



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification ⁶ : C12N 15/12, 5/10, 1/21, C07K 14/47, 16/18, C12Q 1/68, G01N 33/50, A61K 38/17</p>	A1	<p>(11) International Publication Number: WO 99/06558</p> <p>(43) International Publication Date: 11 February 1999 (11.02.99)</p>
<p>(21) International Application Number: PCT/US98/15591</p> <p>(22) International Filing Date: 28 July 1998 (28.07.98)</p> <p>(30) Priority Data: 08/902,853 30 July 1997 (30.07.97) US</p> <p>(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 08/902,853 (CIP) Filed on 30 July 1997 (30.07.97)</p> <p>(71) Applicant (for all designated States except US): INCYTE PHARMACEUTICALS, INC. [US/US]; 3174 Porter Drive, Palo Alto, CA 94304 (US).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): HILLMAN, Jennifer, L. [US/US]; 230 Monroe Drive #12, Mountain View, CA 94040 (US). CORLEY, Neil, C. [US/US]; 1240 Dale Avenue #30, Mountain View, CA 94040 (US). SHAH, Purvi [IN/US]; 1608 Queen Charlotte Drive #5, Sunnyvale, CA 94087 (US). LAL, Preeti [IN/US]; 2382 Lass Drive, Santa Clara, CA 95054 (US).</p>	<p>(74) Agents: BILLINGS, Lucy, J. et al.; Incyte Pharmaceuticals, Inc., 3174 Porter Drive, Palo Alto, CA 94304 (US).</p> <p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report.</i></p>	
<p>(54) Title: HUMAN LONGEVITY-ASSURANCE PROTEIN HOMOLOGS</p> <p>(57) Abstract</p> <p>The invention provides two human longevity-assurance protein homologs, designated individually as LAPH-1 and LAPH-2 and collectively as LAPH, and polynucleotides which identify and encode LAPH. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of LAPH.</p>		

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HUMAN LONGEVITY-ASSURANCE PROTEIN HOMOLOGS

TECHNICAL FIELD

This invention relates to nucleic acid and amino acid sequences of two human longevity-
5 assurance protein homologs and to the use of these sequences in the diagnosis, prevention, and
treatment of disorders associated with dysregulation of cellular homeostasis and disorders
associated with aging.

BACKGROUND OF THE INVENTION

10 Normal growth, differentiation, and survival in multicellular organisms requires a
carefully regulated balance, or homeostasis, between the production and destruction of cells in
tissues throughout the body. Cell division is a carefully coordinated process with numerous
checkpoints and control mechanisms. These mechanisms are designed to regulate DNA
replication and to prevent inappropriate or excessive proliferation. In contrast, apoptosis is a
15 genetically controlled process by which unneeded or damaged cells are eliminated without
causing the tissue destruction and inflammatory responses that are often associated with acute
injury and necrosis.

A variety of ligands and their cellular receptors, enzymes, tumor suppressors, viral gene
products, pharmacological agents, and inorganic ions have important positive or negative roles in
20 regulating and implementing the apoptotic destruction of a cell. Although some specific
components of the apoptotic pathway have been identified and characterized, many interactions
between the proteins involved are undefined, leaving major aspects of the pathway unknown
(Thompson, C.B. (1995) Science 267:1456-1462).

Mammalian cells in culture can be grown for only a limited number of cell divisions after
25 which they cease proliferation and exhibit the morphological changes associated with cellular
senescence. Evidence in support of a genetic determinant for aging has been obtained in various
organisms. For instance, in the yeast Saccharomyces cerevisiae, the patterns of expression of
certain genes change in a specific manner during the life span, and these changed patterns suggest
that the aging process is subject to gene regulation.

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Controlled expression of the transforming gene of Harvey murine sarcoma virus

(v-Ha-ras) was found to extend yeast life span (as measured by the number of cell divisions) nearly two-fold (Jazwinski, S.M. et al. (1993) *Adv. Exp. Med. Biol.* 330: 45-53; Chen, J.B. et al. (1990) *Mol. Microbiol.* 4: 2081-2086). RAS1 and RAS2, which are yeast homologs of the v-Ha-ras oncogene, play central roles in the integration of cell growth and the cell cycle in yeast.

5 The primary role of these RAS proteins in yeast is the GTP-dependent regulation of adenylate cyclase activity. Curiously, mutations in RAS1 and RAS2 have opposite effects on yeast life span. Sun, J. et al. (1994; *J. Biol. Chem* 269:18638-18645) observed that deletion of RAS1 lengthened life span while deletion of RAS2 decreased life span. Elevated expression of yeast RAS2 led to a 30% increase in life span and postponed the senescence-related increase in cell
10 generation time seen during yeast aging. No life span extension was observed by overexpression of RAS1, although both RAS1 and RAS2 mRNA and protein levels declined with replicative age.

D'mello, N.P. et al. (1994; *J. Biol. Chem.* 269:15451-15459) isolated a yeast gene denoted longevity-assurance gene-1 (LAG1). LAG1 expression is highest in young cells and decreases as yeast cells age. The predicted translation product is a 411 amino acid protein
15 (denoted LAG1p or LAP1) which contains clusters of potential phosphorylation sites near the N- and C-termini and multiple potential transmembrane domains. Deletion of LAG1 resulted in a significant increase in the mean and maximum number of cell divisions; the mean life span increased from 17 to 25 cell divisions, and the maximum life span increased from 25 to 37 cell divisions. D'mello et al. (supra) proposed that the expression of LAG1 in young yeast cells may
20 set a threshold which determines the extent to which the cells can divide. Deletion of LAG1 apparently alters this threshold, and a different gene(s) may then become the limiting factor in longevity (D'mello et al., supra). LAC1, described as a virtual copy of LAG1, is found on a different yeast chromosome and also alters longevity (Jazwinski, S.M. (1996) *Science* 273:54-59; Kirchman, P.A. and Jazwinski, S.M., unpublished).

25 Dysregulation of cellular homeostasis is a significant factor in the pathogenesis of human disease. For example, inappropriate cell survival can cause or contribute to diseases such as cancer, autoimmune diseases, and inflammation. In contrast, increased apoptosis can cause or contribute to immunodeficiency diseases such as AIDS, neurodegenerative disorders including Alzheimer's disease, and myelodysplastic syndromes (Thompson, C.B. (1995) *Science* 267:1456-
30 1462).

Furthermore, numerous diseases and disorders are associated with aging. Diseases which

show age-dependent onset of symptoms include Alzheimer's disease, Pick's disease, Huntington's disease, Parkinson's disease, adult onset myotonic dystrophy, multiple sclerosis, adult onset leukodystrophy, diabetes mellitus, arteriosclerosis, and cancer.

Patients who suffer from premature aging syndromes exhibit numerous defects associated with more advanced age groups. Symptoms of Werner's syndrome include scleroderma-like skin changes, cataract, subcutaneous calcification, premature arteriosclerosis, and diabetes mellitus. A striking aspect of Werner's syndrome, presumably arising from the same genetic defect, is a dramatic shortening of the replicative life-span of dermal fibroblasts in vitro (Faragher, R.G. et al. (1993) Proc. Natl. Acad. Sci. USA 90:12030-12034). Fibroblasts from Werner's syndrome patients exit irreversibly from the cell cycle at a faster rate than do normal cells, although they generally start off with a good replicative ability. Faragher, R.G. et al (supra) proposed that the Werner syndrome gene is a "counting" gene which controls the number of times that human cells are able to divide before terminal differentiation.

The discovery of two new human longevity-assurance protein homologs and the polynucleotides encoding them satisfies a need in the art by providing new compositions which are useful in the diagnosis, prevention and treatment of disorders associated with dysregulation of cellular homeostasis and disorders associated with aging.

SUMMARY OF THE INVENTION

The invention features two substantially purified polypeptides, human longevity-assurance protein homologs (designated collectively as LAPH and individually as LAPH-1 and LAPH-2) having the amino acid sequences shown in SEQ ID NO:1 and SEQ ID NO:3, respectively, or fragments thereof.

The invention further provides an isolated and substantially purified polynucleotide sequence encoding the polypeptide comprising the amino acid sequence of SEQ ID NO:1 or fragments thereof and a composition comprising said polynucleotide sequence. The invention also provides a polynucleotide sequence which hybridizes under stringent conditions to the polynucleotide sequence encoding the amino acid sequence SEQ ID NO:1, or fragments of said polynucleotide sequence. The invention further provides a polynucleotide sequence comprising the complement of the polynucleotide sequence encoding the amino acid sequence of SEQ ID NO:1, or fragments or variants of said polynucleotide sequence.

The invention also provides an isolated and purified sequence comprising SEQ ID NO:2 or variants thereof. In addition, the invention provides a polynucleotide sequence which hybridizes under stringent conditions to the polynucleotide sequence of SEQ ID NO:2. The invention also provides a polynucleotide sequence comprising the complement of SEQ ID NO:2, or fragments or variants thereof.

The present invention further provides an expression vector containing at least a fragment of any of the claimed polynucleotide sequences. In yet another aspect, the expression vector containing the polynucleotide sequence is contained within a host cell.

The invention also provides a method for producing a polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment thereof, the method comprising the steps of: a) culturing the host cell containing an expression vector containing at least a fragment of the polynucleotide sequence encoding LAPH-1 under conditions suitable for the expression of the polypeptide; and b) recovering the polypeptide from the host cell culture.

The invention also provides a pharmaceutical composition comprising a substantially purified LAPH-1 having the amino acid sequence of SEQ ID NO:1 in conjunction with a suitable pharmaceutical carrier.

The invention also provides a purified antagonist of the polypeptide of SEQ ID NO:1. In one aspect the invention provides a purified antibody which binds to a polypeptide comprising at least a fragment of the amino acid sequence of SEQ ID NO:1.

Still further, the invention provides a purified agonist of the polypeptide of SEQ ID NO:1.

The invention also provides a method for treating or preventing a disorder associated with dysregulation of cellular homeostasis comprising administering to a subject in need of such treatment an effective amount of an antagonist to LAPH-1.

The invention also provides a method for treating or preventing a disorder associated with aging comprising administering to a subject in need of such treatment an effective amount of an antagonist to LAPH-1.

The invention also provides a method for detecting a polynucleotide which encodes LAPH-1 in a biological sample comprising the steps of: a) hybridizing the complement of the polynucleotide sequence which encodes SEQ ID NO:1 to nucleic acid material of a biological sample, thereby forming a hybridization complex; and b) detecting the hybridization complex, wherein the presence of the complex correlates with the presence of a polynucleotide encoding

LAPH-1 in the biological sample. In one aspect the nucleic acid material of the biological sample is amplified by the polymerase chain reaction prior to hybridization.

The invention further provides an isolated and substantially purified polynucleotide sequence encoding the polypeptide comprising the amino acid sequence of SEQ ID NO:3 or fragments thereof and a composition comprising said polynucleotide sequence. The invention also provides a polynucleotide sequence which hybridizes under stringent conditions to the polynucleotide sequence encoding the amino acid sequence SEQ ID NO:3, or fragments of said polynucleotide sequence. The invention further provides a polynucleotide sequence comprising the complement of the polynucleotide sequence encoding the amino acid sequence of SEQ ID NO:3, or fragments or variants of said polynucleotide sequence.

The invention also provides an isolated and purified sequence comprising SEQ ID NO:4 or variants thereof. In addition, the invention provides a polynucleotide sequence which hybridizes under stringent conditions to the polynucleotide sequence of SEQ ID NO:4. The invention also provides a polynucleotide sequence comprising the complement of SEQ ID NO:4, or fragments or variants thereof.

The present invention further provides an expression vector containing at least a fragment of any of the claimed polynucleotide sequences. In yet another aspect, the expression vector containing the polynucleotide sequence is contained within a host cell.

The invention also provides a method for producing a polypeptide comprising the amino acid sequence of SEQ ID NO:3 or a fragment thereof, the method comprising the steps of: a) culturing the host cell containing an expression vector containing at least a fragment of the polynucleotide sequence encoding LAPH-2 under conditions suitable for the expression of the polypeptide; and b) recovering the polypeptide from the host cell culture.

The invention also provides a pharmaceutical composition comprising a substantially purified LAPH-2 having the amino acid sequence of SEQ ID NO:3 in conjunction with a suitable pharmaceutical carrier.

The invention also provides a purified antagonist of the polypeptide of SEQ ID NO:3. In one aspect the invention provides a purified antibody which binds to a polypeptide comprising the amino acid sequence of SEQ ID NO:3.

Still further, the invention provides a purified agonist of the polypeptide of SEQ ID NO:3.

The invention also provides a method for treating or preventing a disorder associated with dysregulation of cellular homeostasis comprising administering to a subject in need of such treatment an effective amount of an antagonist to LAPH-2.

5 The invention also provides a method for treating or preventing a disorder associated with aging comprising administering to a subject in need of such treatment an effective amount of an antagonist to LAPH-2.

The invention also provides a method for detecting a polynucleotide which encodes LAPH-2 in a biological sample comprising the steps of: a) hybridizing the complement of the polynucleotide sequence which encodes SEQ ID NO:3 to nucleic acid material of a biological sample, thereby forming a hybridization complex; and b) detecting the hybridization complex, 10 wherein the presence of the complex correlates with the presence of a polynucleotide encoding LAPH-2 in the biological sample. In one aspect the nucleic acid material of the biological sample is amplified by the polymerase chain reaction prior to hybridization.

15 BRIEF DESCRIPTION OF THE FIGURES

Figures 1A, 1B, 1C, and 1D show the amino acid sequence (SEQ ID NO:1) and nucleic acid sequence (SEQ ID NO:2) of LAPH-1. The alignment was produced using MacDNASIS PRO™ software (Hitachi Software Engineering Co. Ltd. San Bruno, CA).

20 Figures 2A, 2B, 2C, 2D, 2E and 2F show the amino acid sequence (SEQ ID NO:3) and nucleic acid sequence (SEQ ID NO:4) of LAPH-2.

Figures 3A, 3B, 3C, and 3D show the amino acid sequence identity alignments among LAPH-1 (2516821; SEQ ID NO:1), Caenorhabditis elegans LAP-1 homolog (GI 1123105; SEQ ID NO:5), longevity assurance factor (LAF) from Schizosaccharomyces pombe (GI 1675382; SEQ ID NO:6), and LAP-1 from Saccharomyces cerevisiae (GI 541568; SEQ ID NO:7); aligned 25 amino acids with identical functional groups are boxed. The alignment was produced using the multisequence alignment program of DNASTAR™ software (DNASTAR Inc, Madison WI).

Figures 4A, 4B, 4C, and 4D show the amino acid sequence similarity alignments among LAPH-1 (2516821; SEQ ID NO:1), C. elegans LAP-1 homolog (GI 1123105; SEQ ID NO:5), longevity assurance factor (LAF) from S. pombe (GI 1675382; SEQ ID NO:6), and LAP-1 from 30 S. cerevisiae (GI 541568; SEQ ID NO:7); aligned amino acids with chemically similar functional groups are boxed.

Figures 5A, 5B, 5C, and 5D show the amino acid sequence identity alignments among LAPH-2 (493014; SEQ ID NO:3), C. elegans LAP-1 homolog (GI 1123105; SEQ ID NO:5), longevity assurance factor (LAF) from S. pombe (GI 1675382; SEQ ID NO:6), and LAP-1 from S. cerevisiae (GI 541568; SEQ ID NO:7); aligned amino acids with identical functional groups are boxed.

Figures 6A, 6B, 6C, and 6D show the amino acid sequence similarity alignments among LAPH-2 (493014; SEQ ID NO:3), C. elegans LAP-1 homolog (GI 1123105; SEQ ID NO:5), longevity assurance factor (LAF) from S. pombe (GI 1675382; SEQ ID NO:6), and LAP-1 from S. cerevisiae (GI 541568; SEQ ID NO:7); aligned amino acids with chemically similar functional groups are boxed.

Figures 7A and 7B show the hydrophobicity plots for LAPH-1 (SEQ ID NO: 1) and LAPH-2 (SEQ ID NO: 3), respectively; the positive X axis reflects amino acid position, and the negative Y axis, hydrophobicity (MacDNASIS PRO software).

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DESCRIPTION OF THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular methodology, protocols, cell lines, vectors, and reagents described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms “a”, “an”, and “the” include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to “a host cell” includes a plurality of such host cells, reference to the “antibody” is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and

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materials are now described. All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing the cell lines, vectors, and methodologies which are reported in the publications which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate
5 such disclosure by virtue of prior invention.

DEFINITIONS

LAPH, as used herein, refers to the amino acid sequences of substantially purified LAPH obtained from any species, particularly mammalian, including bovine, ovine, porcine, murine,
10 equine, and preferably human, from any source whether natural, synthetic, semi-synthetic, or recombinant.

The term "agonist", as used herein, refers to a molecule which, when bound to LAPH, increases or prolongs the duration of the effect of LAPH. Agonists may include proteins, nucleic acids, carbohydrates, or any other molecules which bind to and modulate the effect of LAPH.

