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C07K 16/40

(30) 1998/06/01 (09/088,550) US

(54) **HOMOLOGUE DE CARBAMOYL PHOSPHATE SYNTHASE**

(54) **CARBAMOYL PHOSPHATE SYNTHASE HOMOLOG**

(57) La présente invention concerne un homologue de carbamoyl phosphate synthase humain (CPSH) et des polynucléotides identifiant et codant CPSH. L'invention concerne également des vecteurs d'expression, des cellules hôtes, des anticorps, des agonistes et des antagonistes. L'invention concerne enfin des méthodes de diagnostic, de traitement ou de prévention de troubles associés à l'expression de CPSH.

(57) The invention provides a human carbamoyl phosphate synthase homolog (CPSH) and polynucleotides which identify and encode CPSH. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating or preventing disorders associated with expression of CPSH.

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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| <b>(51) International Patent Classification 6 :</b><br><b>C12N 9/00, 15/52, 5/10, A61K 38/43, C07K 16/40, C12Q 1/68</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | <b>A1</b> | <b>(11) International Publication Number:</b> <b>WO 99/63067</b><br><b>(43) International Publication Date:</b> 9 December 1999 (09.12.99) |     |     |     |    |    |    |   |     |     |     |     |     |   |     |     |     |     |     |   |     |     |     |     |     |   |     |     |     |     |     |   |     |     |     |     |     |   |     |     |     |     |     |   |     |     |     |     |     |   |     |     |     |     |     |   |     |     |     |     |     |   |     |     |     |     |     |   |     |     |     |     |     |   |     |     |     |     |     |   |     |     |     |     |     |   |     |     |     |     |     |   |     |     |     |     |     |   |     |     |     |     |     |   |     |     |     |     |     |   |     |     |     |     |     |   |     |     |     |     |     |   |     |     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| <div style="display: flex; justify-content: space-between;"> <div style="width: 48%;"> <b>(21) International Application Number:</b> PCT/US99/11161<br/> <b>(22) International Filing Date:</b> 20 May 1999 (20.05.99)<br/> <b>(30) Priority Data:</b><br/>           09/088,550      1 June 1998 (01.06.98)      US<br/> <b>(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application</b><br/>           US      09/088,550 (CIP)<br/>           Filed on      1 June 1998 (01.06.98)<br/> <b>(71) Applicant (for all designated States except US):</b> INCYTE PHARMACEUTICALS, INC. [US/US]; 3174 Porter Drive, Palo Alto, CA 94304 (US).<br/> <b>(72) Inventors; and</b><br/> <b>(75) Inventors/Applicants (for US only):</b> 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). LAL, Preeti [IN/US]; 2382 Lass Drive, Santa Clara, CA 95054 (US).<br/> <b>(74) Agents:</b> BILLINGS, Lucy, J. et al.; Incyte Pharmaceuticals, Inc., 3174 Porter Drive, Palo Alto, CA 94304 (US).         </div> <div style="width: 48%;"> <b>(81) Designated States:</b> 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, SL, 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).<br/><br/> <b>Published</b><br/> <i>With international search report.</i><br/> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> </div> </div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    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| <b>(54) Title:</b> CARBAMOYL PHOSPHATE SYNTHASE HOMOLOG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                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| <table style="margin: auto; border-collapse: collapse;"> <tr> <td style="text-align: center;">10</td> <td style="text-align: center;">19</td> <td style="text-align: center;">28</td> <td style="text-align: center;">37</td> <td style="text-align: center;">46</td> <td style="text-align: center;">55</td> </tr> <tr> <td style="text-align: center;">G</td> <td style="text-align: center;">TCC</td> <td style="text-align: center;">AGG</td> <td style="text-align: center;">GGG</td> <td style="text-align: center;">TTT</td> <td style="text-align: center;">GTG</td> </tr> <tr> <td style="text-align: center;">G</td> <td style="text-align: center;">TCC</td> <td style="text-align: center;">AGG</td> <td style="text-align: center;">GGG</td> <td style="text-align: center;">TTT</td> <td style="text-align: center;">GTG</td> </tr> <tr> <td style="text-align: center;">G</td> <td style="text-align: center;">TCC</td> <td style="text-align: center;">AGG</td> <td style="text-align: center;">GGG</td> <td 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## CARBAMOYL PHOSPHATE SYNTHASE HOMOLOG

### TECHNICAL FIELD

5 This invention relates to nucleic acid and amino acid sequences of a carbamoyl phosphate synthase homolog and to the use of these sequences in the diagnosis, treatment, and prevention of cancer and metabolic disorders.

### BACKGROUND OF THE INVENTION

10 The urea cycle is the process whereby nitrogenous waste, in the form of ammonia produced mainly in the breakdown of proteins in the liver, is converted to urea for excretion. The first step in this process, catalyzed by carbamoyl phosphate synthase (CPS), is the synthesis of carbamoyl phosphate from carbon dioxide and ammonia. This is a complex reaction, requiring the hydrolysis of two ATP molecules as well as the  
15 allosteric participation of *N*-acetylglutamate. Next, the enzyme ornithine transcarbamoylase attaches the carbamoyl group to ornithine, producing citrulline. These first two steps of the urea cycle occur in the mitochondrial matrix. Subsequent steps are carried out in the cytosol, where citrulline and aspartate are condensed by the enzyme argininosuccinate synthase, producing argininosuccinate. This compound is cleaved by  
20 argininosuccinase to arginine and fumarate, a molecule which participates in the citric acid cycle. Arginine, in turn, is hydrolyzed by arginase to produce urea, which can then be excreted by the kidney. (Lim, A. and Powers-Lee, S. (1996) *J. Biol. Chem.* 271:11400-11409; Stryer, L. (1995) Biochemistry, 4<sup>th</sup> edition, W.H. Freeman and Company, New York, NY pp. 634-636.)

