Lys Arg Phe
```

55 60 65 70

AAG ATG TGAGAGGGTC AGACGCCTGA GGAACCCTTA CAGTAGGAGC CCAGCTCTGA 408
Lys Met

AACCAGTGTT AGGGAAGGGC CTGCCACAGC CTCCCCTGCC AGGGCAGGGC CCCAGGCATT 468

GCCAAGGGCT TTGTTTTGCA CACTTTGCCA TATTTTCACC ATTTGATTAT GTAGCAAAAT 528

ACATGACATT TATTTTTCAT TTAGTTTGAT TATTCAGTGT CACTGGCGAC ACGTAGCAGC 588

TTAGACTAAG GCCATTATTG TACTTGCCTT ATTAGAGTGT CTTTCCACGG AGCCACTCCT 648

CTGACTCAGG GCTCCTGGGT TTTGTATTCT CTGAGCTGTG CAGGTGGGGA GACTGGGCTG 708

AGGGAGCCTG GCCCCATGGT CAGCCCTAGG GTGGAGAGCC ACCAAGAGGG ACGCCTGGGG 768

GTGCCAGGAC CAGTCAACCT GGGCAAAGCC TAGTGAAGGC TTCTCTCTGT GGGATGGGAT 828

GGTGGAGGGC CACATGGGAG GCTCACCCCC TTCTCCATCC ACATGGGAGC CGGGTCTGCC 888

TCTTCTGGGA GGGCAGCAGG GCTACCCTGA GCTGAGGCAG CAGTGTGAGG CCAGGGCAGA 948

GTGAGACCCA GCCCTCATCC CGAGCACCTC CACATCCTCC ACGTTCTGCT CATCATTCTC 1008

TGTCTCATCC ATCATCATGT GTGTCCACGA CTGTCTCCAT GGCCCCGCAA AAGGACTCTC 1068

AGGACCAAAG CTTTCATGTA AACTGTGCAC CAAGCAGGAA ATGAAAATGT CTTGTGTTAC 1128

CTGAAAACAC TGTGCACATC TGTGTCTTGT GTGGAATATT GTCCATTGTC CAATCCTATG 1188

TTTTTGTTCA AAGCCAGCGT CCTCCTCTGT GACCAATGTC TTGATGCATG CACTGTTCCC 1248

CCTGTGCAGC CGCTGAGCGA GGAGATGCTC CTTGGGCCCT TTGAGTGCAG TCCTGATCAG 1308

AGCCGTGGTC CTTTGGGGTG AACTACCTTG GTTCCCCCAC TGATCACAAA AACATGGTGG 1368

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TGGCAATGCC ATCATCTCCT GCCTTCCTCC TGGGCCCTCT CTGCTCTGCG TGTCACCTGT 1668

GCTTCGGGCC CTTCCCACAG GACATTTCTC TAAGAGAACA ATGTGCTATG TGAAGAGTAA 1728

GTCAACCTGC CTGACATTTG GAGTGTTCCC CTCCCACTGA GGGCAGTCGA TAGAGCTGTA 1788

TTAAGCCACT TAAAATGTTC ACTTTTGACA AAGGCAAGCA CTTGTGGGTT TTTGTTTTGT 1848

TTTTCATTCA GTCTTACGAA TACTTTTGCC CTTTGATTAA AGACTCCAGT TAAAAAAAAT 1908

TTTAATGAAG AAAGTGGAAA ACAAGGAAGT CAAAGCAAGG AAACTATGTA ACATGTAGGA 1968
AGTAGGAAGT AAATTATAGT GATGTAATCT TGAATTGTAA CTGTTCGTGA ATTTAATAAT 2028
CTGTAGGGTA ATTAGTAACA TGTGTTAAGT ATTTTCATAA GTATTTCAAA TTGGAGCTTC 2088
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AGTGGGGCAG TCATTATCCA GGTAATCCAA GATATTTTAA AATCTGTCAC GTAGAACTTG 2328
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GAAAATAGAT AAGAGTAGAA ACTGCAGGGA AAATTATTTG CATAACAATT CCTCTACTAA 2508
CAATCAGCTC CTTCCTGGAG ACTGCCCAGC TAAAGCAATA TGCATTTAAA TACAGTCTTC 2568
CATTTGCAAG GGAAAAGTCT CTTGTAATCC GAATCTCTTT TTGCTTTCGA ACTGCTAGTC 2628
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GCCTCTACCT GACACTCCCT TGGGCTCCCT GTAACCTCTT CAGAGGCCCT CGCTGCCAGC 2748
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GAGTCTGTGC CACGTGTATG TGCTGTGGTG TGTCCCCCTC TGTCCAGGCA CTGAGATACC 2868
AGCGAGGAGG CTCCAGAGGG CACTCTGCTT GTTATTAGAG ATTACCTCCT GAGAAAAAAG 2928