15 An "allele" or "allelic sequence", as used herein, is an alternative form of the gene encoding LAPH. Alleles may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or polypeptides whose structure or function may or may not be altered. Any given natural or recombinant gene may have none, one, or many allelic forms. Common mutational changes which give rise to alleles are generally ascribed to natural deletions,
20 additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

"Altered" nucleic acid sequences encoding LAPH as used herein include those with deletions, insertions, or substitutions of different nucleotides resulting in a polynucleotide that encodes the same or a functionally equivalent LAPH. Included within this definition are
25 polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding LAPH, and improper or unexpected hybridization to alleles, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding LAPH. The encoded protein may also be "altered" and contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally
30 equivalent LAPH. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the

residues as long as the biological or immunological activity of LAPH is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid; positively charged amino acids may include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values may include leucine, isoleucine, and valine, glycine and alanine, asparagine and glutamine, serine and threonine, and phenylalanine and tyrosine.

“Amino acid sequence” as used herein refers to an oligopeptide, peptide, polypeptide, or protein sequence, and fragment thereof, and to naturally occurring or synthetic molecules. Fragments of LAPH are preferably about 5 to about 15 amino acids in length and retain the biological activity or the immunological activity of LAPH. Where “amino acid sequence” is recited herein to refer to an amino acid sequence of a naturally occurring protein molecule, amino acid sequence, and like terms, are not meant to limit the amino acid sequence to the complete, native amino acid sequence associated with the recited protein molecule.

“Amplification” as used herein refers to the production of additional copies of a nucleic acid sequence and is generally carried out using polymerase chain reaction (PCR) technologies well known in the art (Dieffenbach, C.W. and G.S. Dveksler (1995) PCR Primer, a Laboratory Manual, Cold Spring Harbor Press, Plainview, NY).

The term “antagonist” as used herein, refers to a molecule which, when bound to LAPH, decreases the amount or the duration of the effect of the biological or immunological activity of LAPH. Antagonists may include proteins, nucleic acids, carbohydrates, antibodies or any other molecules which decrease the effect of LAPH.

As used herein, the term “antibody” refers to intact molecules as well as fragments thereof, such as Fa, F(ab')₂, and Fv, which are capable of binding the epitopic determinant. Antibodies that bind LAPH polypeptides can be prepared using intact polypeptides or fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal can be derived from the translation of RNA or synthesized chemically and can be conjugated to a carrier protein, if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin and thyroglobulin, keyhole limpet hemocyanin. The coupled peptide is then used to immunize the animal (e.g., a mouse, a rat, or a rabbit).

The term “antigenic determinant”, as used herein, refers to that fragment of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or fragment of a

protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to a given region or three-dimensional structure on the protein; these regions or structures are referred to as antigenic determinants. An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The term "antisense", as used herein, refers to any composition containing nucleotide sequences which are complementary to a specific DNA or RNA sequence. The term "antisense strand" is used in reference to a nucleic acid strand that is complementary to the "sense" strand. Antisense molecules include peptide nucleic acids and may be produced by any method including synthesis or transcription. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form duplexes and block either transcription or translation. The designation "negative" is sometimes used in reference to the antisense strand, and "positive" is sometimes used in reference to the sense strand.

The term "biologically active", as used herein, refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic LAPH, or any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The terms "complementary" or "complementarity", as used herein, refer to the natural binding of polynucleotides under permissive salt and temperature conditions by base-pairing. For example, the sequence "A-G-T" binds to the complementary sequence "T-C-A". Complementarity between two single-stranded molecules may be "partial", in which only some of the nucleic acids bind, or it may be complete when total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acids strands and in the design and use of PNA molecules.

A "composition comprising a given polynucleotide sequence" as used herein refers broadly to any composition containing the given polynucleotide sequence. The composition may comprise a dry formulation or an aqueous solution. Compositions comprising polynucleotide sequences encoding LAPH (SEQ ID NO:1 or SEQ ID NO:3) or fragments thereof (e.g., SEQ ID

NO:2, SEQ ID NO:4, and fragments thereof) may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts (e.g., NaCl), detergents (e.g., SDS) and other components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.).

"Consensus", as used herein, refers to a nucleic acid sequence which has been resequenced to resolve uncalled bases, has been extended using XL-PCR™ (Perkin Elmer, Norwalk, CT) in the 5' and/or the 3' direction and resequenced, or has been assembled from the overlapping sequences of more than one Incyte Clone using a computer program for fragment assembly (e.g., GELVIEW™ Fragment Assembly system, GCG, Madison, WI). Some sequences have been both extended and assembled to produce the consensus sequence .

The term "correlates with expression of a polynucleotide", as used herein, indicates that the detection of the presence of ribonucleic acid that is similar to SEQ ID NO:2 or SEQ ID NO:4 by northern analysis is indicative of the presence of mRNA encoding LAPH in a sample and thereby correlates with expression of the transcript from the polynucleotide encoding the protein.

A "deletion", as used herein, refers to a change in the amino acid or nucleotide sequence and results in the absence of one or more amino acid residues or nucleotides.

The term "derivative", as used herein, refers to the chemical modification of a nucleic acid encoding or complementary to LAPH or the encoded LAPH. Such modifications include, for example, replacement of hydrogen by an alkyl, acyl, or amino group. A nucleic acid derivative encodes a polypeptide which retains the biological or immunological function of the natural molecule. A derivative polypeptide is one which is modified by glycosylation, pegylation, or any similar process which retains the biological or immunological function of the polypeptide from which it was derived.

The term "homology", as used herein, refers to a degree of complementarity. There may be partial homology or complete homology (i.e., identity). A partially complementary sequence that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid is referred to using the functional term "substantially homologous." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or northern blot, solution hybridization and the like) under conditions of low stringency. A substantially homologous sequence or hybridization probe will

compete for and inhibit the binding of a completely homologous sequence to the target sequence under conditions of low stringency. This is not to say that conditions of low stringency are such that non-specific binding is permitted; low stringency conditions require that the binding of two sequences to one another be a specific (i.e., selective) interaction. The absence of non-specific
5 binding may be tested by the use of a second target sequence which lacks even a partial degree of complementarity (e.g., less than about 30% identity). In the absence of non-specific binding, the probe will not hybridize to the second non-complementary target sequence.

Human artificial chromosomes (HACs) are linear microchromosomes which may contain DNA sequences of 10K to 10M in size and contain all of the elements required for stable mitotic
10 chromosome segregation and maintenance (Harrington, J.J. et al. (1997) Nat Genet. 15:345-355).

The term "humanized antibody", as used herein, refers to antibody molecules in which amino acids have been replaced in the non-antigen binding regions in order to more closely resemble a human antibody, while still retaining the original binding ability.

The term "hybridization", as used herein, refers to any process by which a strand of
15 nucleic acid binds with a complementary strand through base pairing.

The term "hybridization complex", as used herein, refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary G and C bases and between complementary A and T bases; these hydrogen bonds may be further stabilized by base stacking interactions. The two complementary nucleic acid sequences
20 hydrogen bond in an antiparallel configuration. A hybridization complex may be formed in solution (e.g., C_0t or R_0t analysis) or between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

25 An "insertion" or "addition", as used herein, refers to a change in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively, as compared to the naturally occurring molecule.

"Microarray" refers to an array of distinct polynucleotides or oligonucleotides synthesized on a substrate, such as paper, nylon or other type of membrane, filter, chip, glass slide, or any
30 other suitable solid support.

The term "modulate", as used herein, refers to a change in the activity of LAPH. For

example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional or immunological properties of LAPH.

“Nucleic acid sequence” as used herein refers to an oligonucleotide, nucleotide, or polynucleotide, and fragments thereof, and to DNA or RNA of genomic or synthetic origin which
5 may be single- or double-stranded, and represent the sense or antisense strand. “Fragments” are those nucleic acid sequences which are greater than 60 nucleotides in length, and most preferably includes fragments that are at least 100 nucleotides or at least 1000 nucleotides, and at least 10,000 nucleotides in length.

The term “oligonucleotide” refers to a nucleic acid sequence of at least about 6
10 nucleotides to about 60 nucleotides, preferably about 15 to 30 nucleotides, and more preferably about 20 to 25 nucleotides, which can be used in PCR amplification or a hybridization assay, or a microarray. As used herein, oligonucleotide is substantially equivalent to the terms “amplimers”, “primers”, “oligomers”, and “probes”, as commonly defined in the art.

“Peptide nucleic acid”, PNA as used herein, refers to an antisense molecule or anti-gene
15 agent which comprises an oligonucleotide of at least five nucleotides in length linked to a peptide backbone of amino acid residues which ends in lysine. The terminal lysine confers solubility to the composition. PNAs may be pegylated to extend their lifespan in the cell where they preferentially bind complementary single stranded DNA and RNA and stop transcript elongation (Nielsen, P.E. et al. (1993) *Anticancer Drug Des.* 8:53-63).

20 The term “portion”, as used herein, with regard to a protein (as in “a portion of a given protein”) refers to fragments of that protein. The fragments may range in size from five amino acid residues to the entire amino acid sequence minus one amino acid. Thus, a protein “comprising at least a portion of the amino acid sequence of SEQ ID NO:1” encompasses the full-length LAPH-1 and fragments thereof.

25 The term “sample”, as used herein, is used in its broadest sense. A biological sample suspected of containing nucleic acid encoding LAPH, or fragments thereof, or LAPH itself may comprise a bodily fluid, extract from a cell, chromosome, organelle, or membrane isolated from a cell, a cell, genomic DNA, RNA, or cDNA in solution or bound to a solid support, a tissue, a tissue print, and the like.

30 The terms “specific binding” or “specifically binding”, as used herein, refers to that interaction between a protein or peptide and an agonist, an antibody and an antagonist. The

interaction is dependent upon the presence of a particular structure (i.e., the antigenic determinant or epitope) of the protein recognized by the binding molecule. For example, if an antibody is specific for epitope "A", the presence of a protein containing epitope A (or free, unlabeled A) in a reaction containing labeled "A" and the antibody will reduce the amount of labeled A bound to the antibody.

The terms "stringent conditions" or "stringency", as used herein, refer to the conditions for hybridization as defined by the nucleic acid, salt, and temperature. These conditions are well known in the art and may be altered in order to identify or detect identical or related polynucleotide sequences. Numerous equivalent conditions comprising either low or high stringency depend on factors such as the length and nature of the sequence (DNA, RNA, base composition), nature of the target (DNA, RNA, base composition), milieu (in solution or immobilized on a solid substrate), concentration of salts and other components (e.g., formamide, dextran sulfate and/or polyethylene glycol), and temperature of the reactions (within a range from about 5°C below the melting temperature of the probe to about 20°C to 25°C below the melting temperature). One or more factors may be varied to generate conditions of either low or high stringency different from, but equivalent to, the above listed conditions.

The term "substantially purified", as used herein, refers to nucleic or amino acid sequences that are removed from their natural environment, isolated or separated, and are at least 60% free, preferably 75% free, and most preferably 90% free from other components with which they are naturally associated.

A "substitution", as used herein, refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

"Transformation", as defined herein, describes a process by which exogenous DNA enters and changes a recipient cell. It may occur under natural or artificial conditions using various methods well known in the art. Transformation may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method is selected based on the type of host cell being transformed and may include, but is not limited to, viral infection, electroporation, heat shock, lipofection, and particle bombardment. Such "transformed" cells include stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome. They also include cells which transiently express the inserted DNA or RNA for limited periods of

time.

A "variant" of LAPH, as used herein, refers to an amino acid sequence that is altered by one or more amino acids. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with
5 isoleucine. More rarely, a variant may have "nonconservative" changes, e.g., replacement of a glycine with a tryptophan. Analogous minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing biological or immunological activity may be found using computer programs well known in the art, for example, DNASTAR software.

10

THE INVENTION

The invention is based on the discovery of two new human longevity-assurance protein homologs, hereinafter referred to collectively as "LAPH" and individually as "LAPH-1" and "LAPH-2", the polynucleotides encoding LAPH, and the use of these compositions for the
15 diagnosis, prevention, or treatment of disorders associated with dysregulation of cellular homeostasis and disorders associated with aging.

Nucleic acids encoding the LAPH-1 of the present invention were first identified in Incyte Clone 2516821 from the liver tumor cDNA library (LIVRTUT04) using a computer search for amino acid sequence alignments. A consensus sequence, SEQ ID NO:2, was derived from the
20 following overlapping and/or extended nucleic acid sequences: Incyte Clones 1209284 (BRSTNOT02); 1916270 (PROSTUT04); 2188902 (PROSNOT26); 1442622 and 1442407 (THYRNOT03); 2516821 (LIVRTUT04); and 1500224 (SINTBST01).

In one embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:1, as shown in Figs. 1A, 1B, 1C, and 1D. LAPH-1 is 394 amino acids
25 in length and contains four potential asn-linked glycosylation sites at residues N19, N81, N172 and N364; six potential casein kinase-II phosphorylation sites at residues S4, T21, T23, T288, S342 and S349; and seven potential protein kinase-C phosphorylation sites at residues T74, T114, T130, S137, T174, S294, and T387. LAPH-1 also contains a G-coupled receptor signature sequence pattern comprising residues A46 through L62. As shown in Figs. 3A, 3B, 3C, and 3D
30 and Figs. 4A, 4B, 4C, and 4D, LAPH-1 has chemical and structural homology with C. elegans LAP-1 homolog (GI 1123105; SEQ ID NO:5), longevity assurance factor (LAF) from S. pombe

(GI 1675382; SEQ ID NO:6), and LAPH-1 from *S. cerevisiae* (GI 541568; SEQ ID NO:7). In particular, LAPH-1 and *C. elegans* LAPH-1 homolog share 52% amino acid sequence similarity and 33% amino acid sequence identity; LAPH-1 and *S. pombe* LAF share 35% similarity and 22% identity; and LAPH-1 and *S. cerevisiae* LAPH-1 share 33% similarity and 20% identity.

5 Similar amino acids are defined by chemically similar functional groups as described in Karlin, S. et al. (1985; Proc Natl. Acad. Sci. USA 82:8597-8601). As illustrated by Figure 7A, LAPH-1 contains at least seven potential transmembrane domains. Northern analysis shows the expression of LAPH-1 in various tissues, including fetal kidney and lung; cancers of the breast, liver, lung, prostate, small intestine and thyroid; diseased adrenal gland; Crohn's disease-afflicted
10 small intestine; and Alzheimer's disease-afflicted brain tissues (corpus callosum, hippocampus, and pons). The similarity of LAPH-1 to longevity-assurance protein, the presence of multiple phosphorylation sites and a G-coupled receptor signature pattern in the LAPH-1 protein sequence, and the expression of LAPH-1 in diseases associated with dysregulation of cellular homeostasis suggest that LAPH-1 participates in signal transduction and cell cycle regulation,
15 and may play a role in regulating the balance between cell proliferation and apoptosis. Increased expression of LAPH-1 may increase susceptibility to dysregulation of cellular homeostatic pathways.

Nucleic acids encoding the LAPH-2 of the present invention were first identified in Incyte Clone 493014 from a neuronal precursor teratocarcinoma cell line cDNA library (HNT2NOT01)
20 using a computer search for amino acid sequence alignments. A consensus sequence, SEQ ID NO:4, was derived from the following overlapping and/or extended nucleic acid sequences: Incyte Clones 493014 (HNT2NOT01); 503465 (TMLR3DT02); 2512244 (CONUTUT01); 1979181 (LUNGTUTO3); 2607554 (LUNGTUT07); 1879756 (LEUKNOT03); 126702 (LUNGNOT01); 1682573 (PROSNOT15); 1361323 (LUNGNOT12); 2733890 (OVARTUT04);
25 1865737 (PROSNOT19); and 2474414 (SMCANOT01).

In another embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:3, as shown in Figs. 2A, 2B, 2C, 2D, 2E and 2F. LAPH-2 is 380 amino acids in length and contains two potential asn-linked glycosylation sites at residues N19 and N81; four potential casein kinase-II phosphorylation sites at residues T4, T21, S341, and
30 S349; and six potential protein kinase-C phosphorylation sites at residues S91, S110, S137, S248, T332, and S341. LAPH-2 also contains an ATP/GTP-binding site P-loop motif comprising

residues A355 through S362. As shown in Figs. 5A, 5B, 5C, and 5D and Figs. 6A, 6B, 6C, and 6D, LAPH-2 has chemical and structural homology with C. elegans LAP-1 homolog (GI 1123105; SEQ ID NO:5), longevity assurance factor (LAF) from S. pombe (GI 1675382; SEQ ID NO:6), and LAP-1 from S. cerevisiae (GI 541568; SEQ ID NO:7). In particular, LAPH-2 and C. elegans LAP-1 homolog share 49% amino acid sequence similarity and 32% amino acid sequence identity; LAPH-2 and S. pombe LAF share 36% similarity and 19% identity; and LAPH-2 and S. cerevisiae LAP-1 share 34% similarity and 17% identity. Similar amino acids are defined by chemically similar functional groups as described in Karlin, S. et al. (supra). As illustrated by Figure 7B, LAPH-2 contains at least seven potential transmembrane domains. Northern analysis shows the expression of LAPH-2 in various libraries, including fibroblast, coronary endothelial, neuronal precursor, cardiac smooth muscle and promonocyte cell lines; fetal colon, liver, kidney, lung and placenta; cancers of the bladder, brain, breast, colon, heart, liver, lung, neuro- and paraganglia, ovary, prostate, testicle, thymus, thyroid and uterus; Alzheimer's disease-afflicted brain; and cells and tissues associated with inflammation or the immune response, including macrophages, bone marrow, lung (asthma), small intestine (Crohn's disease) and skin (erythema nodosum). The similarity of LAPH-2 to longevity-assurance protein, the presence of multiple phosphorylation sites and an ATP/GTP-binding site P-loop motif in the LAPH-2 protein sequence, and the expression of LAPH-2 in diseases and disorders associated with dysregulation of cellular homeostasis suggest that LAPH-2 participates in signal transduction and cell cycle regulation, and may play a role in regulating the balance between cell proliferation and apoptosis. Increased expression of LAPH-2 may increase susceptibility to dysregulation of cellular homeostatic pathways.