25 CPS has been implicated in hyperammonemia, a condition of elevated blood ammonia levels that can lead to brain damage, coma, and death. Two forms of hyperammonemia related to CPS deficiency are recognized: a lethal neonatal type associated with an apparent lack of CPS, and a less severe, delayed onset type, associated with a marked reduction in the enzyme. (Wong, L. J. et al. (1994) *Ann Int. Med.* 120:216-217; Suzuki, Y. et al. (1986) *Eur. J. Pediat.* 145:406-408.)  
30

CPS participates in the first committed step of *de novo* pyrimidine biosynthesis by

regulating the activity of aspartate transcarbamoylase and the availability of its substrates. The first step in the biosynthesis of pyrimidines such as thymidine, uridine, and cytosine is the formation of carbamoyl phosphate by the action of CPS on bicarbonate and glutamate. In contrast to the CPS that participates in the urea cycle, this CPS is located in the cytosol.

5 Carbamoyl phosphate and the amino acid aspartate are combined by the enzyme aspartate transcarbamoylase to form the intermediate *N*-carbamoylaspartate. Subsequent modifications of *N*-carbamoylaspartate by the enzymes dihydroorotase, dihydroorotase dehydrogenase, orotate phosphoribosyl transferase and finally orotidylate decarboxylase eventually produce the pyrimidine nucleotide uridylate, precursor to uridine. Synthesis of

10 pyrimidines is essential, because replication of DNA and transcription of RNA in proliferating cells requires significant amounts of nucleic acid precursors, including the pyrimidines. (Stryer, supra.)

The discovery of a new carbamoyl phosphate synthase and the polynucleotides encoding it satisfies a need in the art by providing new compositions which are useful in

15 the diagnosis, treatment, and prevention of cancer and metabolic disorders.

### SUMMARY OF THE INVENTION

The invention is based on the discovery of a new carbamoyl phosphate synthase homolog (CPSH), the polynucleotides encoding CPSH, and the use of these compositions

20 for the diagnosis, treatment, or prevention of cancer and metabolic disorders.

The invention features a substantially purified polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1.

The invention further provides a substantially purified variant having at least 90% amino acid sequence identity to the amino acid sequence of SEQ ID NO:1 or a fragment of

25 SEQ ID NO:1. The invention also provides an isolated and purified polynucleotide encoding the polypeptide comprising the sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1. The invention also includes an isolated and purified polynucleotide variant having at least 90% polynucleotide sequence identity to the polynucleotide encoding the polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ

30 ID NO:1.

The invention further provides an isolated and purified polynucleotide which hybridizes under stringent conditions to the polynucleotide encoding the polypeptide

comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1, as well as an isolated and purified polynucleotide which is complementary to the polynucleotide encoding the polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1.

5       The invention also provides an isolated and purified polynucleotide comprising the polynucleotide sequence of SEQ ID NO:2 or a fragment of SEQ ID NO:2, and an isolated and purified polynucleotide variant having at least 90% polynucleotide sequence identity to the polynucleotide comprising the polynucleotide sequence of SEQ ID NO:2 or a fragment of SEQ ID NO:2. The invention also provides an isolated and purified  
10   polynucleotide having a sequence complementary to the polynucleotide comprising the polynucleotide sequence of SEQ ID NO:2 or a fragment of SEQ ID NO:2.

      The invention further provides an expression vector comprising at least a fragment of the polynucleotide encoding the polypeptide comprising the sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1. In another aspect, the expression vector is contained  
15   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 of SEQ ID NO:1, the method comprising the steps of: (a) culturing the host cell comprising an expression vector containing at least a fragment of a polynucleotide encoding the polypeptide comprising the  
20   amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO: 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 polypeptide having the sequence of SEQ ID NO:1 or a fragment of  
25   SEQ ID NO:1 in conjunction with a suitable pharmaceutical carrier.

      The invention further includes a purified antibody which binds to a polypeptide comprising the sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1, as well as a purified agonist and a purified antagonist of the polypeptide.

      The invention also provides a method for treating or preventing cancer, the method  
30   comprising administering to a subject in need of such treatment an effective amount of an antagonist of the polypeptide having the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1.

The invention also provides a method for treating or preventing a metabolic disorder, the method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising substantially purified polypeptide having the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID  
5 NO:1.

The invention also provides a method for detecting a polynucleotide encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 1 or a fragment of SEQ ID NO: 1 in a biological sample containing nucleic acids, the method comprising the steps of: (a) hybridizing the complement of the polynucleotide encoding the polypeptide  
10 comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1 to at least one of the nucleic acids of the biological sample, thereby forming a hybridization complex; and (b) detecting the hybridization complex, wherein the presence of the hybridization complex correlates with the presence of a polynucleotide encoding the polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ  
15 ID NO:1 in the biological sample. In one aspect, the nucleic acids of the biological sample are amplified by the polymerase chain reaction prior to the hybridizing step.

### BRIEF DESCRIPTION OF THE FIGURES

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

### DESCRIPTION OF THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is  
25 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.

30 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, a reference to "a host cell" includes a plurality of such host cells, and a

reference to "an 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 materials are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, vectors, and methodologies which are reported in the publications and 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 such disclosure by virtue of prior invention.

#### DEFINITIONS

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

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

An "allelic variant," as this term is used herein, is an alternative form of the gene encoding CPSH. Allelic variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in 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 allelic variants are generally ascribed to natural deletions, 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 CPSH, as described herein, include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polynucleotide the same as CPSH or a polypeptide with at least one

functional characteristic of CPSH. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding CPSH, and improper or unexpected hybridization to allelic variants, with a locus other than the normal chromosomal locus for the polynucleotide  
5 sequence encoding CPSH. The encoded protein may also be "altered," and may contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent CPSH. 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  
10 immunological activity of CPSH 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.