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AAGGAGCCCC ATTCCTGGGA AATATTCCCT AGAAACTTCC AAATCCCCTA AGCAGACCAC 3228
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CAGTGAATGA TTCAGTGTTA AATGTGATGA ATACTGTATT TTGTATTGTT TCAAGTGCAT 3348
CTCCCAGATA ATGTGAAAAT GGTCCAGGAG AAGGCCAATT CCTATACGCA GCGTGCTTTA 3408
AAAAATAAAT AAGAAACAAC TCTTTGAGAA ACAACAATTT CTACTTTGAA GTCATACCAA 3468
TGAAAAAATG TATATGCACT TATAATTTTC CTAATAAAGT TCTGTACTCA AATGTAAA 3526

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1797 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

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

GACCACTTTC CCTCTCGGTC CACCTCGGTG TCCTCTTGCT GTCCAGCTCT GCAGCCTCCG 60
GCGCGCCCTC CCGCCCACGC CATGGACGCC AAGGTCGTCG CCGTGCTGGC CCTGGTGCTG 120
GCCGCGCTCT GCATCAGTGA CGGTAAACCA GTCAGCCTGA GCTACCGATG CCCCTGCCGG 180
TTCTTCGAGA GCCACATCGC CAGAGCCAAC GTCAAGCATC TGAAAATCCT CAACACTCCA 240
AACTGTGCCC TTCAGATTGT TGCACGGCTG AAGAACAACA ACAGACAAGT GTGCATTGAC 300
CCGAAATTAA AGTGGATCCA AGAGTACCTG GAGAAAGCTT TAAACAAGTA AGCACAACAG 360
CCCAAAGGAC TTTCCAGTAG ACCCCCGAGG AAGGCTGACA TCCGTGGGAG ATGCAAGGGC 420
AGTGGTGGGG AGGAGGGCCT GAACCCTGGC CAGGATGGCC GGCGGGACAG CACTGACTGG 480
GGTCATGCTA AGGTTTGCCA GCATAAAGAC ACTCCGCCAT AGCATATGGT ACGATATTGC 540
AGCTTATATT CATCCCTGCC CTCGCCCGTG CACAATGGAG CTTTTATAAC TGGGGTTTTT 600
CTAAGGAATT GTATTACCCT AACCAGTTAG CTTCATCCCC ATTCTCCTCA TCCTCATCTT 660
CATTTTAAAA AGCAGTGATT ACTTCAAGGG CTGTATTCAG TTTGCTTTGG AGCTTCTCTT 720
TGCCCTGGGG CCTCTGGGCA CAGTTATAGA CGGTGGCTTT GCAGGGAGCC CTAGAGAGAA 780
ACCTTCCACC AGAGCAGAGT CCGAGGAACG CTGCAGGGCT TGTCCTGCAG GGGGCGCTCC 840
TCGACAGATG CCTTGTCCTG AGTCAACACA AGATCCGGCA GAGGGAGGCT CCTTTATCCA 900
GTTCAGTGCC AGGGTCGGGA AGCTTCCTTT AGAAGTGATC CCTGAAGCTG TGCTCAGAGA 960
CCCTTTCCTA GCCGTTCCTG CTCTCTGCTT GCCTCCAAAC GCATGCTTCA TCTGACTTCC 1020
GCTTCTCACC TCTGTAGCCT GACGGACCAA TGCTGCAATG GAAGGGAGGA GAGTGATGTG 1080
GGGTGCCCCC TCCCTCTCTT CCCTTTGCTT TCCTCTCACT TGGGCCCTTT GTGAGATTTT 1140
TCTTTGGCCT CCTGTAGAAT GGAGCCAGAC CATCCTGGAT AATGTGAGAA CATGCCTAGA 1200
TTTACCCACA AAACACAAGT CTGAGAATTA ATCATAAACG GAAGTTTAAA TGAGGATTTG 1260
GACCTTGGTA ATTGTCCCTG AGTCCTATAT ATTTCAACAG TGGCTCTATG GGCTCTGATC 1320

GAATATCAGT GATGAAAATA ATAATAATAA TAATAATAAC GAATAAGCCA GAATCTTGCC 1380

ATGAAGCCAC AGTGGGGATT CTGGGTTCCA ATCAGAAATG GAGACAAGAT AAAACTTGCA 1440

TACATTCTTA TGATCACAGA CGGCCCTGGT GGTTTTTGGT AACTATTTAC AAGGCATTTT 1500

TTTACATATA TTTTTGTGCA CTTTTTATGT TTCTTTGGAA GACAAATGTA TTTCAGAATA 1560

TATTTGTAGT CAATTCATAT ATTTGAAGTG GAGCCATAGT AATGCCAGTA GATATCTCTA 1620

TGATCTTGAG CTACTGGCAA CTTGTAAAGA AATATATATG ACATATAAAT GTATTGTAGC 1680

TTTCCGGTGT CAGCCACGGT GTATTTTTCC ACTTGGAATG AAATTGTATC AACTGTGACA 1740