The invention also encompasses LAPH variants. A preferred LAPH variant is one having at least 80%, and more preferably at least 90%, amino acid sequence identity to the LAPH amino acid sequence (SEQ ID NO:1 or SEQ ID NO:3) and which retains at least one biological, immunological or other functional characteristic or activity of LAPH. A most preferred LAPH variant is one having at least 95% amino acid sequence identity to SEQ ID NO:1 or SEQ ID NO:3.

The invention also encompasses polynucleotides which encode LAPH. Accordingly, any nucleic acid sequence which encodes the amino acid sequence of LAPH can be used to produce recombinant molecules which express LAPH. In a particular embodiment, the invention

encompasses the polynucleotide comprising the nucleic acid sequence of SEQ ID NO:2 as shown in Figs. 1A, 1B, 1C, and 1D. In another embodiment, the invention encompasses the polynucleotide comprising the nucleic acid sequence of SEQ ID NO:4 as shown in Figs. 2A, 2B, 2C, 2D, 2E and 2F.

5 It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of nucleotide sequences encoding LAPH, some bearing minimal homology to the nucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of nucleotide sequence that could be made by selecting combinations based on possible codon choices. These
10 combinations are made in accordance with the standard triplet genetic code as applied to the nucleotide sequence of naturally occurring LAPH, and all such variations are to be considered as being specifically disclosed.

 Although nucleotide sequences which encode LAPH and its variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring LAPH under
15 appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding LAPH or its derivatives possessing a substantially different codon usage. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular
20 codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding LAPH and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

 The invention also encompasses production of DNA sequences, or fragments thereof, which encode LAPH and its derivatives, entirely by synthetic chemistry. After production, the
25 synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents that are well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding LAPH or any fragment thereof.

 Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed nucleotide sequences, and in particular, those shown in SEQ ID NO:2
30 and in SEQ ID NO:4, under various conditions of stringency as taught in Wahl, G.M. and S.L. Berger (1987; *Methods Enzymol.* 152:399-407) and Kimmel, A.R. (1987; *Methods Enzymol.*

152:507-511).

Methods for DNA sequencing which are well known and generally available in the art and may be used to practice any of the embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, Sequenase® (US Biochemical Corp, Cleveland, OH), Taq polymerase (Perkin Elmer), thermostable T7 polymerase (Amersham, Chicago, IL), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE Amplification System marketed by Gibco/BRL (Gaithersburg, MD). Preferably, the process is automated with machines such as the Hamilton Micro Lab 2200 (Hamilton, Reno, NV), Peltier Thermal Cycler (PTC200; MJ Research, Watertown, MA) and the ABI Catalyst and 373 and 377 DNA Sequencers (Perkin Elmer).

The nucleic acid sequences encoding LAPH may be extended utilizing a partial nucleotide sequence and employing various methods known in the art to detect upstream sequences such as promoters and regulatory elements. For example, one method which may be employed, "restriction-site" PCR, uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar, G. (1993) PCR Methods Applic. 2:318-322). In particular, genomic DNA is first amplified in the presence of primer to a linker sequence and a primer specific to the known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR may also be used to amplify or extend sequences using divergent primers based on a known region (Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186). The primers may be designed using commercially available software such as OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, MN), or another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68°-72° C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Another method which may be used is capture PCR which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119). In this method, multiple restriction enzyme digestions and ligations may also be used to place an engineered

double-stranded sequence into an unknown fragment of the DNA molecule before performing PCR.

Another method which may be used to retrieve unknown sequences is that of Parker, J.D. et al. (1991; Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PromoterFinder™ libraries to walk genomic DNA (Clontech, Palo Alto, CA). This process
5 avoids the need to screen libraries and is useful in finding intron/exon junctions. When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Also, random-primed libraries are preferable, in that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed library may be
10 especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In
15 particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser activated, and detection of the emitted wavelengths by a charge coupled device camera. Output/light intensity may be converted to electrical signal using appropriate software (e.g. Genotyper™ and Sequence Navigator™, Perkin Elmer) and the entire process from loading of samples to computer analysis
20 and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode LAPH may be used in recombinant DNA molecules to direct expression of LAPH,
25 fragments or functional equivalents thereof, in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced, and these sequences may be used to clone and express LAPH.

As will be understood by those of skill in the art, it may be advantageous to produce
30 LAPH-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate

of protein expression or to produce an RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter LAPH encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations, and so forth.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding LAPH may be ligated to a heterologous sequence to encode a fusion protein. For example, to screen peptide libraries for inhibitors of LAPH activity, it may be useful to encode a chimeric LAPH protein that can be recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the LAPH encoding sequence and the heterologous protein sequence, so that LAPH may be cleaved and purified away from the heterologous moiety.

In another embodiment, sequences encoding LAPH may be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers, M.H. et al. (1980) Nucl. Acids Res. Symp. Ser. 215-223, Horn, T. et al. (1980) Nucl. Acids Res. Symp. Ser. 225-232). Alternatively, the protein itself may be produced using chemical methods to synthesize the amino acid sequence of LAPH, or a fragment thereof. For example, peptide synthesis can be performed using various solid-phase techniques (Roberge, J.Y. et al. (1995) Science 269:202-204) and automated synthesis may be achieved, for example, using the ABI 431A Peptide Synthesizer (Perkin Elmer).

The newly synthesized peptide may be substantially purified by preparative high performance liquid chromatography (e.g., Creighton, T. (1983) Proteins, Structures and Molecular Principles, WH Freeman and Co., New York, NY). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; Creighton, supra). Additionally, the amino acid sequence of LAPH, or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

In order to express a biologically active LAPH, the nucleotide sequences encoding LAPH or functional equivalents, may be inserted into appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence.

5 Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding LAPH and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described in Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, 10 Plainview, NY, and Ausubel, F.M. et al. (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY.

A variety of expression vector/host systems may be utilized to contain and express sequences encoding LAPH. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression 15 vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transformed with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems.

The invention is not limited by the host cell employed.

20 The "control elements" or "regulatory sequences" are those non-translated regions of the vector--enhancers, promoters, 5' and 3' untranslated regions--which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be 25 used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the Bluescript® phagemid (Stratagene, LaJolla, CA) or pSport1™ plasmid (Gibco BRL) and the like may be used. The baculovirus polyhedrin promoter may be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (e.g., heat shock, RUBISCO; and storage protein genes) or from plant viruses (e.g., viral promoters or leader 30 sequences) may be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell

line that contains multiple copies of the sequence encoding LAPH, vectors based on SV40 or EBV may be used with an appropriate selectable marker.

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for LAPH. For example, when large quantities of LAPH are needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be used. Such vectors include, but are not limited to, the multifunctional E. coli cloning and expression vectors such as Bluescript® (Stratagene), in which the sequence encoding LAPH may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β -galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509); and the like. pGEX vectors (Promega, Madison, WI) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems may be designed to include heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast, Saccharomyces cerevisiae, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH may be used. For reviews, see Ausubel et al. (supra) and Grant et al. (1987) Methods Enzymol. 153:516-544.

In cases where plant expression vectors are used, the expression of sequences encoding LAPH may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV may be used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) EMBO J. 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used (Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105). These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (see, for example, Hobbs, S. or Murry, L.E. in McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York, NY; pp. 191-196.

An insect system may also be used to express LAPH. For example, in one such system,

Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in Spodoptera frugiperda cells or in Trichoplusia larvae. The sequences encoding LAPH may be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of LAPH will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses may then be used to infect, for example, S. frugiperda cells or Trichoplusia larvae in which LAPH may be expressed (Engelhard, E.K. et al. (1994) Proc. Nat. Acad. Sci. 91:3224-3227).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding LAPH may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain a viable virus which is capable of expressing LAPH in infected host cells (Logan, J. and Shenk, T. (1984) Proc. Natl. Acad. Sci. 81:3655-3659). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained and expressed in a plasmid. HACs of 6 to 10M are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes.

Specific initiation signals may also be used to achieve more efficient translation of sequences encoding LAPH. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding LAPH, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used, such as those described in the literature (Scharf, D. et al. (1994) Results Probl.

Cell Differ. 20:125-162).

In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding and/or function. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC; Bethesda, MD) and may be chosen to ensure the correct modification and processing of the foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express LAPH may be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler, M. et al. (1977) Cell 11:223-32) and adenine phosphoribosyltransferase (Lowy, I. et al. (1980) Cell 22:817-23) genes which can be employed in tk⁻ or aprt⁻ cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr which confers resistance to methotrexate (Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. 77:3567-70); npt, which confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin, F. et al (1981) J. Mol. Biol. 150:1-14) and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, supra). Additional selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine (Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. 85:8047-51). Recently, the use of visible markers has gained

popularity with such markers as anthocyanins, β glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes, C.A. et al. (1995) *Methods Mol. Biol.* 55:121-131).

5 Although the presence/absence of marker gene expression suggests that the gene of interest is also present, its presence and expression may need to be confirmed. For example, if the sequence encoding LAPH is inserted within a marker gene sequence, transformed cells containing sequences encoding LAPH can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding LAPH under the
10 control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

 Alternatively, host cells which contain the nucleic acid sequence encoding LAPH and express LAPH may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and
15 protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein.

 The presence of polynucleotide sequences encoding LAPH can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or fragments or fragments of
20 polynucleotides encoding LAPH. Nucleic acid amplification based assays involve the use of oligonucleotides or oligomers based on the sequences encoding LAPH to detect transformants containing DNA or RNA encoding LAPH.

 A variety of protocols for detecting and measuring the expression of LAPH, using either polyclonal or monoclonal antibodies specific for the protein are known in the art. Examples
25 include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on LAPH is preferred, but a competitive binding assay may be employed. These and other assays are described, among other
places, in Hampton, R. et al. (1990; *Serological Methods, a Laboratory Manual*, APS Press, St Paul, MN) and Maddox, D.E. et al. (1983; *J. Exp. Med.* 158:1211-1216).

30 A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled

hybridization or PCR probes for detecting sequences related to polynucleotides encoding LAPH include oligolabeling, nick translation, end-labeling or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding LAPH, or any fragments thereof may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes *in vitro* by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits (Pharmacia & Upjohn, (Kalamazoo, MI); Promega (Madison WI); and U.S. Biochemical Corp., Cleveland, OH). Suitable reporter molecules or labels, which may be used for ease of detection, include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding LAPH may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode LAPH may be designed to contain signal sequences which direct secretion of LAPH through a prokaryotic or eukaryotic cell membrane. Other constructions may be used to join sequences encoding LAPH to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, WA). The inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen, San Diego, CA) between the purification domain and LAPH may be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing LAPH and a nucleic acid encoding 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on IMAC (immobilized metal ion affinity chromatography as described in Porath, J. et al. (1992, Prot. Exp. Purif. 3: 263-281) while the enterokinase cleavage site provides a means for purifying LAPH from the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll, D.J. et al. (1993; DNA

Cell Biol. 12:441-453).

In addition to recombinant production, fragments of LAPH may be produced by direct peptide synthesis using solid-phase techniques Merrifield J. (1963) J. Am. Chem. Soc. 85:2149-2154). Protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Various fragments of LAPH may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

THERAPEUTICS

Chemical and structural homology exists among LAPH, *C. elegans* LAP-1 homolog (GI 1123105), *S. pombe* LAF (GI 1675382), and *S. cerevisiae* LAP-1 (GI 541568). In addition, LAPH is expressed in tumors, fetal tissues, cell lines, cells and tissues involved in inflammation, and Alzheimer's disease-afflicted brain. Increased expression of LAPH appears to increase susceptibility to dysregulation of cellular homeostatic pathways. Therefore, LAPH appears to play a role in disorders associated with dysregulation of cellular homeostasis and disorders associated with aging.

Therefore, in one embodiment, an antagonist of LAPH may be administered to a subject to prevent or treat a disorder associated with dysregulation of cellular homeostasis. Such disorders include, but are not limited to, cancers such as adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, and teratocarcinoma, and particularly cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; disorders associated with inflammation or autoimmune disease including AIDS, Addison's disease, adult respiratory distress syndrome, allergies, anemia, asthma, atherosclerosis, bronchitis, cholecystitis, Crohn's disease, ulcerative colitis, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, erythema nodosum, atrophic gastritis, glomerulonephritis, gout, Graves' disease, hypereosinophilia, irritable bowel syndrome, lupus erythematosus, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, rheumatoid arthritis, scleroderma, Sjögren's syndrome, and autoimmune thyroiditis; complications of cancer, hemodialysis, and extracorporeal circulation; viral, bacterial, fungal, parasitic, protozoal, and

helminthic infections; and trauma; neurodegenerative disorders including akathisia, Alzheimer's disease, amnesia, amyotrophic lateral sclerosis, bipolar disorder, catatonia, dementia, depression, Down's syndrome, tardive dyskinesia, dystonias, epilepsy, Huntington's disease, multiple sclerosis, neurofibromatosis, Parkinson's disease, paranoid psychoses, schizophrenia, and
5 Tourette's disorder; and myelodysplastic disorders such as aplastic anemia and pancytopenia. In one aspect, an antibody which specifically binds LAPH may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissue which express LAPH.

In another embodiment, a vector expressing the complement of the polynucleotide
10 encoding LAPH may be administered to a subject to treat or prevent a disorder associated with dysregulation of cellular homeostasis including, but not limited to, those described above.

In another embodiment, an antagonist of LAPH may be administered to a subject to prevent or treat a disorder associated with aging. Such disorders include, but are not limited to, Alzheimer's disease, Pick's disease, Huntington's disease, Parkinson's disease, myotonic
15 dystrophy, multiple sclerosis, adult onset leukodystrophy, diabetes mellitus, arteriosclerosis, scleroderma, cataract, osteosarcoma, osteoporosis, erythroidmacrocytosis, and premature aging syndromes including Werner's and Okamoto's syndromes. In one aspect, an antibody which specifically binds LAPH may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissue which express LAPH.

In another embodiment, a vector expressing the complement of the polynucleotide
20 encoding LAPH may be administered to a subject to treat or prevent a disorder associated with aging including, but not limited to, those described above.

In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences or vectors of the invention may be administered in combination with
25 other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for
30 adverse side effects.

An antagonist of LAPH may be produced using methods which are generally known in

the art. In particular, purified LAPH may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind LAPH.

Antibodies to LAPH may be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, single
5 chain, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies, (i.e., those which inhibit dimer formation) are especially preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others, may be immunized by injection with LAPH or any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants
10 may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to
15 LAPH have an amino acid sequence consisting of at least five amino acids and more preferably at least 10 amino acids. It is also preferable that they are identical to a portion of the amino acid sequence of the natural protein, and they may contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of LAPH amino acids may be fused with those of
20 another protein such as keyhole limpet hemocyanin and antibody produced against the chimeric molecule.

Monoclonal antibodies to LAPH may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-
25 hybridoma technique (Kohler, G. et al. (1975) Nature 256:495-497; Kozbor, D. et al. (1985) J. Immunol. Methods 81:31-42; Cote, R.J. et al. (1983) Proc. Natl. Acad. Sci. 80:2026-2030; Cole, S.P. et al. (1984) Mol. Cell Biol. 62:109-120).

In addition, techniques developed for the production of "chimeric antibodies", the splicing
30 of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can be used (Morrison, S.L. et al. (1984) Proc. Natl. Acad. Sci. 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; Takeda, S. et al. (1985) Nature

314:452-454). Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce LAPH-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries (Burton D.R. 5 (1991) Proc. Natl. Acad. Sci. 88:11120-3).

Antibodies may also be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. 86: 3833-3837; Winter, G. et al. (1991) Nature 349:293-299).

10 Antibody fragments which contain specific binding sites for LAPH may also be generated. For example, such fragments include, but are not limited to, the F(ab')₂ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal 15 Fab fragments with the desired specificity (Huse, W.D. et al. (1989) Science 254:1275-1281).

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between 20 LAPH and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering LAPH epitopes is preferred, but a competitive binding assay may also be employed (Maddox, supra).

In another embodiment of the invention, the polynucleotides encoding LAPH, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, the 25 complement of the polynucleotide encoding LAPH may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding LAPH. Thus, complementary molecules or fragments may be used to modulate LAPH activity, or to achieve regulation of gene function. Such technology is now well known in the art, and sense or antisense oligonucleotides or larger 30 fragments, can be designed from various locations along the coding or control regions of sequences encoding LAPH.

Expression vectors derived from retro viruses, adenovirus, herpes or vaccinia viruses, or from various bacterial plasmids may be used for delivery of nucleotide sequences to the targeted organ, tissue or cell population. Methods which are well known to those skilled in the art can be used to construct vectors which will express nucleic acid sequence which is complementary to the polynucleotides of the gene encoding LAPH. These techniques are described both in
5 Sambrook et al. (supra) and in Ausubel et al. (supra).