15 The terms "amino acid" or "amino acid sequence," as used herein, refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. In this context, "fragments," "immunogenic fragments," or "antigenic fragments" refer to fragments of CPSH which are preferably about 5 to about 15 amino acids in length, most preferably 14 amino acids, and which  
20 retain some biological activity or immunological activity of CPSH. 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.

25 "Amplification," as used herein, relates to the production of additional copies of a nucleic acid sequence. Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art. (See, e.g., Dieffenbach, C.W. and G.S. Dveksler (1995) PCR Primer, a Laboratory Manual, Cold Spring Harbor Press, Plainview, NY, pp.1-5.)

30 The term "antagonist," as it is used herein, refers to a molecule which, when bound to CPSH, decreases the amount or the duration of the effect of the biological or immunological activity of CPSH. Antagonists may include proteins, nucleic acids,

carbohydrates, antibodies, or any other molecules which decrease the effect of CPSH.

As used herein, the term "antibody" refers to intact molecules as well as to fragments thereof, such as Fab, F(ab')<sub>2</sub>, and Fv fragments, which are capable of binding the epitopic determinant. Antibodies that bind CPSH polypeptides can be prepared using  
5 intact polypeptides or using fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) 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, thyroglobulin, and  
10 keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

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 a fragment of a protein is used to immunize a host animal, numerous regions of the  
15 protein may induce the production of antibodies which bind specifically to antigenic determinants (given regions or three-dimensional structures on the protein). 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 a  
20 nucleic acid sequence which is complementary to the "sense" strand of a specific nucleic acid sequence. Antisense molecules 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 to block either transcription or translation. The designation "negative" can refer to the antisense strand, and the  
25 designation "positive" can refer to the sense strand.

As used herein, the term "biologically active," 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 CPSH, or of any oligopeptide thereof, to induce a specific immune response in appropriate  
30 animals or cells and to bind with specific antibodies.

The terms "complementary" or "complementarity," as used herein, refer to the natural binding of polynucleotides 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," such that only some of the nucleic acids bind, or it may be "complete," such that total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant  
5 effects on the efficiency and strength of the hybridization between the 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 peptide nucleic acid (PNA) molecules.

A "composition comprising a given polynucleotide sequence" or a "composition  
10 comprising a given amino acid sequence," as these terms are used herein, refer broadly to any composition containing the given polynucleotide or amino acid sequence. The composition may comprise a dry formulation, an aqueous solution, or a sterile composition. Compositions comprising polynucleotide sequences encoding CPSH or fragments of CPSH may be employed as hybridization probes. The probes may be stored  
15 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., sodium dodecyl sulfate (SDS), and other components, e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.

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

As used herein, the term "correlates with expression of a polynucleotide" indicates that the detection of the presence of nucleic acids, the same or related to a nucleic acid sequence encoding CPSH, by Northern analysis is indicative of the presence of nucleic acids encoding CPSH in a sample, and thereby correlates with expression of the transcript  
30 from the polynucleotide encoding CPSH.

A "deletion," as the term is used herein, refers to a change in the amino acid or nucleotide sequence that 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 polypeptide sequence, or a polynucleotide sequence. Chemical modifications of a polynucleotide sequence can include, for example, replacement of hydrogen by an alkyl, acyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains at least one biological or immunological function of the natural molecule. A derivative polypeptide is one modified by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

10 The term "similarity," as used herein, refers to a degree of complementarity. There may be partial similarity or complete similarity. The word "identity" may substitute for the word "similarity." A partially complementary sequence that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid is referred to as "substantially similar." The inhibition of hybridization of the completely complementary  
15 sequence to the target sequence may be examined using a hybridization assay (Southern or Northern blot, solution hybridization, and the like) under conditions of reduced stringency. A substantially similar sequence or hybridization probe will compete for and inhibit the binding of a completely similar (identical) sequence to the target sequence under conditions of reduced stringency. This is not to say that conditions of reduced stringency  
20 are such that non-specific binding is permitted, as reduced stringency conditions require that the binding of two sequences to one another be a specific (i.e., a selective) interaction. The absence of non-specific 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% similarity or identity). In the absence of non-specific binding, the substantially similar sequence or  
25 probe will not hybridize to the second non-complementary target sequence.

The phrases "percent identity" or "% identity" refer to the percentage of sequence similarity found in a comparison of two or more amino acid or nucleic acid sequences. Percent identity can be determined electronically, e.g., by using the MegAlign™ program (DNASTAR, Inc., Madison WI). The MegAlign™ program can create alignments  
30 between two or more sequences according to different methods, e.g., the clustal method. (See, e.g., Higgins, D.G. and P.M. Sharp (1988) Gene 73:237-244.) The clustal algorithm groups sequences into clusters by examining the distances between all pairs. The clusters

are aligned pairwise and then in groups. The percentage similarity between two amino acid sequences, e.g., sequence A and sequence B, is calculated by dividing the length of sequence A, minus the number of gap residues in sequence A, minus the number of gap residues in sequence B, into the sum of the residue matches between sequence A and  
5 sequence B, times one hundred. Gaps of low or of no similarity between the two amino acid sequences are not included in determining percentage similarity. Percent identity between nucleic acid sequences can also be counted or calculated by other methods known in the art, e.g., the Jotun Hein method. (See, e.g., Hein, J. (1990) *Methods Enzymol.* 183:626-645.) Identity between sequences can also be determined by other methods  
10 known in the art, e.g., by varying hybridization conditions.

“Human artificial chromosomes” (HACs), as described herein, are linear microchromosomes which may contain DNA sequences of about 6 kb to 10 Mb in size, and which contain all of the elements required for stable mitotic chromosome segregation and maintenance. (See, e.g., Harrington, J.J. et al. (1997) *Nat Genet.* 15:345-355.)

15 The term “humanized antibody,” as used herein, refers to antibody molecules in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

“Hybridization,” as the term is used herein, refers to any process by which a strand  
20 of nucleic acid binds with a complementary strand through base pairing.