TTATATGCAC TAGCAATAAA ATGCTAATTG TTTCATGCTG TAAAAAAAAA AAAAAAA 1797

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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

AATTCGCGGC CGCT 14

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /label= phosphorylated

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

AGCGGCCGCG 10

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

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

GTAATACGAC TCACTATAGG GGAGAGCT 28

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

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

CTCCCCTATA GTGAGTCGTA TTACTGCA 28

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

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

CTAGTCTATA GTGTCACCTA AATCGTGGGT AC 32

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

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

CACGATTTAG GTGACACTAT AGA 23

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

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

AATATAGTCG ACCACCATGA ACGCCAAGGT CGTGGTCGTG CTGG 44

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

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

CGGCGGACTA GTTTACTTGT TTAAAGCTTT CTCCAGG 37

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 55 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

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

GCCGCCACTA GTTTAGTGGT GGTGGTGGTG GTGCTTGTTT AAAGCTTTCT CCAGG 55

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

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

CGGCGGACTA GTTCACATCT TGAACCTCTT GTTTAAAGC 39

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GCCGCCACTA GTTCAGTGGT GGTGGTGGTG GTGCATCTTG AACCTCTTGT TTAAAGC 57

What is claimed is:

1 . A polypeptide having the amino acid sequence shown in SEQ ID No. 1 in substantially purified form.

2. DNA encoding a polypeptide according to claim 1.

3. DNA according to claim 2 having the nucleotide sequence shown in SEQ ID No. 2.

4. DNA according to claim 2 having the nucleotide sequence shown in SEQ ID No. 3.

5. A replication and expression vector comprising DNA according to any one of claims 2 to 4.

6. Host cells transformed or tranfected with a replication and expression vector according to claim 5.

7. A method of producing a polypeptide which comprises culturing host cells according to claim 6 under conditions effective to express a polypeptide according to claim 1.

8. A pharmaceutical composition containing a polypeptide according to claim 1 in association with a pharmaceutically acceptable diluent and/or carrier.