Genes encoding LAPH can be turned off by transforming a cell or tissue with expression vectors which express high levels of a polynucleotide or fragment thereof which encodes LAPH. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell.
10 Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector and even longer if appropriate replication elements are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by designing
15 complementary sequences or antisense molecules (DNA, RNA, or PNA) to the control, 5' or regulatory regions of the gene encoding LAPH (signal sequence, promoters, enhancers, and introns). Oligonucleotides derived from the transcription initiation site, e.g., between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the
20 ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature (Gee, J.E. et al. (1994) In: Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing Co., Mt. Kisco, NY). The complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the
25 transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples which may be used include engineered hammerhead motif ribozyme molecules that
30 can specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding LAPH.

Specific ribozyme cleavage sites within any potential RNA target are initially identified

by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Complementary ribonucleic acid molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding LAPH. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize complementary RNA constitutively or inducibly can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections or polycationic amino polymers (Goldman, C.K. et al. (1997) Nature Biotechnology 15:462-66; incorporated herein by reference) may be achieved using methods which are well known in the art.

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

An additional embodiment of the invention relates to the administration of a pharmaceutical composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of LAPH, antibodies to LAPH, mimetics, agonists, antagonists, or inhibitors of LAPH. The compositions may be administered alone or in combination with at least one other agent, such as stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs or hormones.

The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, PA).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired,

disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the

corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder which may contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of LAPH, such labeling would include amount, frequency, and method of administration.

Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells, or in animal models, usually mice, rabbits, dogs, or pigs. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example LAPH or fragments thereof, antibodies of LAPH, agonists, antagonists or inhibitors of LAPH, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED₅₀ (the dose therapeutically effective in 50% of the population) and LD₅₀ (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD₅₀/ED₅₀. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, general health of the subject, age, weight, and

gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation.

5 Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to
10 particular cells, conditions, locations, etc.

DIAGNOSTICS

In another embodiment, antibodies which specifically bind LAPH may be used for the diagnosis of conditions or diseases characterized by expression of LAPH, or in assays to monitor
15 patients being treated with LAPH, agonists, antagonists or inhibitors. The antibodies useful for diagnostic purposes may be prepared in the same manner as those described above for therapeutics. Diagnostic assays for LAPH include methods which utilize the antibody and a label to detect LAPH in human body fluids or extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by joining them, either covalently or non-
20 covalently, with a reporter molecule. A wide variety of reporter molecules which are known in the art may be used, several of which are described above.

A variety of protocols including ELISA, RIA, and FACS for measuring LAPH are known in the art and provide a basis for diagnosing altered or abnormal levels of LAPH expression. Normal or standard values for LAPH expression are established by combining body fluids or cell
25 extracts taken from normal mammalian subjects, preferably human, with antibody to LAPH under conditions suitable for complex formation. The amount of standard complex formation may be quantified by various methods, but preferably by photometric, means. Quantities of LAPH expressed in subject, control and disease, samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the
30 parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding LAPH may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide

sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantitate gene expression in biopsied tissues in which expression of LAPH may be correlated with disease. The diagnostic assay may be used to distinguish between absence, presence, and excess expression of LAPH, and to monitor regulation of LAPH levels
5 during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding LAPH or closely related molecules, may be used to identify nucleic acid sequences which encode LAPH. The specificity of the probe, whether it is made from a highly specific region, e.g., 10 unique nucleotides in the 5'
10 regulatory region, or a less specific region, e.g., especially in the 3' coding region, and the stringency of the hybridization or amplification (maximal, high, intermediate, or low) will determine whether the probe identifies only naturally occurring sequences encoding LAPH, alleles, or related sequences.

Probes may also be used for the detection of related sequences, and should preferably
15 contain at least 50% of the nucleotides from any of the LAPH encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and derived from the nucleotide sequence of SEQ ID NO:2 or SEQ ID NO:4 or from genomic sequence including promoter, enhancer elements, and introns of the naturally occurring LAPH.

Means for producing specific hybridization probes for DNAs encoding LAPH include the
20 cloning of nucleic acid sequences encoding LAPH or LAPH derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, commercially available, and may be used to synthesize RNA probes *in vitro* by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, radionuclides such as ³²P or ³⁵S, or enzymatic labels,
25 such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding LAPH may be used for the diagnosis of conditions or disorders which are associated with expression of LAPH. Examples of such conditions or disorders include cancers such as adenocarcinoma, leukemia, lymphoma, melanoma, myeloma,
30 sarcoma, and teratocarcinoma, and particularly cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver,

lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; disorders associated with inflammation or autoimmune disease including AIDS, Addison's disease, adult respiratory distress syndrome, allergies, anemia, asthma, atherosclerosis, bronchitis, cholecystitis, Crohn's disease, ulcerative colitis, atopic
5 dermatitis, dermatomyositis, diabetes mellitus, emphysema, erythema nodosum, atrophic gastritis, glomerulonephritis, gout, Graves' disease, hypereosinophilia, irritable bowel syndrome, lupus erythematosus, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, rheumatoid arthritis, scleroderma, Sjögren's syndrome, and autoimmune thyroiditis; complications of cancer,
10 hemodialysis, and extracorporeal circulation; viral, bacterial, fungal, parasitic, protozoal, and helminthic infections; and trauma; neurodegenerative disorders including akathisia, Alzheimer's disease, amnesia, amyotrophic lateral sclerosis, bipolar disorder, catatonia, dementia, depression, Down's syndrome, tardive dyskinesia, dystonias, epilepsy, Huntington's disease, multiple sclerosis, neurofibromatosis, Parkinson's disease, paranoid psychoses, schizophrenia, and
15 Tourette's disorder; myelodysplastic disorders such as aplastic anemia and pancytopenia; and disorders associated with aging including Alzheimer's disease, Pick's disease, Huntington's disease, Parkinson's disease, myotonic dystrophy, multiple sclerosis, adult onset leukodystrophy, diabetes mellitus, arteriosclerosis, scleroderma, cataract, osteosarcoma, osteoporosis, erythroidmacrocytosis, and premature aging syndromes including Werner's and Okamoto's
20 syndromes. The polynucleotide sequences encoding LAPH may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; or in dipstick, pin, ELISA assays or microarrays utilizing fluids or tissues from patient biopsies to detect altered LAPH expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding LAPH may be useful in assays
25 that detect activation or induction of various cancers, particularly those mentioned above. The nucleotide sequences encoding LAPH may be labeled by standard methods, and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantitated and compared with a standard value. If the amount of signal in the biopsied or extracted sample
30 is significantly altered from that of a comparable control sample, the nucleotide sequences have hybridized with nucleotide sequences in the sample, and the presence of altered levels of

nucleotide sequences encoding LAPH in the sample indicates the presence of the associated disease. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or in monitoring the treatment of an individual patient.

5 In order to provide a basis for the diagnosis of disease associated with expression of LAPH, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, which encodes LAPH, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the
10 values obtained from normal subjects with those from an experiment where a known amount of a substantially purified polynucleotide is used. Standard values obtained from normal samples may be compared with values obtained from samples from patients who are symptomatic for disease. Deviation between standard and subject values is used to establish the presence of disease.

15 Once disease is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to evaluate whether the level of expression in the patient begins to approximate that which is observed in the normal patient. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

20 With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

25 Additional diagnostic uses for oligonucleotides designed from the sequences encoding LAPH may involve the use of PCR. Such oligomers may be chemically synthesized, generated enzymatically, or produced *in vitro*. Oligomers will preferably consist of two nucleotide sequences, one with sense orientation (5'→3') and another with antisense (3'←5'), employed under optimized conditions for identification of a specific gene or condition. The same two
30 oligomers, nested sets of oligomers, or even a degenerate pool of oligomers may be employed under less stringent conditions for detection and/or quantitation of closely related DNA or RNA

sequences.

Methods which may also be used to quantitate the expression of LAPH include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and standard curves onto which the experimental results are interpolated (Melby, P.C. et al. (1993) J.

5 Immunol. Methods, 159:235-244; Duplaa, C. et al. (1993) Anal. Biochem. 229-236). The speed of quantitation of multiple samples may be accelerated by running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

In further embodiments, an oligonucleotide derived from any of the polynucleotide
10 sequences described herein may be used as a target in a microarray. The microarray can be used to monitor the expression level of large numbers of genes simultaneously (to produce a transcript image), and to identify genetic variants, mutations and polymorphisms. This information will be useful in determining gene function, understanding the genetic basis of disease, diagnosing disease, and in developing and monitoring the activity of therapeutic agents (Heller, R. et al.
15 (1997) Proc. Natl. Acad. Sci. 94:2150-55).

In one embodiment, the microarray is prepared and used according to the methods described in PCT application WO95/11995 (Chee et al.), Lockhart, D. J. et al. (1996; Nat. Biotech. 14: 1675-1680) and Schena, M. et al. (1996; Proc. Natl. Acad. Sci. 93: 10614-10619), all of which are incorporated herein in their entirety by reference.

20 The microarray is preferably composed of a large number of unique, single-stranded nucleic acid sequences, usually either synthetic antisense oligonucleotides or fragments of cDNAs, fixed to a solid support. The oligonucleotides are preferably about 6-60 nucleotides in length, more preferably 15-30 nucleotides in length, and most preferably about 20-25 nucleotides in length. For a certain type of microarray, it may be preferable to use oligonucleotides which are
25 only 7-10 nucleotides in length. The microarray may contain oligonucleotides which cover the known 5', or 3', sequence, sequential oligonucleotides which cover the full length sequence; or unique oligonucleotides selected from particular areas along the length of the sequence. Polynucleotides used in the microarray may be oligonucleotides that are specific to a gene or genes of interest in which at least a fragment of the sequence is known or that are specific to one
30 or more unidentified cDNAs which are common to a particular cell type, developmental or disease state.

In order to produce oligonucleotides to a known sequence for a microarray, the gene of interest is examined using a computer algorithm which starts at the 5' or more preferably at the 3' end of the nucleotide sequence. The algorithm identifies oligomers of defined length that are unique to the gene, have a GC content within a range suitable for hybridization, and lack
5 predicted secondary structure that may interfere with hybridization. In certain situations it may be appropriate to use pairs of oligonucleotides on a microarray. The "pairs" will be identical, except for one nucleotide which preferably is located in the center of the sequence. The second oligonucleotide in the pair (mismatched by one) serves as a control. The number of oligonucleotide pairs may range from two to one million. The oligomers are synthesized at
10 designated areas on a substrate using a light-directed chemical process. The substrate may be paper, nylon or other type of membrane, filter, chip, glass slide or any other suitable solid support.

In another aspect, an oligonucleotide may be synthesized on the surface of the substrate by using a chemical coupling procedure and an ink jet application apparatus, as described in PCT
15 application WO95/251116 (Baldeschweiler et al.) which is incorporated herein in its entirety by reference. In another aspect, a "gridded" array analogous to a dot (or slot) blot may be used to arrange and link cDNA fragments or oligonucleotides to the surface of a substrate using a vacuum system, thermal, UV, mechanical or chemical bonding procedures. An array, such as those described above, may be produced by hand or by using available devices (slot blot or dot
20 blot apparatus), materials (any suitable solid support), and machines (including robotic instruments), and may contain 8, 24, 96, 384, 1536 or 6144 oligonucleotides, or any other number between two and one million which lends itself to the efficient use of commercially available instrumentation.

In order to conduct sample analysis using a microarray, the RNA or DNA from a
25 biological sample is made into hybridization probes. The mRNA is isolated, and cDNA is produced and used as a template to make antisense RNA (aRNA). The aRNA is amplified in the presence of fluorescent nucleotides, and labeled probes are incubated with the microarray so that the probe sequences hybridize to complementary oligonucleotides of the microarray. Incubation conditions are adjusted so that hybridization occurs with precise complementary matches or with
30 various degrees of less complementarity. After removal of nonhybridized probes, a scanner is used to determine the levels and patterns of fluorescence. The scanned images are examined to

determine degree of complementarity and the relative abundance of each oligonucleotide sequence on the microarray. The biological samples may be obtained from any bodily fluids (such as blood, urine, saliva, phlegm, gastric juices, etc.), cultured cells, biopsies, or other tissue preparations. A detection system may be used to measure the absence, presence, and amount of hybridization for all of the distinct sequences simultaneously. This data may be used for large scale correlation studies on the sequences, mutations, variants, or polymorphisms among samples.

In another embodiment of the invention, the nucleic acid sequences which encode LAPH may also be used to generate hybridization probes which are useful for mapping the naturally occurring genomic sequence. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome or to artificial chromosome constructions, such as human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions or single chromosome cDNA libraries as reviewed in Price, C.M. (1993) *Blood Rev.* 7:127-134, and Trask, B.J. (1991) *Trends Genet.* 7:149-154.

Fluorescent in situ hybridization (FISH as described in Verma et al. (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York, NY) may be correlated with other physical chromosome mapping techniques and genetic map data. Examples of genetic map data can be found in various scientific journals or at Online Mendelian Inheritance in Man (OMIM). Correlation between the location of the gene encoding LAPH on a physical chromosomal map and a specific disease, or predisposition to a specific disease, may help delimit the region of DNA associated with that genetic disease. The nucleotide sequences of the subject invention may be used to detect differences in gene sequences between normal, carrier, or affected individuals.

In situ hybridization of chromosomal preparations and physical mapping techniques such as linkage analysis using established chromosomal markers may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms, or parts thereof, by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or

syndrome has been crudely localized by genetic linkage to a particular genomic region, for example, AT to 11q22-23 (Gatti, R.A. et al. (1988) Nature 336:577-580), any sequences mapping to that area may represent associated or regulatory genes for further investigation. The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal
5 location due to translocation, inversion, etc. among normal, carrier, or affected individuals.

In another embodiment of the invention, LAPH, its catalytic or immunogenic fragments or oligopeptides thereof, can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of
10 binding complexes, between LAPH and the agent being tested, may be measured.

Another technique for drug screening which may be used provides for high throughput screening of compounds having suitable binding affinity to the protein of interest as described in published PCT application WO84/03564. In this method, as applied to LAPH large numbers of different small test compounds are synthesized on a solid substrate, such as plastic pins or some
15 other surface. The test compounds are reacted with LAPH, or fragments thereof, and washed. Bound LAPH is then detected by methods well known in the art. Purified LAPH can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which
20 neutralizing antibodies capable of binding LAPH specifically compete with a test compound for binding LAPH. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with LAPH.

In additional embodiments, the nucleotide sequences which encode LAPH may be used in
25 any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

The examples below are provided to illustrate the subject invention and are not included for the purpose of limiting the invention.

30

EXAMPLES

I cDNA Library Construction

LIVRTUT04

The LIVRTUT04 cDNA library was prepared from cancerous liver tissue obtained from a 50-year-old Caucasian male during a partial hepatectomy. Pathology indicated a grade 3-4
5 hepatoma forming a mass. Surgical margins were free of tumor. A 1.5 cm cavity of gel foam was present at the capsular surface. No lymphovascular invasion was seen. The adjacent liver showed mild portal fibrosis with lymphoid aggregates and mild steatosis. Patient history included benign hypertension and hepatitis at the age of 16. The patient was classified as a carrier because patient was positive for antibody to hepatitis B core antigen and hepatitis B surface antigen, while
10 negative for HBV DNA.

The frozen tissue was homogenized and lysed in Trizol reagent (1 gm tissue/10 ml Trizol; Cat. #10296-028; Gibco/BRL), a monoplastic solution of phenol and guanidine isothiocyanate, using a Brinkmann Homogenizer Polytron PT-3000 (Brinkmann Instruments, Westbury, NY). After a brief incubation on ice, chloroform was added (1:5 v/v) and the lysate was centrifuged.
15 The upper chloroform layer was removed to a fresh tube and the RNA extracted with isopropanol, resuspended in DEPC-treated water, and treated with DNase for 25 min at 37°C. The RNA was re-extracted once with acid phenol-chloroform pH 4.7 and precipitated using 0.3M sodium acetate and 2.5 volumes ethanol. The mRNA was then isolated with the Qiagen Oligotex kit (QIAGEN, Inc., Chatsworth, CA) and used to construct the cDNA library.

The mRNA was handled according to the recommended protocols in the SuperScript Plasmid System for cDNA Synthesis and Plasmid Cloning (Cat. #18248-013, Gibco/BRL). LIVRTUT04 cDNAs were fractionated on a Sepharose CL4B column (Cat. #275105-01; Pharmacia & Upjohn), and those cDNAs exceeding 400 bp were ligated into pINCY 1. The plasmid pINCY 1 was subsequently transformed into DH5aTM competent cells (Cat. #18258-012;
25 Gibco/BRL).

HNT2NOT01

The hNT2 cell line derives from a human teratocarcinoma and exhibits characteristics of a committed neuronal precursor cell at an early stage of development. The HNT2NOT01 cDNA library prepared from this untreated cell line was obtained from Stratagene (Cat. No. 937230).
30 The cDNA library was constructed by essentially the following procedure. cDNAs were primed using oligo d(T) and size fractionated to isolate fragments of 500 bp and larger. Synthetic adapter

oligonucleotides were ligated onto the cDNA molecules enabling them to be inserted into the Uni-ZAP™ vector system (Stratagene). The quality of the cDNA library was screened using DNA probes, and then, the pBluescript phagemid (Stratagene) was excised. Subsequently, the custom-constructed library phage particles were infected into *E. coli* host strain XL1-Blue®
5 (Stratagene).