As used herein, the term “hybridization complex” refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed in solution (e.g.,  $C_0t$  or  $R_0t$  analysis) or formed between one nucleic acid sequence present in solution and another  
25 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).

The words “insertion” or “addition,” as used herein, refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or  
30 nucleotides, respectively, to the sequence found in the naturally occurring molecule.

“Immune response” can refer to conditions associated with inflammation, trauma, immune disorders, or infectious or genetic disease, etc. These conditions can be

characterized by expression of various factors, e.g., cytokines, chemokines, and other signaling molecules, which may affect cellular and systemic defense systems.

The term "microarray," as used herein, refers to an arrangement of distinct polynucleotides arrayed on a substrate, e.g., paper, nylon or any other type of membrane, 5 filter, chip, glass slide, or any other suitable solid support.

The terms "element" or "array element" as used herein in a microarray context, refer to hybridizable polynucleotides arranged on the surface of a substrate.

The term "modulate," as it appears herein, refers to a change in the activity of CPSH. For example, modulation may cause an increase or a decrease in protein activity, 10 binding characteristics, or any other biological, functional, or immunological properties of CPSH.

The phrases "nucleic acid" or "nucleic acid sequence," as used herein, refer to a nucleotide, oligonucleotide, polynucleotide, or any fragment thereof. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or 15 double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material. In this context, "fragments" refers to those nucleic acid sequences which, when translated, would produce polypeptides retaining some functional characteristic, e.g., antigenicity, or structural domain characteristic, e.g., ATP-binding site, of the full-length polypeptide.

20 The terms "operably associated" or "operably linked," as used herein, refer to functionally related nucleic acid sequences. A promoter is operably associated or operably linked with a coding sequence if the promoter controls the translation of the encoded polypeptide. While operably associated or operably linked nucleic acid sequences can be contiguous and in the same reading frame, certain genetic elements, e.g., repressor genes, 25 are not contiguously linked to the sequence encoding the polypeptide but still bind to operator sequences that control expression of the polypeptide.

The term "oligonucleotide," as used herein, refers to a nucleic acid sequence of at least about 6 nucleotides to 60 nucleotides, preferably about 15 to 30 nucleotides, and most preferably about 20 to 25 nucleotides, which can be used in PCR amplification or in 30 a hybridization assay or microarray. As used herein, the term "oligonucleotide" is substantially equivalent to the terms "amplimer," "primer," "oligomer," and "probe," as these terms are commonly defined in the art.

“Peptide nucleic acid” (PNA), as used herein, refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers solubility to the composition. PNAs preferentially bind complementary  
5 single stranded DNA or RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell. (See, e.g., Nielsen, P.E. et al. (1993) Anticancer Drug Des. 8:53-63.)

The term “sample,” as used herein, is used in its broadest sense. A biological sample suspected of containing nucleic acids encoding CPSH, or fragments thereof, or  
10 CPSH itself, may comprise a bodily fluid; an 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; etc.

As used herein, the terms “specific binding” or “specifically binding” refer to that interaction between a protein or peptide and an agonist, an antibody, or an antagonist. The  
15 interaction is dependent upon the presence of a particular structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding molecule. For example, if an antibody is specific for epitope “A,” the presence of a polypeptide containing the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

20 As used herein, the term “stringent conditions” refers to conditions which permit hybridization between polynucleotides and the claimed polynucleotides. Stringent conditions can be defined by salt concentration, the concentration of organic solvent (e.g., formamide), temperature, and other conditions well known in the art. In particular, stringency can be increased by reducing the concentration of salt, increasing the  
25 concentration of formamide, or raising the hybridization temperature.

For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and most preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent,  
30 e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and most preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30°C, more

preferably of at least about 37°C, and most preferably of at least about 42°C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by  
5 combining these various conditions as needed. In a preferred embodiment, hybridization will occur at 30°C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37°C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 µg/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42°C in 250 mM  
10 NaCl, 25 mM trisodium citrate, 1% SDS, 50 % formamide, and 200 µg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

The washing steps which follow hybridization can also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing  
15 temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include temperature of at least about 25°C, more preferably of at least about 42°C, and most preferably of at least about 68°C. In a preferred  
20 embodiment, wash steps will occur at 25°C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a most preferred embodiment, wash steps will occur at 68°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the  
25 art.

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

30 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. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation 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. The term "transformed" cells includes 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, as well as transiently transformed cells which express the inserted DNA or RNA for limited periods of time.

A "variant" of CPSH, 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 isoleucine). More rarely, a variant may have "nonconservative" changes (e.g., replacement of glycine with 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, LASERGENE™ software.

## THE INVENTION

The invention is based on the discovery of a new carbamoyl phosphate synthase homolog (CPSH), the polynucleotides encoding CPSH, and the use of these compositions for the diagnosis, treatment, or prevention of cancer and metabolic disorders.

Nucleic acids encoding the CPSH of the present invention were first identified in Incyte Clone 2045605 from the promonocyte cell line cDNA library (THP1T7T01) using a computer search, e.g., BLAST, for amino acid sequence alignments. A consensus sequence, SEQ ID NO:2, was derived from the following overlapping and/or extended nucleic acid sequences: Incyte Clones 2045605H1 (THP1T7T01), 1419560F1 (KIDNNOT09), 1450306F1 (PENITUT01), 1447977F1 (PLACNOT02), 1298934F1 (BRSTNOT07), and 2819342H1 (BRSTNOT14).