9. A polypeptide having the amino acid sequence shown in SEQ ID No. 5 in substantially purified form.

10. DNA encoding a polypeptide according to claim 9.

11. DNA according to claim 10 having the nucleotide sequence shown in SEQ ID No. 6.

12. DNA according to claim 10 having the nucleotide sequence shown in SEQ ID No. 7.

13. A replication and expression vector comprising DNA according to any one of claims 10 to 12.

14. Host cells transformed or tranfected with a replication and expression vector according to claim 13.

15. A method of producing a polypeptide which comprises culturing host cells according to claim 14 under conditions effective to express a polypeptide according to claim 9.

16. A pharmaceutical composition containing a polypeptide according to claim 9 in association with a pharmaceutically acceptable diluent and/or carrier.

17. A stromal derived factor-lα (SDF-1α) polypeptide having the amino acid sequence as shown in SEQ. ID NO:1.

18. A stromal derived factor-1β (SDF-1 β) polypeptide having the amino acid sequence as shown in SEQ. ID NO:5.

19. A mature peptide of the stromal derived factor-l-α (SDF-1α) having amino acid sequence as follows:

Lys Pro Val Ser Leu Ser Tyr Arg Cys Pro Cys Arg Phe Phe Glu Ser
1 5 10 15

His Val Ala Arg Ala Asn Val Lys His Leu Lys Ile Leu Asn Thr Pro
20 25 30

Asn Cys Ala Leu Gln Ile Val Ala Arg Leu Lys Asn Asn Asn Arg Gln
35 40 45

Val Cys Ile Asp Pro Lys Leu Lys Trp Ile Gln Glu Tyr Leu Glu Lys
50 55 60

Ala Leu Asn Lys.