II Isolation and Sequencing of cDNA Clones

Plasmid or phagemid DNA was purified using the Miniprep Kit (Catalogue # 77468, Advanced Genetic Technologies Corporation, Gaithersburg MD), a 96-well block kit with
10 reagents for 960 purifications. The recommended protocol included with the kit was employed except for the following changes. Each of the 96 wells was filled with only 1 ml of sterile Terrific Broth (Catalog # 22711, GIBCO/BRL) with carbenicillin at 25 mg/L and glycerol at 0.4%. After the wells were inoculated, the bacteria were cultured for 24 hours and lysed with 60
15 μ l of lysis buffer. A centrifugation step (Beckman GS-6R @2900 rpm for 5 min; Beckman Instruments) was performed before the contents of the block were added to the primary filter plate. The optional step of adding isopropanol to TRIS buffer was not routinely performed. After the last step in the protocol, samples were transferred to a Beckman 96-well block for storage.

The cDNAs were sequenced by the method of Sanger F. and A.R. Coulson (1975; J Mol
20 Biol 94:441f), using a Hamilton Micro Lab 2200 (Hamilton, Reno NV) in combination with four Peltier Thermal Cyclers (PTC200 from MJ Research, Watertown MA) and Applied Biosystems 377 or 373 DNA Sequencing Systems (Perkin Elmer), and the reading frame was determined.

III Homology Searching of cDNA Clones and Their Deduced Proteins

25 The nucleotide sequences of the Sequence Listing or amino acid sequences deduced from them were used as query sequences against databases such as GenBank, SwissProt, BLOCKS, and Pima II. These databases which contain previously identified and annotated sequences were searched for regions of homology (similarity) using BLAST, which stands for Basic Local
Alignment Search Tool (Altschul, S.F. (1993) J. Mol. Evol. 36:290-300; Altschul et al. (1990) J.
30 Mol. Biol. 215:403-410).

BLAST produces alignments of both nucleotide and amino acid sequences to determine

sequence similarity. Because of the local nature of the alignments, BLAST is especially useful in determining exact matches or in identifying homologs which may be of prokaryotic (bacterial) or eukaryotic (animal, fungal or plant) origin. Other algorithms such as the one described in Smith R.F. and T.F. Smith (1992; Protein Engineering 5:35-51), incorporated herein by reference, can be used when dealing with primary sequence patterns and secondary structure gap penalties. As disclosed in this application, the sequences have lengths of at least 49 nucleotides, and no more than 12% uncalled bases (where N is recorded rather than A, C, G, or T).

The BLAST approach, as detailed in Karlin, S. and S.F. Altschul (1993; Proc. Nat. Acad. Sci. 90:5873-7) and incorporated herein by reference, searches for matches between a query sequence and a database sequence, to evaluate the statistical significance of any matches found, and to report only those matches which satisfy the user-selected threshold of significance. In this application, threshold was set at 10^{-25} for nucleotides and 10^{-14} for peptides.

Incyte nucleotide sequences were searched against the GenBank databases for primate (pri), rodent (rod), and mammalian sequences (mam), and deduced amino acid sequences from the same clones are searched against GenBank functional protein databases, mammalian (mamp), vertebrate (vrtp) and eukaryote (eukp), for homology.

IV Northern Analysis

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound (Sambrook et al., supra).

Analogous computer techniques using BLAST (Altschul, S.F. (1993) J. Mol. Evol. 36:290-300; Altschul, S.F. et al. (1990) J. Mol. Evol. 215:403-410) are used to search for identical or related molecules in nucleotide databases such as GenBank or the LIFESEQ™ database (Incyte Pharmaceuticals). This analysis is much faster than multiple, membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or homologous.

The basis of the search is the product score which is defined as:

$$\frac{\% \text{ sequence identity} \times \% \text{ maximum BLAST score}}{100}$$

The product score takes into account both the degree of similarity between two sequences and the

length of the sequence match. For example, with a product score of 40, the match will be exact within a 1-2% error; and at 70, the match will be exact. Homologous molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules.

5 The results of northern analysis are reported as a list of libraries in which the transcript encoding LAPH occurs. Abundance and percent abundance are also reported. Abundance directly reflects the number of times a particular transcript is represented in a cDNA library, and percent abundance is abundance divided by the total number of sequences examined in the cDNA library.

10

V **Extension of LAPH Encoding Polynucleotides**

The nucleic acid sequence of Incyte Clone 2516821 or of Incyte Clone 493014 was used to design oligonucleotide primers for extending a partial nucleotide sequence to full length. One primer was synthesized to initiate extension in the antisense direction, and the other was
15 synthesized to extend sequence in the sense direction. Primers were used to facilitate the extension of the known sequence "outward" generating amplicons containing new, unknown nucleotide sequence for the region of interest. The initial primers were designed from the cDNA using OLIGO 4.06 (National Biosciences), or another appropriate program, to be about 22 to about 30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target
20 sequence at temperatures of about 68° to about 72° C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries (Gibco/BRL) were used to extend the sequence. If more than one extension is necessary or desired, additional sets of primers are designed to further extend the known region.

25 High fidelity amplification was obtained by following the instructions for the XL-PCR kit (Perkin Elmer) and thoroughly mixing the enzyme and reaction mix. Beginning with 40 pmol of each primer and the recommended concentrations of all other components of the kit, PCR was performed using the Peltier Thermal Cycler (PTC200; M.J. Research, Watertown, MA) and the following parameters:

30	Step 1	94° C for 1 min (initial denaturation)
	Step 2	65° C for 1 min
	Step 3	68° C for 6 min

	Step 4	94° C for 15 sec
	Step 5	65° C for 1 min
	Step 6	68° C for 7 min
	Step 7	Repeat step 4-6 for 15 additional cycles
5	Step 8	94° C for 15 sec
	Step 9	65° C for 1 min
	Step 10	68° C for 7:15 min
	Step 11	Repeat step 8-10 for 12 cycles
	Step 12	72° C for 8 min
10	Step 13	4° C (and holding)

A 5-10 μ l aliquot of the reaction mixture was analyzed by electrophoresis on a low concentration (about 0.6-0.8%) agarose mini-gel to determine which reactions were successful in extending the sequence. Bands thought to contain the largest products were excised from the gel, purified using QIAQuick™ (QIAGEN Inc., Chatsworth, CA), and trimmed of overhangs using Klenow enzyme to facilitate religation and cloning.

After ethanol precipitation, the products were redissolved in 13 μ l of ligation buffer, 1 μ l T4-DNA ligase (15 units) and 1 μ l T4 polynucleotide kinase were added, and the mixture was incubated at room temperature for 2-3 hours or overnight at 16° C. Competent *E. coli* cells (in 40 μ l of appropriate media) were transformed with 3 μ l of ligation mixture and cultured in 80 μ l of SOC medium (Sambrook et al., supra). After incubation for one hour at 37° C, the *E. coli* mixture was plated on Luria Bertani (LB)-agar (Sambrook et al., supra) containing 2x Carb. The following day, several colonies were randomly picked from each plate and cultured in 150 μ l of liquid LB/2x Carb medium placed in an individual well of an appropriate, commercially-available, sterile 96-well microtiter plate. The following day, 5 μ l of each overnight culture was transferred into a non-sterile 96-well plate and after dilution 1:10 with water, 5 μ l of each sample was transferred into a PCR array.

For PCR amplification, 18 μ l of concentrated PCR reaction mix (3.3x) containing 4 units of rTth DNA polymerase, a vector primer, and one or both of the gene specific primers used for the extension reaction were added to each well. Amplification was performed using the following conditions:

	Step 1	94° C for 60 sec
	Step 2	94° C for 20 sec
	Step 3	55° C for 30 sec
35	Step 4	72° C for 90 sec
	Step 5	Repeat steps 2-4 for an additional 29 cycles
	Step 6	72° C for 180 sec

Step 7 4° C (and holding)

Aliquots of the PCR reactions were run on agarose gels together with molecular weight markers. The sizes of the PCR products were compared to the original partial cDNAs, and appropriate clones were selected, ligated into plasmid, and sequenced.

In like manner, the nucleotide sequence of SEQ ID NO:2 or of SEQ ID NO:4 is used to obtain 5' regulatory sequences using the procedure above, oligonucleotides designed for 5' extension, and an appropriate genomic library.

10 **VI Labeling and Use of Individual Hybridization Probes**

Hybridization probes derived from SEQ ID NO:2 or SEQ ID NO:4 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base-pairs, is specifically described, essentially the same procedure is used with larger nucleotide fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 (National Biosciences), labeled by combining 50 pmol of each oligomer and 250 μ Ci of [γ -³²P] adenosine triphosphate (Amersham) and T4 polynucleotide kinase (DuPont NEN[®], Boston, MA). The labeled oligonucleotides are substantially purified with Sephadex G-25 superfine resin column (Pharmacia & Upjohn). A aliquot containing 10⁷ counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA digested with one of the following endonucleases (Ase I, Bgl II, Eco RI, Pst I, Xba I, or Pvu II; DuPont NEN[®]).

The DNA from each digest is fractionated on a 0.7 percent agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham, NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under increasingly stringent conditions up to 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. After XOMAT AR™ film (Kodak, Rochester, NY) is exposed to the blots in a Phosphorimager cassette (Molecular Dynamics, Sunnyvale, CA) for several hours, hybridization patterns are compared visually.

30 **VII Microarrays**

To produce oligonucleotides for a microarray, one of the nucleotide sequences described herein is examined using a computer algorithm which starts at the 3' end of the nucleotide

sequence. The algorithm identifies oligomers of defined length that are unique to the gene, have a GC content within a range suitable for hybridization, and lack predicted secondary structure that would interfere with hybridization. The algorithm identifies 20 sequence-specific oligonucleotides of 20 nucleotides in length (20-mers). A matched set of oligonucleotides is
5 created in which one nucleotide in the center of each sequence is altered. This process is repeated for each gene in the microarray, and double sets of twenty 20 mers are synthesized and arranged on the surface of the silicon chip using a light-directed chemical process (Chee, M. et al., PCT/WO95/11995, incorporated herein by reference).

In the alternative, a chemical coupling procedure and an ink jet device are used to
10 synthesize oligomers on the surface of a substrate (Baldeschweiler, J.D. et al., PCT/WO95/25116, incorporated herein by reference). In another alternative, a "gridded" array analogous to a dot (or slot) blot is used to arrange and link cDNA fragments or oligonucleotides to the surface of a substrate using a vacuum system, thermal, UV, mechanical or chemical bonding procedures. An array may be produced by hand or using available materials and
15 machines and contain grids of 8 dots, 24 dots, 96 dots, 384 dots, 1536 dots or 6144 dots. After hybridization, the microarray is washed to remove nonhybridized probes, and a scanner is used to determine the levels and patterns of fluorescence. The scanned images are examined to determine degree of complementarity and the relative abundance of each oligonucleotide sequence on the micro-array.

20

VIII Complementary Polynucleotides

Sequence complementary to the LAPH-encoding sequence, or any part thereof, is used to decrease or inhibit expression of naturally occurring LAPH. Although use of oligonucleotides comprising from about 15 to about 30 base-pairs is described, essentially the same procedure is
25 used with smaller or larger sequence fragments. Appropriate oligonucleotides are designed using Oligo 4.06 software and the coding sequence of LAPH, SEQ ID NO:1 or SEQ ID NO:3. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the LAPH-encoding
30 transcript.

IX Expression of LAPH

Expression of LAPH is accomplished by subcloning the cDNAs into appropriate vectors and transforming the vectors into host cells. In this case, the cloning vector is also used to express LAPH in *E. coli*. Upstream of the cloning site, this vector contains a promoter for β -galactosidase, followed by sequence containing the amino-terminal Met, and the subsequent
5 β -galactosidase. Immediately following these eight residues is a bacteriophage promoter useful for transcription and a linker containing a number of unique restriction sites.

Induction of an isolated, transformed bacterial strain with IPTG using standard methods produces a fusion protein which consists of the first eight residues of β -galactosidase, about 5 to
10 15 residues of linker, and the full length protein.

X Demonstration of LAPH Activity

The effect of LAPH on apoptosis may be assayed by quantitating apoptosis-associated DNA fragmentation using the Fluorescein Apoptosis Detection System (Catalog #G3250,
15 Promega). This kit utilizes the Terminal Deoxynucleotidyl Transferase (TdT)-mediated dUTP Nick End-Labeling (TUNEL) technique.

PC12 cells cultured in the presence of 100 ng/ml nerve growth factor (NGF) for at least 2 weeks undergo apoptosis within 48 hours once NGF is removed from the culture medium (Wolozin, B. et al. (1996) Science 274:1710-1713). PC12 cells cultured in the presence of NGF
20 are transfected with a eukaryotic expression vector encoding LAPH or with the vector alone. Cells are cultured for 48 hours in the presence or absence of NGF. Apoptosis is assayed by the incorporation of fluorescein-12-dUTP at the 3'-hydroxyl ends of fragmented DNA according to the directions supplied by Promega. Cells harboring fluorescein-dUTP-labeled DNA are
25 visualized directly by fluorescence microscopy or quantitated by fluorescence activated cell sorting (FACS).

XI Production of LAPH Specific Antibodies

LAPH that is substantially purified using PAGE electrophoresis (Sambrook, supra), or other purification techniques, is used to immunize rabbits and to produce antibodies using
30 standard protocols. The amino acid sequence deduced from SEQ ID NO:2 or SEQ ID NO:4 is analyzed using DNASTAR software (DNASTAR Inc) to determine regions of high

immunogenicity and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions, is described by Ausubel et al. (supra), and others.

Typically, the oligopeptides are 15 residues in length, synthesized using an Applied Biosystems Peptide Synthesizer Model 431A using fmoc-chemistry, and coupled to keyhole limpet hemocyanin (KLH, Sigma, St. Louis, MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS; Ausubel et al., supra). Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. The resulting antisera are tested for anti-peptide activity, for example, by binding the peptide to plastic, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio iodinated, goat anti-rabbit IgG.

XII Purification of Naturally Occurring LAPH Using Specific Antibodies

Naturally occurring or recombinant LAPH is substantially purified by immunoaffinity chromatography using antibodies specific for LAPH. An immunoaffinity column is constructed by covalently coupling LAPH antibody to an activated chromatographic resin, such as CNBr-activated Sepharose (Pharmacia & Upjohn). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing LAPH is passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of LAPH (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/LAPH binding (eg, a buffer of pH 2-3 or a high concentration of a chaotrope, such as urea or thiocyanate ion), and LAPH is collected.

XIII Identification of Molecules Which Interact with LAPH

LAPH or biologically active fragments thereof are labeled with ¹²⁵I Bolton-Hunter reagent (Bolton et al. (1973) Biochem. J. 133: 529). Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled LAPH, washed and any wells with labeled LAPH complex are assayed. Data obtained using different concentrations of LAPH are used to calculate values for the number, affinity, and association of LAPH with the candidate molecules.

All publications and patents mentioned in the above specification are herein incorporated

by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited
5 to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

What is claimed is:

1. A substantially purified human longevity-assurance protein homolog comprising the amino acid sequence of SEQ ID NO:1 or fragments thereof.
- 5
2. A substantially purified variant of human longevity-assurance protein homolog having at least 90% amino acid identity to SEQ ID NO:1 and which retains at least one functional characteristic of human longevity-assurance protein homolog.
- 10
3. An isolated and purified polynucleotide sequence encoding the human longevity-assurance protein homolog of claim 1 or fragments or variants of said polynucleotide sequence.
4. A composition comprising the polynucleotide sequence of claim 3.
- 15
5. A polynucleotide sequence which hybridizes to the polynucleotide sequence of claim 3.
6. A polynucleotide sequence which is complementary to the polynucleotide sequence of claim 3 or fragments or variants thereof.
- 20
7. An isolated and purified polynucleotide sequence comprising SEQ ID NO:2 or fragments or variants thereof.
8. A polynucleotide sequence which is complementary to the polynucleotide
- 25
- sequence of claim 7.
9. An expression vector containing at least a fragment of the polynucleotide sequence of claim 3.
- 30
10. A host cell containing the vector of claim 9.

11. A method for producing a polypeptide comprising the amino acid sequence of SEQ ID NO:1, or a fragment thereof, the method comprising the steps of:

a) culturing the host cell of claim 10 under conditions suitable for the expression of the polypeptide; and

5 b) recovering the polypeptide from the host cell culture.

12. A pharmaceutical composition comprising a substantially purified human longevity-assurance protein homolog having the amino acid sequence of SEQ ID NO:1 in conjunction with a suitable pharmaceutical carrier.

10

13. A purified antibody which specifically binds to the polypeptide of claim 1.

14. A purified agonist of the polypeptide of claim 1.

15 15. A purified antagonist of the polypeptide of claim 1.

16. A method for treating a disorder associated with dysregulation of cellular homeostasis comprising administering to a subject in need of such treatment an effective amount of the antagonist of claim 15.

20

17. A method for treating a disorder associated with aging comprising administering to a subject in need of such treatment an effective amount of the antagonist of claim 15.