In one embodiment, the invention encompasses a polypeptide comprising the

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amino acid sequence of SEQ ID NO:1, as shown in Figures 1A, 1B, 1C, 1D, and 1E. CPSH is 469 amino acids in length and has two potential glycosylation sites at residues N<sub>187</sub> and N<sub>268</sub>. In addition, CPSH has two potential cAMP- and cGMP-dependent protein kinase phosphorylation sites at residues T<sub>129</sub> and S<sub>250</sub>, six potential casein kinase II phosphorylation sites at residues S<sub>15</sub>, T<sub>58</sub>, S<sub>203</sub>, T<sub>289</sub>, S<sub>332</sub>, and S<sub>357</sub>, five potential protein kinase C phosphorylation sites at residues S<sub>203</sub>, T<sub>207</sub>, T<sub>225</sub>, S<sub>253</sub>, and S<sub>344</sub>, and a potential tyrosine kinase phosphorylation site at residue Y<sub>288</sub>. PRINTS analysis identified CPSH as a carbamoyl-phosphate synthase protein (PR00098), which the algorithm defines using seven regions designated PR00098A through PR00098G. The region from residue S<sub>352</sub> through residue A<sub>366</sub> received a score of 1115 on a strength of 1477, and was supported by the presence of region PR00098E with a *P* value less than 0.012. Northern analysis shows the expression of this sequence in various libraries, at least 60% of which are immortalized or cancerous and at least 29% of which involve immune response. In addition, 23% of the libraries expressing CPSH were from reproductive tissue, while 15% were from gastrointestinal, hematopoietic/immune, or nervous tissue. Of particular note is the expression of CPSH in breast and ovarian cancers. The fragment of SEQ ID NO:2 from about nucleotide 128 through about nucleotide 188 is useful, e.g., as a hybridization probe.

The invention also encompasses CPSH variants. A preferred CPSH variant is one which has at least about 80%, more preferably at least about 90%, and most preferably at least about 95% amino acid sequence identity to the CPSH amino acid sequence, and which contains at least one functional or structural characteristic of CPSH.

The invention also encompasses polynucleotides which encode CPSH. In a particular embodiment, the invention encompasses a polynucleotide sequence comprising the sequence of SEQ ID NO:2, which encodes an CPSH.

The invention also encompasses a variant of a polynucleotide sequence encoding CPSH. In particular, such a variant polynucleotide sequence will have at least about 80%, more preferably at least about 90%, and most preferably at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding CPSH. A particular aspect of the invention encompasses a variant of SEQ ID NO:2 which has at least about 80%, more preferably at least about 90%, and most preferably at least about 95% polynucleotide sequence identity to SEQ ID NO:2. Any one of the polynucleotide variants described above can encode an amino acid sequence which contains at least one functional or

structural characteristic of CPSH.

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

10 Although nucleotide sequences which encode CPSH and its variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring CPSH under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding CPSH or its derivatives possessing a substantially different codon usage, e.g., inclusion of non-naturally occurring codons. Codons may be selected to  
15 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 codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding CPSH 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,  
20 than transcripts produced from the naturally occurring sequence.

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

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID NO:2, or a fragment of SEQ ID NO:2, under various conditions of stringency.  
30 (See, e.g., Wahl, G.M. and S.L. Berger (1987) Methods Enzymol. 152:399-407; Kimmel, A.R. (1987) Methods Enzymol. 152:507-511.)

Methods for DNA sequencing 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 (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).

10       The nucleic acid sequences encoding CPSH may be extended utilizing a partial nucleotide sequence and employing various PCR-based 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 and nested primers to amplify unknown sequence from genomic DNA within a cloning vector. (See, e.g.,  
15   Sarkar, G. (1993) PCR Methods Applic. 2:318-322.) Another method, inverse PCR, uses primers that extend in divergent directions to amplify unknown sequence from a circularized template. The template is derived from restriction fragments comprising a known genomic locus and surrounding sequences. (See, e.g., Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186.) A third method, capture PCR, involves PCR amplification  
20   of DNA fragments adjacent to known sequences in human and yeast artificial chromosome DNA. (See, e.g., Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119.) In this method, multiple restriction enzyme digestions and ligations may be used to insert an engineered double-stranded sequence into a region of unknown sequence before performing PCR. Other methods which may be used to retrieve unknown sequences are  
25   known in the art. (See, e.g., Parker, J.D. et al. (1991) Nucleic Acids Res. 19:3055-306). Additionally, one may use PCR, nested primers, and PromoterFinder™ libraries to walk genomic DNA (Clontech, Palo Alto, CA). This procedure avoids the need to screen libraries and is useful in finding intron/exon junctions. For all PCR-based methods, primers may be designed using commercially available software, such as OLIGO™ 4.06  
30   Primer Analysis software (National Biosciences Inc., Plymouth, MN) or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the template at temperatures of about 68°C to 72°C.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. In addition, random-primed libraries, which often include sequences containing the 5' regions of genes, are preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be  
5 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 particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different nucleotide-specific, laser-stimulated fluorescent dyes, and a  
10 charge coupled device camera for detection of the emitted wavelengths. 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 and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for sequencing small DNA  
15 fragments which may be present in limited amounts in a particular sample.

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

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter CPSH-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing,  
25 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, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, and so forth.

30 In another embodiment, sequences encoding CPSH may be synthesized, in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) Nucl. Acids Res. Symp. Ser. 215-223, and Horn, T. et al. (1980) Nucl. Acids Res.

Symp. Ser. 225-232.) Alternatively, CPSH itself or a fragment thereof may be synthesized using chemical methods. For example, peptide synthesis can be performed using various solid-phase techniques. (See, e.g., Roberge, J.Y. et al. (1995) Science 269:202-204.)

Automated synthesis may be achieved using the ABI 431A Peptide Synthesizer (Perkin Elmer). Additionally, the amino acid sequence of CPSH, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

The peptide may be substantially purified by preparative high performance liquid chromatography. (See, e.g., Chiez, R.M. and F.Z. Regnier (1990) Methods Enzymol. 182:392-421.) The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing. (See, e.g., Creighton, T. (1984) Proteins, Structures and Molecular Properties, WH Freeman and Co., New York, NY.)