65

20. A mature peptide of the stromal derived factor-lβ (SDF-1β) having amino acid sequence as follows:

Lys Pro Val Ser Leu Ser Tyr Arg Cys Pro Cys Arg Phe Phe Glu Ser
1 5 10 15

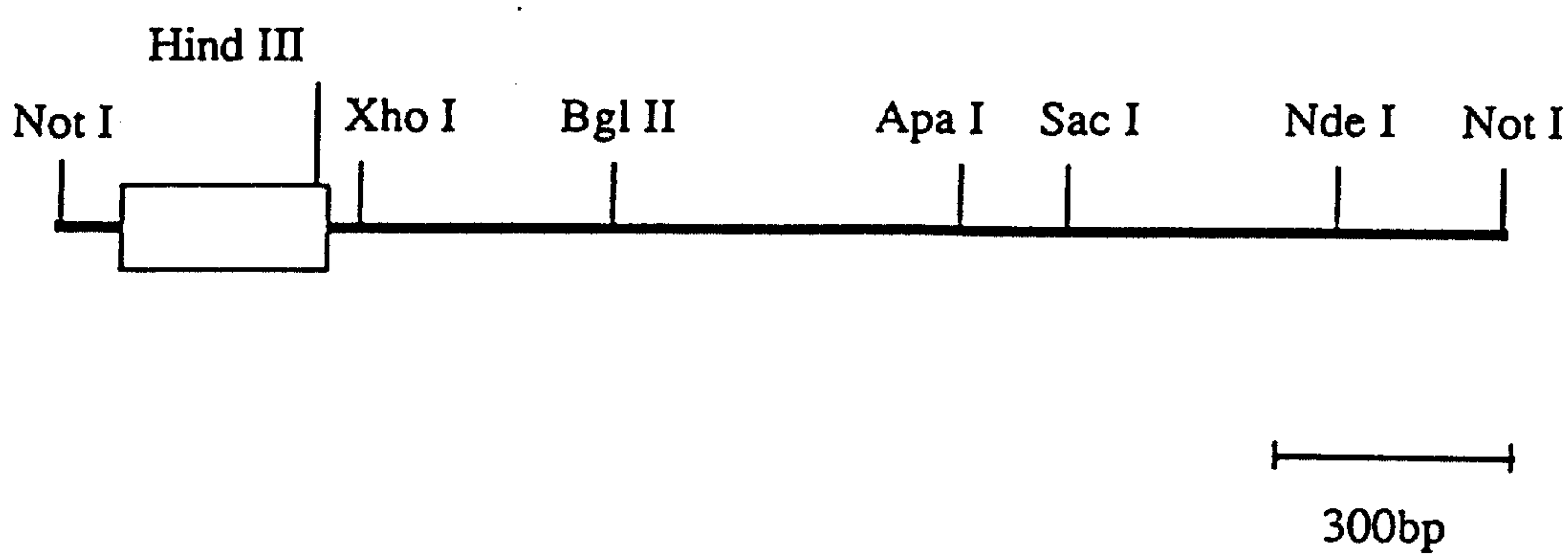
His Val Ala Arg Ala Asn Val Lys His Leu Lys Ile Leu Asn Thr Pro
20 25 30

Asn Cys Ala Leu Gln Ile Val Ala Arg Leu Lys Asn Asn Asn Arg Gln
35 40 45

Val Cys Ile Asp Pro Lys Leu Lys Trp Ile Gln Glu Tyr Leu Glu Lys
50 55 60

Ala Leu Asn Lys Arg Phe Lys Met.
65 70

Figure 1



Box is indecated open reading frame.

Figure 2

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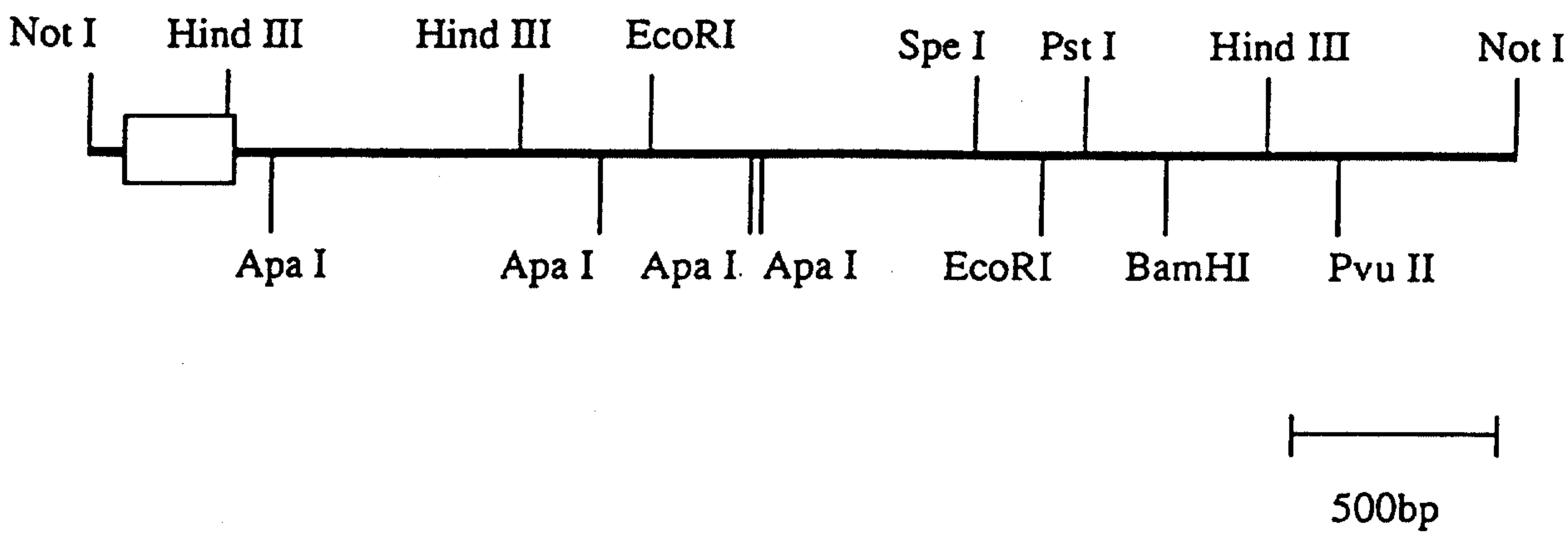
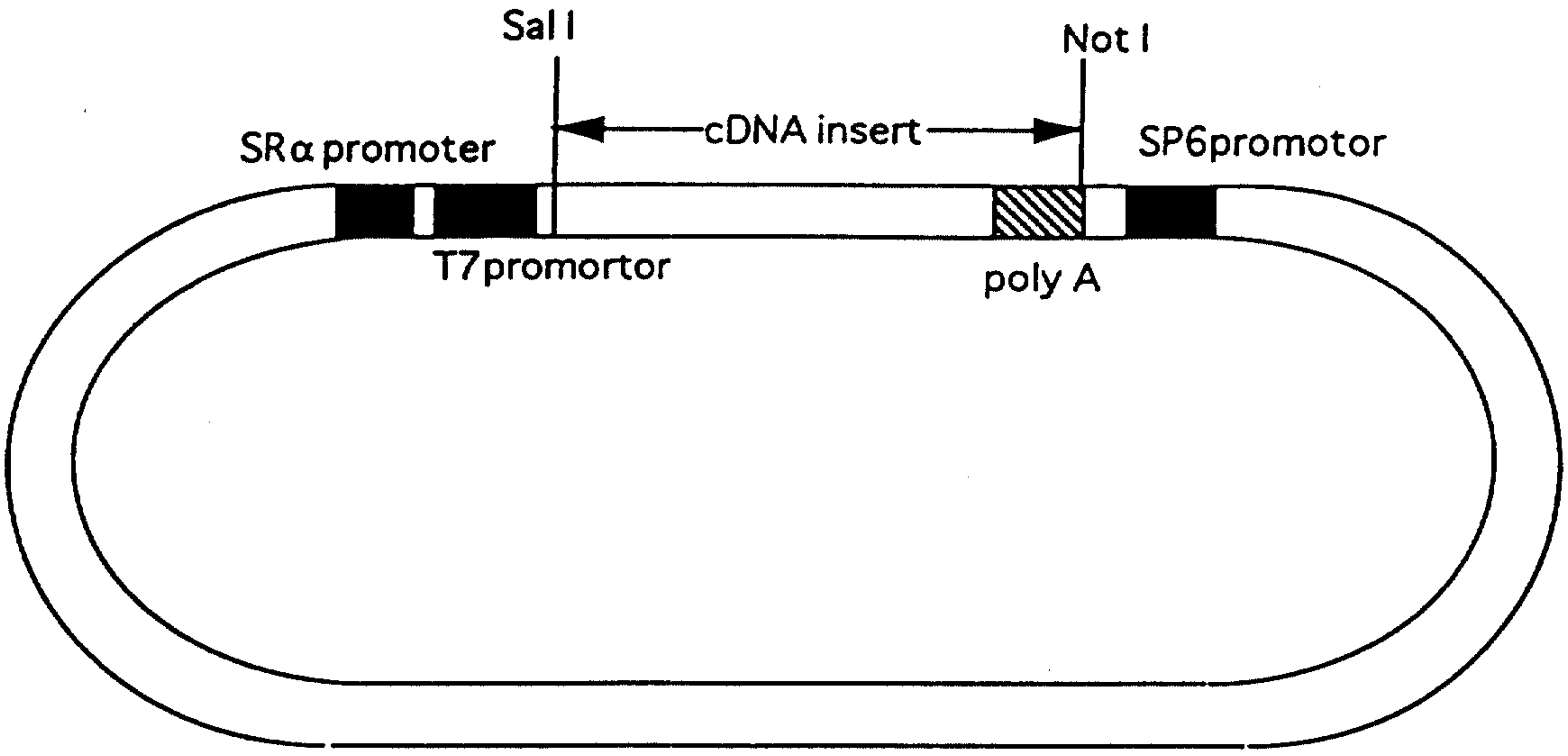


Box is indecated open reading frame.

Figure 3



pUCSRαML2 Vector