18. A method for detecting a polynucleotide which encodes human longevity-assurance protein homolog in a biological sample comprising the steps of:

25

a) hybridizing the polynucleotide of claim 6 to nucleic acid material of a biological sample, thereby forming a hybridization complex; and

b) detecting said hybridization complex, wherein the presence of said complex correlates with the presence of a polynucleotide encoding human longevity-assurance protein homolog in said biological sample.

30

19. The method of claim 18 wherein the nucleic acid material is amplified by the polymerase chain reaction prior to hybridization.

20. A substantially purified human longevity-assurance protein homolog comprising
5 the amino acid sequence of SEQ ID NO:3 or fragments thereof.

21. A substantially purified variant of human longevity-assurance protein homolog having at least 90% amino acid identity to SEQ ID NO:3 and which retains at least one functional characteristic of human longevity-assurance protein homolog.
10

22. An isolated and purified polynucleotide sequence encoding the human longevity-assurance protein homolog of claim 20 or fragments or variants of said polynucleotide sequence.

23. A composition comprising the polynucleotide sequence of claim 22.
15

24. A polynucleotide sequence which hybridizes to the polynucleotide sequence of claim 22.

25. A polynucleotide sequence which is complementary to the polynucleotide
20 sequence of claim 22 or fragments or variants thereof.

26. An isolated and purified polynucleotide sequence comprising SEQ ID NO:4 or fragments or variants thereof.

27. A polynucleotide sequence which is complementary to the polynucleotide
25 sequence of claim 26.

28. An expression vector containing at least a fragment of the polynucleotide
sequence of claim 22.
30

29. A host cell containing the vector of claim 28.

30. A method for producing a polypeptide comprising the amino acid sequence of SEQ ID NO:3, or a fragment thereof, the method comprising the steps of:

a) culturing the host cell of claim 29 under conditions suitable for the expression of the polypeptide; and

5 b) recovering the polypeptide from the host cell culture.

31. A pharmaceutical composition comprising a substantially purified human longevity-assurance protein homolog having the amino acid sequence of SEQ ID NO:3 in conjunction with a suitable pharmaceutical carrier.

10

32. A purified antibody which specifically binds to the polypeptide of claim 20.

33. A purified agonist of the polypeptide of claim 20.

15

34. A purified antagonist of the polypeptide of claim 20.

35. A method for treating a disorder associated with dysregulation of cellular homeostasis comprising administering to a subject in need of such treatment an effective amount of the antagonist of claim 34.

20

36. A method for treating a disorder associated with aging comprising administering to a subject in need of such treatment an effective amount of the antagonist of claim 34.

37. A method for detecting a polynucleotide which encodes human longevity-assurance protein homolog in a biological sample comprising the steps of:

25

a) hybridizing the polynucleotide of claim 25 to nucleic acid material of a biological sample, thereby forming a hybridization complex; and

b) detecting said hybridization complex, wherein the presence of said complex correlates with the presence of a polynucleotide encoding human longevity-assurance protein homolog in said biological sample.

30

38. The method of claim 37 wherein the nucleic acid material is amplified by the polymerase chain reaction prior to hybridization.

<110> INCYTE PHARMACEUTICALS, INC.
 HILLMAN, Jennifer L.
 CORLEY, Neil C.
 SHAH, Purvi
 LAL, Preeti

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FIG. 1A

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	Q T Q CAG CGA TGG TTC CGG AGA CGC CGG AAC CAG GAT CGA CCC CAG CTG				
441	ACC AAG AAG TTC TGT GAG GCC AGC TGG AGG TTT CTC TTC TAC CTG TCC TCC TTC	459	468	477	486
	T K K F C E A S W R F L F Y L S S F				
495	GTG GGC GGC CTC TCG GTC CCA AAC CAG ACT CTG AAG CCA TCC CTG TGG GCA CCA GTA ATG	513	522	531	540
	V G G L S V L Y H E S W L W A P V M				
549	TGC TGG GAC AGG TAC CCA AAC CAG ACT CTG AAG CCA TCC CTG TAC TGG TGG TAC	567	576	585	594
	C W D R Y P N Q T L K P S L Y W Y				
603	CTC TTG GAG CTG GGT TTC TAC CTC TCA CTG CTA ATC AGG CTG CCC TTT GAT GTC	621	630	639	648
	L L E L G F Y L S L L I R L P F D V				
657	AAG CGC AAG GAT TTC AAG GAG CAG GTG ATA CAC CAC TTC GTG GCG GTC ATC CTG	675	684	693	702
	K R K D F K E Q V I H H F V A V I L				
711	ATG ACC TTC TCC TAC AGT GCC AAC CTG CTG CGC ATT GGC TCT CTG GTG CTG CTG	729	738	747	756
	M T F S Y S A N L L R I G S L V L L				

FIG. 1B

765	774	783	792	801	810
TTA CAC GAT TCC TCT GAC TAC CTG CTG GAG GCC TGT AAG ATG GTC AAC TAC ATG					
L H D S S D Y L L L E A C K M V N Y M					
819	828	837	846	855	864
CAG TAT CAG CAA GTG TGC GAC GCT CTC TTC CTC ATC TTC TCC TTT GTC TTC TTC					
Q Y Q Q V C D A L F L I F S F V F F					
873	882	891	900	909	918
TAC ACC CGA CTG GTC CTC TTT CCC ACC CAG ATC CTC TAC ACC ACA TAC TAC GAG					
Y T R L V L F P T Q I L Y T T Y Y E					
927	936	945	954	963	972
TCC ATC AGC AAC AGG GGC CCC TTC TTC GGC TAC TAC TTC AAC GGC CTT CTG					
S I S N R G G P F F G Y Y F F N G L L					
981	990	999	1008	1017	1026
ATG TTG CTG CAG CTG CTG CAC GTG TTC TGG TCT TGC CTC ATT CTG CGC ATG CTC					
M L L Q L L H V F F W S C L I L R M L					
1035	1044	1053	1062	1071	1080
TAT AGC TTC ATG AAG AAG GGC CAG ATG GAG AAG GAC ATT CGT AGT GAT GTA GAA					
Y S F M K K G Q M E K D I R S D V E					
1089	1098	1107	1116	1125	1134
GAA TCA GAC TCC AGT GAG GAG GCG GCG GCG GAG GAA CCT CTG CAG CTA AAG					
E S D S S E E A A A A Q E P L Q L K					

FIG. 1C

1143 1152 1161 1170 1179 1188
 AAC GGG ACA GCT GGA GGG CCC AGG CCA GCC CCC ACT GAT GGC CCT CGG AGC CGG
 N G T A G G G P R P A P T D G P R S R

 1197 1206 1215 1224 1233 1242
 GTG GCC GGG CGT CTG ACC AAC AGG CAC ACA ACA GCC ACA TAG CCG GGC GGG GCT
 V A G R L T N R H T A T

 1251 1260 1269 1278 1287 1296
 GGC TGT AAG GGG TTG CCC CCC CGC CAG TGC CTT GGA TAT TTC TGG GGT GAC TGG

 1305 1314 1323 1332 1341 1350
 ACT GGC GCC CCT GGG CCA CCT TTC TGG AGA CAG GGA GGG CCC CAC CCG GGG TGG

 1359 1368 1377 1386 1395 1404
 GTG GGA AGG CTG ATG ATC TGT CTC CAG CCC CTT CCT TCT GCC CAC CCG CCC TTC

 1413 1422 1431 1440 1449 1458
 TTC CCT CTG GGC AAC TGG ACA GAT CTG GGA GCC AGC AGC TGG ATG CTG TGG CTG

 1467 1476 1485 1494 1503 1512
 GCC AGA GAC ACC TCC AGG CTG TAG CCT GGG GGC TGG GGG GAG CCC CAG GCT GAA

 1521 1530 1539 1548
 AAG GGT CCA ATT AAA ACA AAT GGA GCC AAA AAA AGA A

9	18	27	36	45	54
A GGG AGG AGA GGC GCG GGG AGC CAG GCC TCG GGG CCT CGG AGC AAC CAC CCG					
63	72	81	90	99	108
AGC AGA CGG AGT ACA CGG AGC AGC GGC CCC GGC AAC GCT GCC GCC GCG GGG					
117	126	135	144	153	162
ATG CTC CAG ACC TTG TAT GAT TAC TTC TGG TGG GAA CGT CTG TGG CTG CCT GTG					
M L Q T L Y D Y F W W E R L W L P V					
171	180	189	198	207	216
AAC TTG ACC TGG GCC GAT CTA GAA GAC CGA GAT GGA CGT GTC TAC GCC AAA GCC					
N L T W A D L E D R D G R V Y A K A					
225	234	243	252	261	270
TCA GAT CTC TAT ATC ACG CTG CCC CTG GCC TCG CTC TTC CTC ATC GTT CGA TAC					
S D L Y I T L P L A L L F L I V R Y					
279	288	297	306	315	324
TTC TTT GAG CTG TAC GTG GCT ACA CCA CTG GCT GCC CTT TTG AAC ATA AAG GAG					
F F E L Y V A T P L A A L L N I K E					
333	342	351	360	369	378
AAA ACT CGG CTG CGG GCA CCT CCC AAC GCC ACC TTG GAA CAT TTC TAC CTG ACC					
K T R L R A P P N A T L E H F Y L T					

FIG. 2A

387	AGT GGC AAG CAG CCC AAG CAG GTG GAA GTA GAG CTT TTG TCC CGG CAG AGC GGG	414	423	432
	S G K Q Q P K Q V E V E L L S R Q S G			
396		405		
441	CTC TCT GGC CGC CAG GTA GAG CGT TGG TTC CGT CGC CGC AAC CAG GAC CGG	468	477	486
	L S G R Q V E R E R W F R R R N Q D R			
450		459		
495	CCC AGT CTC CTC AAG AAG TTC CGA GAA GCC AGC TGG AGA TTC ACA TTT TAC CTG	522	531	540
	P S L L K K AAG TTC CGA GAA GCC AGC TGG AGA TTC ACA TTT TAC CTG			
504		513		
549	ATT GCC TTC ATT GCC GGC ATG GCC GTC ATT GTG GAT AAA CCC TGG TTC TAT GAC	576	585	594
	I A F I A G M A V I V D K P W F Y D			
558		567		
603	ATG AAG AAA GTT TGG GAG GGA TAT CCC ATA CAG AGC ACT ATC CCT TCC CAG TAT	621	630	648
	M K K V V W E G Y P I Q S T I P S Q Y			
612		621		
657	TGG TAC ATG ATT GAA CTT TCC TTC TAC TGG TCC CTG CTC TTC AGC ATT GCC	684	693	702
	W Y Y M I E L S F Y W S L L F S I A			
666		675		
711	TCT GAT GTC AAG CGA AAG GAT TTC AAG GAA CAG ATC ATC CAC CAT GTG GCC ACC	729	738	756
	S D V K K R K D F F K E Q I I H H V A T			

FIG. 2B

765	ATC ATT CTC ATC AGC TTT TCC TGG TTT GCC AAT TAC ATC CGA GCT GGG ACT CTA	774	774	783	792	801	810
	I I L I S F S W F F TCC TGG TTT GCC AAT TAC ATC CGA GCT GGG ACT CTA						
819	ATC ATG GCT CTG CAT GAC TCT TCC GAT TAC CTG CTG GAG TCA GCC AAG ATG TTT	828	828	837	846	855	864
	I M A L H D S S D D Y L L E S A K M F						
873	AAC TAC GCG GGA TGG AAG AAC ACC TGC AAC AAC ATC TTC ATC GTC TTC GCC ATT	882	882	891	900	909	918
	N Y A G W K N T C N N I F I V F A I						
927	GTT TTT ATC ATC ACC CGA CTG GTC ATC CTG CCC TTC TGG ATC CTG CAT TGC ACC	936	936	945	954	963	972
	V F I I T R L V I I L P F W I L H C T						
981	CTG GTG TAC CCA CTG GAG CTC TAT CCT CCT GGC TTC TTT GGC TAT TAC TTC TTC AAT	990	990	999	1008	1017	1026
	L V Y P L E L Y P A F F G Y Y F F N						
1035	TCC ATG ATG GGA GTT CTA CAG CTG CTG CAT ATC TTC TGG GCC TAC CTC ATT TTG	1044	1044	1053	1062	1071	1080
	S M M G V L Q L L L H I F W A Y L I L						
1089	CGC ATG GCC CAC AAG TTC ATA ACT GGA AAG CTG GTA GAA GAT GAA CGC AGT GAC	1098	1098	1107	1116	1125	1134
	R M A H K F I T G G K L V E D E R S D						

FIG. 2C

1143	1152	1161	1170	1179	1188
CGG GAA GAA ACA GAG AGC TCA GAG GGG GAG GCT GCA GCT GGG GGA GGA GCA					
R E E T E S E S E G E E E A A A G G A					
1197	1206	1215	1224	1233	1242
AAG AGC CGG CCC CTA GCC AAT GGC CAC CCC ATC CTC AAT AAC AAC CAT CGT AAG					
K S R P L A N G H P I L N N N H R K					
1251	1260	1269	1278	1287	1296
AAT GAC TGA ACC ATT ATT CCA GCT GCC TCC CAG ATT AAT GCA TAA AGC CAA GGA					
N D					
1305	1314	1323	1332	1341	1350
ACT ACC CCG CTC CCT CCT GCG CTA TAG GGT CAC TTT AAG CTC TGG GGA AAA AGG AGA					
1359	1368	1377	1386	1395	1404
AAG TGA GAG GAG AGT TCT CTG CAT CCT CCC TCC TTG CTT GTC ACC CAG TTG CCT					
1413	1422	1431	1440	1449	1458
TTA AAC CAA ATT CTA ACC AGC CTA TCC CCA GGT AGG GGG ACG TTG GTT ATA TTC					
1467	1476	1485	1494	1503	1512
TGT TAG AGG GGG ACG GTC GTA TTT TCC TCC CTA CCC GCC AAG TCA TCC TTT CTA					
1521	1530	1539	1548	1557	1566
CTG CTT TTG AGG CCC TCC CTC AGC TCT CTG TGG GTA GGG GTT ACA ATT CAC ATT					

FIG. 2D

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1575	1584	1593	1602	1611	1620
CCT TAT TCT GAG AAT TTG GCC CCA GCT GTT TGC CTT TGA CTC CCT GAC CTC CAG					
1629	1638	1647	1656	1665	1674
AGC CAG GGT TGT GCC TTA TTG TCC CAT CTG TGG GCC TCA TTC TGC CAA AGC TGG					
1683	1692	1701	1710	1719	1728
ACC AAG GCT AAC CTT TCT AAG CTC CCT AAC TTG GGC CAG AAA CCA AAG CTG AGC					
1737	1746	1755	1764	1773	1782
TTT TAA CTT TCT CCC TCT ATG ACA CAA ATG AAT TGA GGG TAG GAG GAG GGT GCA					
1791	1800	1809	1818	1827	1836
CAT AAC CCT TAC CCT ACC TCT GCC AAA AAG TGG GGG CTG TAC TGG GGA CTG CTC					
1845	1854	1863	1872	1881	1890
GGA TGA TCT TTC TTA GTG CTA CTT CTT TCA GCT GTC CCT GTA GCG ACA GGT CTA					
1899	1908	1917	1926	1935	1944
AGA TCT GAC TGC CTC CTC CTT TCT CTG GCC TCT TCC CCC TTC CCT CTT CTC TTC					
1953	1962	1971	1980	1989	1998
AGC TAG GCT AGC TGG TTT GGA GTA GAA TGG CAA CTA ATT CTA ATT TTT ATT TAT					
2007	2016	2025	2034	2043	2052
TAA ATA TTT GGG GTT TTG GTT TTA AAG CCA GAA TTA CGG CTA GCA CCT AGC ATT					

FIG. 2E

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2061 2070 2079 2088 2097 2106
TCA GCA GAG GGA CCA TTT TAG ACC AAA ATG TAC TGT TAA TGG GTT TTT TTT TAA

2115 2124 2133 2142 2151 2160
AAT TAA AAG ATT AAA TAA AAA ATA TTA AAA AAA AAA AAA AAA AAG GGG GGG

GG

FIG. 2F

1 ML - - - - - SSFNEWFWQD 2516821
1 MW - - - - - RM - - SYFW HE GI 1123105
1 MSNRKADEKHHMSSSSLTND GI 1675382
1 MTSATDKSIDRL - - - - VVNA GI 541568

13 RFWLP P - NVTWTELEDRDGR 2516821
11 PYWLP R - NVTWPE VPAK - - - GI 1123105
21 RSYIRNLSNRKT SISRKVP I GI 1675382
17 KTRRRNS S V GKI D L G D TVPG GI 541568

32 VYPHPQDLLAALPLALLVLLA 2516821
27 - FV - - - DLL VPIYLA IP L VI GI 1123105
41 TRTLEDPSNFVAKDGTK L V - GI 1675382
37 FAM P ESAASKNEAKKRMA GI 541568

52 MRLAFERFIGLPLSRWL GVR 2516821
43 IRILWE STIG VT - - - YL - - - GI 1123105
60 - - - - - QAP L F L V W - - QK GI 1675382
57 LTGDSKKDS D L LWK V W FSY R GI 541568

72 DQTRRQ - - - - - 2516821
57 - - - - - GI 1123105
71 EICLS - - - - - IIAIC - FA GI 1675382
77 EMNY R HSWLT PF FI LC VYS GI 541568