In order to express a biologically active CPSH, the nucleotide sequences encoding CPSH or derivatives thereof may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. These elements include regulatory sequences, such as enhancers, constitutive and inducible promoters, and 5' and 3' untranslated regions in the vector and in polynucleotide sequences encoding CPSH. Such elements may vary in their strength and specificity. Specific initiation signals may also be used to achieve more efficient translation of sequences encoding CPSH. Such signals include the ATG initiation codon and adjacent sequences, e.g. the Kozak sequence. In cases where sequences encoding CPSH and its initiation codon and upstream regulatory 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 an in-frame ATG initiation codon should be provided by the vector. 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 appropriate for the particular host cell system used. (See, e.g., Scharf, D. et al. (1994) Results Probl. Cell Differ. 20:125-162.)

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding CPSH and appropriate transcriptional

and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. (See, e.g., Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview, NY, ch. 4, 8, and 16-17; and Ausubel, F.M. et al. (1995, and periodic  
5 supplements) Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY, ch. 9, 13, and 16.)

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

15 In bacterial systems, a number of cloning and expression vectors may be selected depending upon the use intended for polynucleotide sequences encoding CPSH. For example, routine cloning, subcloning, and propagation of polynucleotide sequences encoding CPSH can be achieved using a multifunctional E. coli vector such as Bluescript® (Stratagene) or pSport1™ plasmid (GIBCO BRL). Ligation of sequences  
20 encoding CPSH into the vector's multiple cloning site disrupts the *lacZ* gene, allowing a colorimetric screening procedure for identification of transformed bacteria containing recombinant molecules. In addition, these vectors may be useful for in vitro transcription, dideoxy sequencing, single strand rescue with helper phage, and creation of nested deletions in the cloned sequence. (See, e.g., Van Heeke, G. and S.M. Schuster (1989) J.  
25 Biol. Chem. 264:5503-5509.) When large quantities of CPSH are needed, e.g. for the production of antibodies, vectors which direct high level expression of CPSH may be used. For example, vectors containing the strong, inducible T5 or T7 bacteriophage promoter may be used.

Yeast expression systems may be used for production of CPSH. A number of  
30 vectors containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH, may be used in the yeast Saccharomyces cerevisiae or Pichia pastoris. In addition, such vectors direct either the secretion or intracellular retention of expressed

proteins and enable integration of foreign sequences into the host genome for stable propagation. (See, e.g., Ausubel, supra; and Grant et al. (1987) *Methods Enzymol.* 153:516-54; Scorer, C. A. et al. (1994) *Bio/Technology* 12:181-184.)

Plant systems may also be used for expression of CPSH. Transcription of  
 5 sequences encoding CPSH may be driven viral promoters, e.g., the 35S and 19S promoters of CaMV 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. (See, e.g., Coruzzi, G. et al. (1984) *EMBO J.* 3:1671-1680; Broglie, R. et al. (1984) *Science* 224:838-843; and  
 10 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. (See, e.g., Hobbs, S. or Murry, L.E. in McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York, NY; pp. 191-196.)

In mammalian cells, a number of viral-based expression systems may be utilized.  
 15 In cases where an adenovirus is used as an expression vector, sequences encoding CPSH 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 infective virus which expresses CPSH in host cells. (See, e.g., Logan, J. and T. Shenk (1984) *Proc. Natl. Acad. Sci.* 81:3655-3659.) In  
 20 addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells. SV40 or EBV-based vectors may also be used for high-level protein expression.

Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained in and expressed from a plasmid. HACs of about  
 25 6 kb to 10 Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes.

For long term production of recombinant proteins in mammalian systems, stable expression of CPSH in cell lines is preferred. For example, sequences encoding CPSH can be transformed into cell lines using expression vectors which may contain viral origins of  
 30 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 about 1 to 2 days in enriched media before being switched to selective

media. The purpose of the selectable marker is to confer resistance to a selective agent, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

5 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 and adenine phosphoribosyltransferase genes, for use in *tk* or *apr*<sup>-</sup> cells, respectively. (See, e.g., Wigler, M. et al. (1977) Cell 11:223-232; and Lowy, I. et al. (1980) Cell 22:817-823.) Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for  
10 selection. For example, *dhfr* confers resistance to methotrexate; *neo* confers resistance to the aminoglycosides neomycin and G-418; and *als* or *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g., Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. 77:3567-3570; Colbere-Garapin, F. et al (1981) J. Mol. Biol. 150:1-14; and Murry, supra.) Additional selectable genes have been described, e.g.,  
15 *trpB* and *hisD*, which alter cellular requirements for metabolites. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. 85:8047-8051.) Visible markers, e.g., anthocyanins, green fluorescent proteins (GFP) (Clontech, Palo Alto, CA),  $\beta$  glucuronidase and its substrate  $\beta$ -D-glucuronoside, or luciferase and its substrate luciferin may be used. These markers can be used not only to identify transformants, but also to  
20 quantify the amount of transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, C.A. et al. (1995) Methods Mol. Biol. 55:121-131.)

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding CPSH is inserted within a marker gene sequence,  
25 transformed cells containing sequences encoding CPSH can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding CPSH under the 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.

30 In general, host cells that contain the nucleic acid sequence encoding CPSH and that express CPSH 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, PCR amplification, and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

Immunological methods for detecting and measuring the expression of CPSH  
5 using either specific polyclonal or monoclonal antibodies are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on CPSH is preferred, but a competitive binding assay may be  
10 employed. These and other assays are well known in the art. (See, e.g., Hampton, R. et al. (1990) Serological Methods, a Laboratory Manual, APS Press, St Paul, MN, Section IV; Coligan, J. E. et al. (1997 and periodic supplements) Current Protocols in Immunology, Greene Pub. Associates and Wiley-Interscience, New York, NY; and Maddox, D.E. et al. (1983) J. Exp. Med. 158:1211-1216).