78 - - - - - VKPNATLEKHF LTE 2516821
57 - - - - - - - - - - - YFR T N GI 1123105
83 CLLSPSLR P YA EP - - - FI FL GI 1675382
97 AYFLSGNRTESNPLHM F V AI GI 541568

92 GHRPKEPQ - - - - - 2516821
62 AYASRK - N - - - - - GI 1123105
100 SYK - QPDG SYGKGP KDACFP GI 1675382
117 SYQVDGTD SYAKGI KDLS VF GI 541568

FIG. 3A

100 - - - L S L L A A Q C G L T L Q Q T Q R 2516821
 69 - - - I T L L G - - C - - - - - - - M GI 1123105
 119 I F W V I V F T A F R V I V M D Y V F R GI 1675382
 137 F F Y M I F F T F L R E F L M D V V I R GI 541568

117 W F R R R R N Q D R P Q L T K K F C E A 2516821
 76 W E H M T G G F A S V S R A K K I L E C GI 1123105
 139 P F V L N W G V R N R K V I I R F C E Q GI 1675382
 157 P F T V Y L N V T S E H R Q K R M L E Q GI 541568

137 S W R F L F Y L S S F V G G L S V L Y H 2516821
 96 F W R F S Y Y T F A F L Y G L Y V M K N GI 1123105
 159 G Y S F F Y Y L C F W F L G L Y I Y R S GI 1675382
 177 M Y A I F Y C G V S G P F G L Y I M Y H GI 541568

157 E S - W L W A P V M C W D R Y P N Q T L 2516821
 116 S S - W L Y D V K Q C W I G Y P F H P V GI 1123105
 179 S N Y W S N E E K - L F E D Y P Q Y Y M GI 1675382
 197 S D L W L F K T K P M Y R T Y P V I T N GI 541568

176 K P S L Y W W Y L L E L G F Y L S L - - 2516821
 135 P D T I W W Y Y M I E T G F Y Y S L - - GI 1123105
 198 S P L F K A Y Y L I Q L G F W L Q Q - - GI 1675382
 217 P F L F K I F Y L G Q A A F W A Q Q A C GI 541568

194 L I R L P F D V K R K D F K E Q V I H H 2516821
 153 L I G S T F D V R R S D F W Q L M V H H GI 1123105
 216 I L V L H L E Q R R A D H W Q M F A H H GI 1675382
 237 V L V L Q L E K P R K D Y K E L V F H H GI 541568

214 F V A V I L M T F S Y S A N L L R I G S 2516821
 173 V I T I F L L S S S W T I N F V R V G T GI 1123105
 236 I V T C A L I I L S Y G F N F L R V G N GI 1675382
 257 I V T L L L I W S S Y V F H F T K M G L GI 541568

FIG. 3B

234 L V L L L H D S S D Y L L E A C K M V N 2516821
 193 L I L L S H D V S D V F L E G G K L V R GI 1123105
 256 A I L Y I F D L S D Y I L S G G K M L K GI 1675382
 277 A I Y I T M D V S D F F L S L S K T L N GI 541568

254 Y - M O Y Q Q V C D A L F L I F S F V F 2516821
 213 Y D A H N K N M T N F M F V L F F S S W GI 1123105
 276 Y - L G F G K I C D Y L F G I F V A S W GI 1675382
 297 Y - L N - S V F T P F V F G L F V F F W GI 541568

273 F Y T R L V L F P T Q I L Y T T Y Y E S 2516821
 233 V A T R L I Y Y P F I V I R S A V T E A GI 1123105
 295 V Y S R H Y L F S - K I L R V V V T N A GI 1675382
 315 I Y L R H V V N I - R I L W S V L T E F GI 541568

293 I S N R G P - - - F - - - - F G Y - - - 2516821
 253 A A L I Q P D Y I L - - - - W D Y Q L S GI 1123105
 314 P E I I G - G F H L D V P - N G Y I F N GI 1675382
 334 R H E - G - N Y V L N F A T Q Q Y K C W GI 541568

303 - - Y - - - F F N G L L M L L Q L L H V 2516821
 269 P P Y A P R L I V F A L I L L F F L H I GI 1123105
 332 K P I Y I A F I I - L L F T L Q L L I Y GI 1675382
 352 I S L P I V F V - - L I A A L Q L V N L GI 541568

318 F W S C L I L R M L Y S F M K K G Q M E 2516821
 289 F W T F I I L R I A Y R - T S T G G Q A GI 1123105
 351 I W F G M I V K V A Y R V F S - G E E A GI 1675382
 370 Y W L F L I L R I L Y R L I W - Q G I Q GI 541568

338 K D I R S D V E E S D S S E E A A A A Q 2516821
 308 K D V R S D - S D S D Y D E E E M A R R GI 1123105
 370 T D S R S D - D E G G R R G G E F N - - GI 1675382
 389 K D E R S D - S D S D E S A E N E E S K GI 541568

FIG. 3C

358	<u>E P L Q L</u> - - <u>K N G T A G G P R P A P T</u>	2516821
327	<u>E</u> <u>R T R</u> <u>L</u> L K <u>K</u> <u>K K N K V</u> <u>G</u> S L N C R S	GI 1123105
387	- - - - -	GI 1675382
408	<u>E</u> K - - - - -	GI 541568
376	<u>D G P R S R V A G R L T N R H T T A T</u>	2516821
347	K I N F M K I T A F V S <u>N</u> - - - F C Q	GI 1123105
387	- - - - -	GI 1675382
410	- - - - -	GI 541568

FIG. 3D

1 M L - - - - - S S F N E W F W Q D 2516821
1 M W - - - - - R M - - S Y F W H E GI 1123105
1 M S N R K A D E K H H M S S S S L T N D GI 1675382
1 M T S A T D K S I D R L - - - - V V N A GI 541568

13 R F W L P P - N V T W T E L E D R D G R 2516821
11 P Y W L P R - N V T W P E V P A K - - - GI 1123105
21 R S Y I R N L S N R K T S I S R K V P I GI 1675382
17 K T R R R N S S V G K I D L G D T V P G GI 541568

32 V Y P H P Q D L L A A L P L A L V L L A 2516821
27 - F V - - - D L L V P I Y L A I P L V I GI 1123105
41 T R T L E D P S N F V A K D G T K L V - GI 1675382
37 F A A M P E S A A S K N E A K K R M K A GI 541568

52 M R L A F E R F I G L P L S R W L G V R 2516821
43 I R I L W E S T I G V T - - - Y L - - - GI 1123105
60 - - - - - Q A P L F L L V W - - Q K GI 1675382
57 L T G D S K K D S D L L W K V W F S Y R GI 541568

72 D Q T R R Q - - - - - 2516821
57 - - - - - GI 1123105
71 E I C L S - - - - - I I A I C - F A GI 1675382
77 E M N Y R H S W L T P F F I L V C V Y S GI 541568

78 - - - - - V K P N A T L E K H F L T E 2516821
57 - - - - - Y F R T N GI 1123105
83 C L L S P S L R P Y A E P - - - F I F L GI 1675382
97 A Y F L S G N R T E S N P L H M F V A I GI 541568

92 G H R P K E P Q - - - - - 2516821
62 A Y A S R K - N - - - - - GI 1123105
100 S Y K - Q P D G S Y G K G P K D A C F P GI 1675382
117 S Y Q V D G T D S Y A K G I K D L S F V GI 541568

FIG. 4A

100	- - -	L S L L A A Q C G L T L Q Q T Q R	2516821
69	- - -	I T L L G - - C - - - - - - - M	GI 1123105
119	I F W	V I V F T A F R V I V M D Y V F R	GI 1675382
137	F F Y M I F F T F L R E F L M D V V I R		GI 541568
117		W F R R R R N Q D R P Q L T K K F C E A	2516821
76	W E H	M T G G F A S V S R A K K I L E C	GI 1123105
139	P F V L N W G V R N R K V I I R F C E Q		GI 1675382
157	P F T V Y L N V T S E H R Q K R M L E Q		GI 541568
137		S W R F L F Y L S S F V G G L S V L Y H	2516821
96	F W R F S Y Y T F A F L Y G L Y V M K N		GI 1123105
159	G Y S E F Y Y L C F W F L G L Y I Y R S		GI 1675382
177	M Y A I F Y C G V S G P F G L Y I M Y H		GI 541568
157	E S -	W L W A P V M C W D R Y P N Q T L	2516821
116	S S -	W L Y D V K Q C W I G Y P F H P V	GI 1123105
179	S N Y W S N E E K - L F E D Y P Q Y Y M		GI 1675382
197	S D L W L F K T K P M Y R T Y P V I T N		GI 541568
176		K P S L Y W W Y L L E L G F Y L S L - -	2516821
135	P D T I W W Y Y M I E T G F Y Y S L - -		GI 1123105
198	S P L F K A Y Y L I Q L G F W L Q Q - -		GI 1675382
217	P F L F K I F Y L G Q A A F W A Q Q A C		GI 541568
194		L I R L P F D V K R K D F K E Q V I H H	2516821
153	L I G S T F D V R R S D F W Q L M V H H		GI 1123105
216	I L V L H L E Q R R A D H W Q M F A H H		GI 1675382
237	V L V L Q L E K P R K D Y K E L V F H H		GI 541568
214		F V A V I L M T F S Y S A N L L R I G S	2516821
173	V I T I F L L S S S W T I N F V R V G T		GI 1123105
236	I V T C A L I I L S Y G F N F L R V G N		GI 1675382
257	I V T L L L I W S S Y V F H F T K M G L		GI 541568

FIG. 4B

234	L V L L L H D S S D Y L L E A C K M V N	2516821
193	L I L L S H D V S D V F L E G G K L V R	GI 1123105
256	A I L Y I F D L S D Y I L S G G K M L K	GI 1675382
277	A I Y I T M D V S D F F L S L S K T L N	GI 541568
254	Y - M Q Y Q Q V C D A L F L I F S F V F	2516821
213	Y D A H N K N M T N F M F V L F F S S W	GI 1123105
276	Y - L G F G K I C D Y L F G I F V A S W	GI 1675382
297	Y - L N - S V F T P F V F G L F V F F W	GI 541568
273	F Y T R L V L F P T Q I L Y T T Y Y E S	2516821
233	V A T R L I Y Y P F I V I R S A V T E A	GI 1123105
295	V Y S R H Y L F S - K I L R V V V T N A	GI 1675382
315	I Y L R H V V N I - R I L W S V L T E F	GI 541568
293	I S N R G P - - - F - - - - F G Y - - -	2516821
253	A A L I Q P D Y I L - - - - W D Y Q L S	GI 1123105
314	P E I I G - G F H L D V P - N G Y I F N	GI 1675382
334	R H E - G - N Y V L N F A T Q Q Y K C W	GI 541568
303	- - Y - - - F F N G L L M L L Q L L H V	2516821
269	P P Y A P R L I V F A L I L L F F L H I	GI 1123105
332	K P I Y I A F I I - L L F T L Q L L I Y	GI 1675382
352	I S L P I V F V - - L I A A L Q L V N L	GI 541568
318	F W S C L I L R M L Y S F M K K G Q M E	2516821
289	F W T F I I L R I A Y R - T S T G G Q A	GI 1123105
351	I W F G M I V K V A Y R V F S - G E E A	GI 1675382
370	Y W L F L I L R I L Y R L I W - Q G I Q	GI 541568
338	K D I R S D V E E S D S S E E A A A A Q	2516821
308	K D V R S D - S D S D Y D E E E M A R R	GI 1123105
370	T D S R S D - D E G G R R G G E F N - -	GI 1675382
389	K D E R S D - S D S D E S A E N E E S K	GI 541568

FIG. 4C

1	M L	- - - - -	493014
1	M W	- - - - -	GI 1123105
1	M S N R K A D E K H H M S S S S L T N D		GI 1675382
1	M T S A T D K S I D R L - - - - V V N A		GI 541568
3	Q T L Y D Y F W W E R L W L P V N L T	-	493014
3	R M - - S Y F W H E P Y W L P R N V T	-	GI 1123105
21	R S Y I R N L S N R K T S I S R K V P I		GI 1675382
17	K T R R R N S S V G K I D L G D T V P G		GI 541568
22	W A D L E D R D G R V Y A	- - - - -	493014
20	W P E V P - - - - - A	- - - - -	GI 1123105
41	T R T L E D P S N F V A K D G T K L V -		GI 1675382
37	F A A M P E S A A S K N E A K K R M K A		GI 541568
35	- - - - - K A S D L Y I T L P L A L L		493014
26	- - - - - K F V D L L V P I Y L A I P		GI 1123105
60	- - - - - Q A P L F L L V W - - Q K		GI 1675382
57	L T G D S K K D S D L L W K V W F S Y R		GI 541568
49	F L I V R Y F F - - E L Y V A T P L A A		493014
40	L V I I R I L W - - E - - - - -		GI 1123105
71	E I C L S - - - - - I I A I C - F A		GI 1675382
77	E M N Y R H S W L T P F F I L V C V Y S		GI 541568
67	L L N I K E K T R L R A P P N A T L E H		493014
49	- - - - - - - - - - S T I G V T Y		GI 1123105
83	C L L S P S L R P Y A E P - - - F I F L		GI 1675382
97	A Y F L S G N R T E S N P L H M F V A I		GI 541568
87	F Y L - - T S G K Q P K Q V E - - - - -		493014
56	L Y F - - R T N A Y A S R K N - - - - -		GI 1123105
100	S Y K - Q P D G S Y G K G P K D A C F P		GI 1675382
117	S Y Q V D G T D S Y A K G I K D L S F V		GI 541568

FIG. 5A

100 - - - V E L L S R O S G L S G R Q V E R 493014
 69 - - - I T L L G C - - - - - - - - M GI 1123105
 119 I F W V I V F T A F R V I V M D Y V F R GI 1675382
 137 F F Y M I F F T F L R E F L M D V V I R GI 541568

117 W F R R R R N Q D R P S L L K K F R E A 493014
 76 W E H M T G G F A S V S R A K K I L E C GI 1123105
 139 P F V L N W G V R N R K V I I R F C E Q GI 1675382
 157 P F T V Y L N V T S E H R Q K R M L E Q GI 541568

137 S W R F T F Y L I A F I A G M A V I V D 493014
 96 F W R F S Y Y T F A F L Y G L Y V M K N GI 1123105
 159 G Y S F F Y Y L C F W F L G L Y I Y R S GI 1675382
 177 M Y A I F Y C G V S G P F G L Y I M Y H GI 541568

157 K P - W F Y D M K K V W E G Y P I Q S T 493014
 116 S S - W L Y D V K Q C W I G Y P F H P V GI 1123105
 179 S N Y W S N E E K - L F E D Y P Q Y Y M GI 1675382
 197 S D L W L F K T K P M Y R T Y P V I T N GI 541568

176 I P S Q Y W Y Y M I E L S F Y W S L - - 493014
 135 P D T I W W Y Y M I E T G F Y Y S L - - GI 1123105
 198 S P L F K A Y Y L I Q L G F W L Q Q - - GI 1675382
 217 P F L F K I F Y L G Q A A F W A Q Q A C GI 541568

194 L F S I A S D V K R K D F K E Q I I H H 493014
 153 L I G S T F D V R R S D F W Q L M V H H GI 1123105
 216 I L V L H L E Q R R A D H W Q M F A H H GI 1675382
 237 V L V L Q L E K P R K D Y K E L V F H H GI 541568

214 V A T I I L I S F S W F A N Y I R A G T 493014
 173 V I T I F L L S S S W T I N F V R V G T GI 1123105
 236 I V T C A L I I L S Y G F N F L R V G N GI 1675382
 257 I V T L L L I W S S Y V F H F T K M G L GI 541568

FIG. 5B

234 L I M A L H D S S D Y L L E S A K M F N 493014
 193 L I L L S H D V S D V F L E G G K L V R GI 1123105
 256 A I L Y I F D L S D Y I L S G G K M L K GI 1675382
 277 A I Y I T M D V S D F F L S L S K T L N GI 541568

254 Y A G W - K N T C N N I F I V F A I V F 493014
 213 Y D A H N K N M T N F M F V L F F S S W GI 1123105
 276 Y L G F G K - I C D Y L F G I F V A S W GI 1675382
 297 Y L N - S V - F T P F V F G L F V F F W GI 541568

273 I I T R L V I L P - - F W I L H C T L V 493014
 233 V A T R L I Y Y P - - F I V I R S A V T GI 1123105
 295 V Y S R H Y L F S K I L R V V V T N A P GI 1675382
 315 I Y L R H V V N I R I L W S V L T E F R GI 541568

291 Y P L E L Y - P A F F G Y Y F - - - - 493014
 251 E A A A L I Q P D Y I L W D Y Q L S P P GI 1123105
 315 E I I G G F H L D V P - N G Y I F N K P GI 1675382
 335 H E - G N Y V L N F A T Q Q Y K C W I S GI 541568

305 F N S M M G V L Q L - - - - - L H I F W 493014
 271 Y A P R L I V F A L I L L F F L H I F W GI 1123105
 334 I Y I A F I I L - L F T L Q L L I Y I W GI 1675382
 354 L P I V F V - L - I A A L Q L V N L Y W GI 541568

320 A Y L I L R M A H K F I T G K L V E D E 493014
 291 T F I I L R I A Y R T S T G G Q A K D V GI 1123105
 353 F G M I V K V A Y R V F S G E E A T D S GI 1675382
 372 L F L I L R I L Y R L I W Q G I Q K D E GI 541568