15 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 CPSH include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding  
20 CPSH, 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, such as those provided by Pharmacia & Upjohn  
25 (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 CPSH may be cultured  
30 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 retained 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 CPSH may be designed to contain signal sequences which direct secretion of CPSH through a prokaryotic or eukaryotic cell membrane.

In addition, a host cell strain may be chosen for its ability to modulate expression  
5 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 specify protein targeting, folding, and/or activity. Different host cells which have specific cellular machinery and  
10 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.

In another embodiment of the invention, natural, modified, or recombinant nucleic  
15 acid sequences encoding CPSH may be ligated to a heterologous sequence resulting in translation of a fusion protein in any of the aforementioned host systems. For example, a chimeric CPSH protein containing a heterologous moiety that can be recognized by a commercially available antibody may facilitate the screening of peptide libraries for inhibitors of CPSH activity. Heterologous protein and peptide moieties may also facilitate  
20 purification of fusion proteins using commercially available affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose binding protein (MBP), thioredoxin (Trx), calmodulin binding peptide (CBP), 6-His, FLAG, *c-myc*, and hemagglutinin (HA). GST, MBP, Trx, CBP, and 6-His enable purification of their cognate fusion proteins on immobilized glutathione, maltose, phenylarsine oxide,  
25 calmodulin, and metal-chelate resins, respectively. FLAG, *c-myc*, and hemagglutinin (HA) enable immunoaffinity purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize these epitope tags. A fusion protein may also be engineered to contain a proteolytic cleavage site located between the CPSH encoding sequence and the heterologous protein sequence, so that  
30 CPSH may be cleaved away from the heterologous moiety following purification. Methods for fusion protein expression and purification are discussed in Ausubel, F. M. et al. (1995 and periodic supplements) Current Protocols in Molecular Biology, John Wiley

& Sons, New York, NY, ch 10. A variety of commercially available kits may also be used to facilitate expression and purification of fusion proteins.

In a further embodiment of the invention, synthesis of radiolabeled CPSH may be achieved in vitro using the TNT<sup>TM</sup> rabbit reticulocyte lysate or wheat germ extract systems  
5 (Promega, Madison, WI). These systems couple transcription and translation of protein-coding sequences operably associated with the T7, T3, or SP6 promoters. Translation takes place in the presence of a radiolabeled amino acid precursor, preferably <sup>35</sup>S-methionine.

Fragments of CPSH may be produced not only by recombinant production, but  
10 also by direct peptide synthesis using solid-phase techniques. (See, e.g., Creighton, supra pp. 55-60.) Protein synthesis may be performed by manual techniques or by automation. Automated synthesis may be achieved, for example, using the Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Various fragments of CPSH may be synthesized separately and then combined to produce the full length molecule.

15

#### THERAPEUTICS

Protein sequence analysis identifies CPSH as a carbamoyl phosphate synthase homolog. In addition, CPSH is expressed in cancerous tissues. Therefore, CPSH appears to play a role in cancer and metabolic disorders.

20 In one embodiment, CPSH or a fragment or derivative thereof may be administered to a subject to treat or prevent a metabolic disorder. Such disorders can include, but are not limited to, Addison's disease, cystic fibrosis, diabetes, fatty hepatocirrhosis, galactosemia, goiter, hyperammonemia, hyperadrenalism, hypoadrenalism, hyperparathyroidism, hypoparathyroidism, hypercholesterolemia, hyperthyroidism,  
25 hypothyroidism hyperlipidemia, hyperlipemia, lipid myopathies, obesity, lipodystrophies, and phenylketonuria.

In another embodiment, a vector capable of expressing CPSH or a fragment or derivative thereof may be administered to a subject to treat or prevent a metabolic disorder including, but not limited to, those described above.

30 In a further embodiment, a pharmaceutical composition comprising a substantially purified CPSH in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a metabolic disorder including, but not limited to, those

provided above.

In still another embodiment, an agonist which modulates the activity of CPSH may be administered to a subject to treat or prevent a metabolic disorder including, but not limited to, those listed above.

5 In a further embodiment, an antagonist of CPSH may be administered to a subject to treat or prevent cancer. Such a cancer may include, but is not limited to, adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle,  
10 ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus. In one aspect, an antibody which specifically binds CPSH 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 CPSH.

In an additional embodiment, a vector expressing the complement of the  
15 polynucleotide encoding CPSH may be administered to a subject to treat or prevent a cancer 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 other appropriate therapeutic agents. Selection of the appropriate agents for use in  
20 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 adverse side effects.

25 An antagonist of CPSH may be produced using methods which are generally known in the art. In particular, purified CPSH may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind CPSH. Antibodies to CPSH may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and  
30 single chain antibodies, 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 CPSH or with any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants  
5 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, KLH, 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  
10 antibodies to CPSH have an amino acid sequence consisting of at least about 5 amino acids, and, more preferably, of at least about 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein and contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of CPSH amino acids may be fused with  
15 those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to CPSH 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  
20 hybridoma technique, and the EBV-hybridoma technique. (See, e.g., 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; and Cole, S.P. et al. (1984) Mol. Cell Biol. 62:109-120.)

In addition, techniques developed for the production of "chimeric antibodies," such  
25 as the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. (See, e.g., Morrison, S.L. et al. (1984) Proc. Natl. Acad. Sci. 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; and Takeda, S. et al. (1985) Nature 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be  
30 adapted, using methods known in the art, to produce CPSH-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries.

(See, e.g., Burton D.R. (1991) Proc. Natl. Acad. Sci. 88:10134-10137.)

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. (See, e.g., Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. 86: 3833-3837; and Winter, G. et al. (1991) Nature 349:293-299.)

Antibody fragments which contain specific binding sites for CPSH may also be generated. For example, such fragments include, but are not limited to, F(ab')<sub>2</sub> fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the F(ab')<sub>2</sub> fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse, W.D. et al. (1989) Science 246:1275-1281.)