340 R S D R E E T E S S E G E E A A A G G G 493014
 311 R S D S D - - - - S D Y D E E E M A R R GI 1123105
 373 R S D D E G G R R G G E F N - - - - - GI 1675382
 392 R S D S D S D E S A E N E E S - - - - - GI 541568

FIG. 5C

360	A K S R P L A N G H P I L N N N H R K N	493014
327	E R T R L L K K K K N K V G S L N C R S	GI 1123105
387	- -	GI 1675382
407	- [K] E	GI 541568
380	- [D]	493014
347	K I N F M K I T A F V S N F C Q	GI 1123105
387	- E	GI 1675382
409	K - C E	GI 541568

FIG. 5D

1	M L	- - - - -	493014
1	M W	- - - - -	GI 1123105
1	M S N R K A D E K H H M S S S S L T N D		GI 1675382
1	M T S A T D K S I D R L - - - - V V N A		GI 541568
3	Q T L Y D Y F W W E R L W L P V N L T	-	493014
3	R M - - S Y F W H E P Y W L P R N V T	-	GI 1123105
21	R S Y I R N L S N R K T S I S R K V P I		GI 1675382
17	K T R R R N S S V G K I D L G D T V P G		GI 541568
22	W A D L E D R D G R V Y A	- - - - -	493014
20	W P E V P - - - - - A	- - - - -	GI 1123105
41	T R T L E D P S N F V A K D G T K L V	-	GI 1675382
37	F A A M P E S A A S K N E A K K R M K A		GI 541568
35	- - - - - K A S D L Y I T L P L A L L		493014
26	- - - - - K F V D L L V P I Y L A I P		GI 1123105
60	- - - - - Q A P L F L L V W - - Q K		GI 1675382
57	L T G D S K K D S D L L W K V W F S Y R		GI 541568
49	F L I V R Y F F - - E L Y V A T P L A A		493014
40	L V I I R I L W - - E - - - - -		GI 1123105
71	E I C L S - - - - - I I A I C - F A		GI 1675382
77	E M N Y R H S W L T P F F I L V C V Y S		GI 541568
67	L L N I K E K T R L R A P P N A T L E H		493014
49	- - - - - S T I G V T Y		GI 1123105
83	C L L S P S L R P Y A E P - - - F I F L		GI 1675382
97	A Y F L S G N R T E S N P L H M F V A I		GI 541568
87	F Y L - - T S G K Q P K Q V E - - - - -		493014
56	L Y F - - R T N A Y A S R K N - - - - -		GI 1123105
100	S Y K - Q P D G S Y G K G P K D A C F P		GI 1675382
117	S Y Q V D G T D S Y A K G I K D L S F V		GI 541568

FIG. 6A

100	- - -	V E L L S R Q S G L S G R Q V E R	493014
69	- - -	I T L L G C - - - - - - - - - M	GI 1123105
119	I F W	V I V F T A F R V I V M D Y V F R	GI 1675382
137	F F Y M I F F	T F L R E F L M D V V I R	GI 541568
117		W F R R R R N Q D R P S L L K K F R E A	493014
76	W E H	M T G G F A S V S R A K K I L E C	GI 1123105
139	P F V L N W G V R N R K	V I I R F C E Q	GI 1675382
157	P F T V Y L N	V T S E H R Q K R M L E Q	GI 541568
137		S W R F T F Y L I A F I A G M A V I V D	493014
96	F W R F S Y Y	T F A F L Y G L Y V M K N	GI 1123105
159	G Y S F F Y Y L C F W F L G L Y I Y R S		GI 1675382
177	M Y A I F Y C G V S G P F G L Y I M Y H		GI 541568
157	K P -	W F Y D M K K V W E G Y P I Q S T	493014
116	S S -	W L Y D V K Q C W I G Y P F H P V	GI 1123105
179	S N Y W S N E E K -	L F E D Y P Q Y Y M	GI 1675382
197	S D L W L F K T K P M Y R T Y P V I T N		GI 541568
176		I P S Q Y W Y Y M I E L S F Y W S L - -	493014
135	P D T I W W Y Y M I E T G F Y Y S L - -		GI 1123105
198	S P L F K A Y Y L I Q L G F W L Q Q - -		GI 1675382
217	P F L F K I F Y L G Q A A F W A Q Q A C		GI 541568
194		L F S I A S D V K R K D F K E Q I I H H	493014
153	L I G S T F D V R R S D F W Q L M V H H		GI 1123105
216	I L V L H L E Q R R A D H W Q M F A H H		GI 1675382
237	V L V L Q L E K P R K D Y K E L V F H H		GI 541568
214		V A T I I L I S F S W F A N Y I R A G T	493014
173	V I T I F L L S S S W T I N F V R V G T		GI 1123105
236	I V T C A L I I L S Y G F N F L R V G N		GI 1675382
257	I V T L L L I W S S Y V F H F T K M G L		GI 541568

FIG. 6B

234	L I M A L H D S S D Y L L E S A K M F N	493014
193	L I L L S H D V S D V F L E G G K L V R	GI 1123105
256	A I L Y I F D L S D Y I L S G G K M L K	GI 1675382
277	A I Y I T M D V S D F F L S L S K T L N	GI 541568
254	Y A G W - K N T C N N I F I V F A I V F	493014
213	Y D A H N K N M T N F M F V L F F S S W	GI 1123105
276	Y L G F G K - I C D Y L F G I F V A S W	GI 1675382
297	Y L N - S V - F T P F V F G L F V F F W	GI 541568
273	I I T R L V I L P - - F W I L H C T L V	493014
233	V A T R L I Y Y P - - F I V I R S A V T	GI 1123105
295	V Y S R H Y L F S K I L R V V V T N A P	GI 1675382
315	I Y L R H V V N I R I L W S V L T E F R	GI 541568
291	Y P L E L Y - P A F F G Y Y F - - - - -	493014
251	E A A A L I Q P D Y I L W D Y Q L S P P	GI 1123105
315	E I I G G F H L D V P - N G Y I F N K P	GI 1675382
335	H E - G N Y V L N F A T Q Q Y K C W I S	GI 541568
305	F N S M M G V L Q L - - - - - L H I F W	493014
271	Y A P R L I V F A L I L L F F L H I F W	GI 1123105
334	I Y I A F I I L - L F T L Q L L I Y I W	GI 1675382
354	L P I V F V - L - I A A L Q L V N L Y W	GI 541568
320	A Y L I L R M A H K F I T G K L V E D E	493014
291	T F I I L R I A Y R T S T G G Q A K D V	GI 1123105
353	F G M I V K V A Y R V F S G E E A T D S	GI 1675382
372	L F L I L R I L Y R L I W Q G I Q K D E	GI 541568
340	R S D R E E T E S S E G E E A A A G G G	493014
311	R S D S D - - - - S D Y D E E E M A R R	GI 1123105
373	R S D D E G G R R G G E F N - - - - -	GI 1675382
392	R S D S D S D E S A E N E E S - - - - -	GI 541568

FIG. 6C

360	A	K	S	R	P	L	A	N	G	H	P	I	L	N	N	N	H	R	K	N		493014	
327	E	R	T	R	L	L	K	K	K	K	N	K	V	G	S	L	N	C	R	S		GI 1123105	
387	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	GI 1675382	
407	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	K	E	GI 541568	
380	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D	493014	
347	K	I	N	F	M	K	I	T	A	F	V	S	N	F	C	Q						GI 1123105	
387	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	E	GI 1675382	
409	K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	E	GI 541568

FIG. 6D

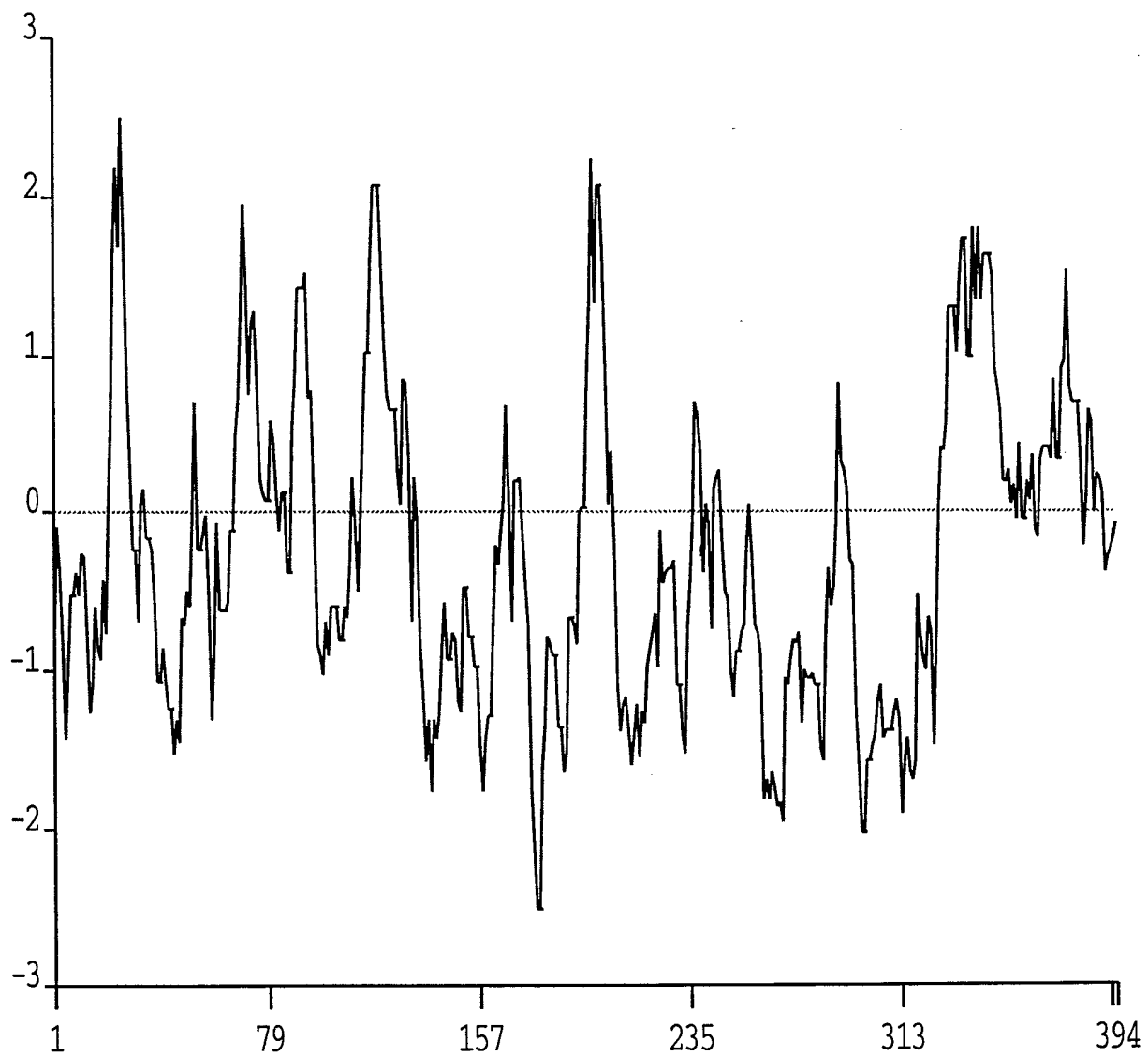


FIG. 7A

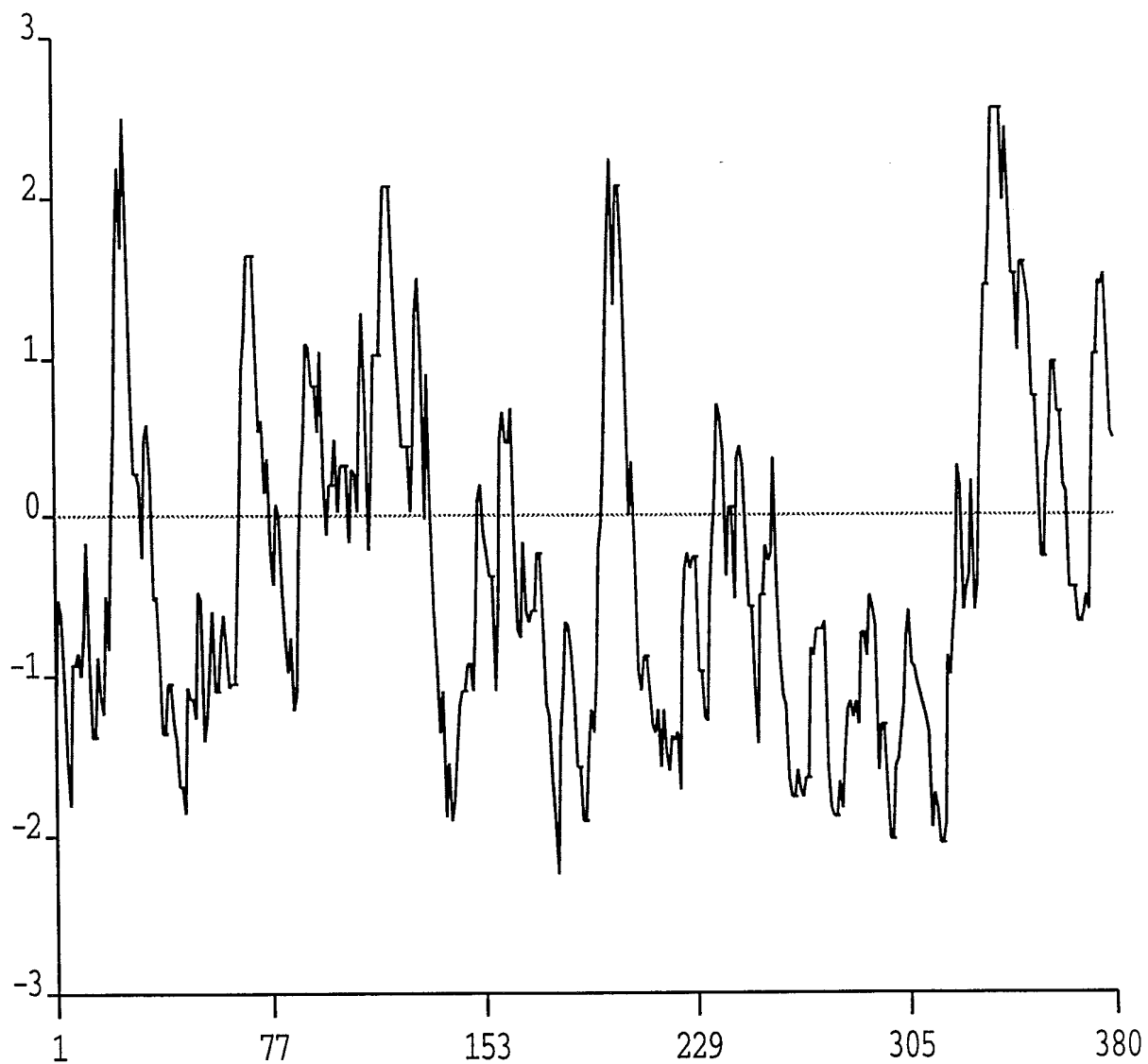


FIG. 7B

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 98/15591

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 C12N15/12 C12N5/10 C12N1/21 C07K14/47 C07K16/18
 C12Q1/68 G01N33/50 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 6 C12N C07K C12Q G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBEST 12 E.M.B.L. Databases Accession Number: AA469020, 18 June 1997 NATIONAL CANCER INSTITUTE: "Homo sapiens cDNA clone IMAGE:783607 similar to WP:C09G4.1" XP002084174	3-11
Y	see abstract <div style="text-align: center;">--- -/--</div>	1, 2, 12, 13, 18, 19

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

12 November 1998

24/11/1998

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Authorized officer

Lonnoy, 0

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/15591

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE EMBEST 12 E.M.B.L. Databases Accession Number: AA181797, 8 January 1997 HILLIER L ET AL: "Homo sapiens cDNA clone 613400 5' similar to WP:C09G4.1" XP002084175</p>	22-30
Y	<p>see abstract</p>	20,21, 31,32, 37,38
Y	<p>--- WO 95 33834 A (RES CORP TECHNOLOGIES INC) 14 December 1995</p> <p>see the whole document in general, and example 10 in particular</p>	1,2,12, 13, 18-21, 31,32, 37,38
A	<p>--- DATABASE EMINV E.M.B.L. Databases Accession Number: U42438, 17 December 1995 MILLER N ET AL: "The sequence of C. elegans cosmid C09G4" XP002084176 see abstract</p>	
A	<p>--- HODES R ET AL: "Longevity assurance genes: how do they influence aging and life span?" J. AM. GERIATR. SOC., vol. 44, no. 8, August 1996, pages 988-991, XP002084173</p> <p>-----</p>	

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/15591

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 16, 17, 35 and 36
are directed to methods of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. Claims Nos.: 14-17 33-36
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Claims Nos.: 14-17 and 33-36

These claims, respectively relating to agonists/antagonists of the polypeptides of claim 1 or 20, pharmaceutical compositions comprising said antagonists, and uses of the latter, could not be searched completely as their subject-matters were insufficiently disclosed.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/15591

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9533834 A	14-12-1995	US 5817782 A	06-10-1998
		CA 2191104 A	14-12-1995
		EP 0760854 A	12-03-1997