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 CPSH and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering CPSH epitopes is preferred, but a competitive binding assay may also be employed. (Maddox, supra.)

In another embodiment of the invention, the polynucleotides encoding CPSH, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, the complement of the polynucleotide encoding CPSH 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 CPSH. Thus, complementary molecules or fragments may be used to modulate CPSH activity, or to achieve regulation of gene function. Such technology is now well known in the art, and sense or antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions of sequences encoding CPSH.

Expression vectors derived from retroviruses, adenoviruses, or 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 to express nucleic acid sequences complementary to the polynucleotides encoding CPSH. (See, e.g., Sambrook, supra; and Ausubel, supra.)

Genes encoding CPSH can be turned off by transforming a cell or tissue with  
5 expression vectors which express high levels of a polynucleotide, or fragment thereof, encoding CPSH. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. 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  
10 vector, and may last even longer if appropriate replication elements are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by designing complementary sequences or antisense molecules (DNA, RNA, or PNA) to the control, 5', or regulatory regions of the gene encoding CPSH. Oligonucleotides derived  
15 from the transcription initiation site, e.g., between about 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 ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex  
20 DNA have been described in the literature. (See, e.g., Gee, J.E. et al. (1994) in Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing Co., Mt. Kisco, NY, pp. 163-177.) A complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

25 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. For example, engineered hammerhead motif ribozyme molecules may specifically and efficiently catalyze endonucleolytic cleavage of sequences  
30 encoding CPSH.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, including 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  
5 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  
10 phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding CPSH. 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  
15 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  
20 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  
25 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 by polycationic amino polymers may be achieved using methods which are well known in the art. (See, e.g., Goldman, C.K. et al. (1997) Nature Biotechnology 15:462-466.)

30 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 or sterile composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of CPSH, antibodies to CPSH, and mimetics, agonists, antagonists, or inhibitors of CPSH. The compositions may be administered alone or in combination with at least one other agent, such as a 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 combining active compounds with solid excipient and processing the resultant mixture of granules (optionally, after grinding) to obtain tablets or dragee cores. Suitable auxiliaries can be added, if desired. Suitable excipients include carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, and 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, and 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,  
5 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  
10 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 fillers 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  
15 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  
20 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, triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain  
25 suitable stabilizers or agents to increase the solubility of the compounds and 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.

30 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, and succinic acid. Salts tend to be more soluble in aqueous or other protonic  
5 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 mM to 50 mM histidine, 0.1% to 2% sucrose, and 2% to 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  
10 appropriate container and labeled for treatment of an indicated condition. For administration of CPSH, 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  
15 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 such as mice, rats, rabbits, dogs, or pigs. An animal model may also be used to determine the appropriate  
20 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 CPSH or fragments thereof, antibodies of CPSH, and agonists, antagonists or inhibitors of CPSH, which ameliorates the symptoms or condition. Therapeutic efficacy  
25 and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating the  $ED_{50}$  (the dose therapeutically effective in 50% of the population) or  $LD_{50}$  (the dose lethal to 50% of the population) statistics. The dose ratio of therapeutic to toxic effects is the therapeutic index, and it can be expressed as the  $ED_{50}/LD_{50}$  ratio. Pharmaceutical compositions which exhibit large  
30 therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used to formulate a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that includes

the ED<sub>50</sub> with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, the 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 requiring treatment. Dosage and administration are adjusted to provide  
5 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, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. Long-acting  
10 pharmaceutical compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from about 0.1  $\mu$ g to 100,000  $\mu$ g, up to a total dose of about 1 gram, 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  
15 formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

#### DIAGNOSTICS

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

A variety of protocols for measuring CPSH, including ELISAs, RIAs, and FACS,  
30 are known in the art and provide a basis for diagnosing altered or abnormal levels of CPSH expression. Normal or standard values for CPSH expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, preferably

human, with antibody to CPSH under conditions suitable for complex formation. The amount of standard complex formation may be quantitated by various methods, preferably by photometric means. Quantities of CPSH expressed in subject, control, and disease samples from biopsied tissues are compared with the standard values. Deviation between  
5 standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding CPSH 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  
10 which expression of CPSH may be correlated with disease. The diagnostic assay may be used to determine absence, presence, and excess expression of CPSH, and to monitor regulation of CPSH levels during therapeutic intervention.

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

Probes may also be used for the detection of related sequences, and should preferably have at least 50% sequence identity to any of the CPSH encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from the sequence of SEQ ID NO:2 or from genomic sequences including  
25 promoters, enhancers, and introns of the CPSH gene.

Means for producing specific hybridization probes for DNAs encoding CPSH include the cloning of polynucleotide sequences encoding CPSH or CPSH derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by means of  
30 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, by radionuclides such as  $^{32}\text{P}$  or  $^{35}\text{S}$ , or by enzymatic labels, such as alkaline phosphatase

coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding CPSH may be used for the diagnosis of a disorder associated with expression of CPSH. Examples of such a disorder include, but are not limited to, cancers such as adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, 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, and metabolic disorders such as Addison's disease, cystic fibrosis, diabetes, fatty hepatocirrhosis, galactosemia, goiter, hyperammonemia, hyperadrenalism, hypoadrenalism, hyperparathyroidism, hypoparathyroidism, hypercholesterolemia, hyperthyroidism, hypothyroidism, hyperlipidemia, hyperlipemia, lipid myopathies, obesity, lipodystrophies, and phenylketonuria. The polynucleotide sequences encoding CPSH may be used in Southern or Northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and ELISA assays; and in microarrays utilizing fluids or tissues from patients to detect altered CPSH expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding CPSH may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding CPSH 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 patient sample is significantly altered in comparison to a control sample then the presence of altered levels of nucleotide sequences encoding CPSH in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor the treatment of an individual patient.

In order to provide a basis for the diagnosis of a disorder associated with expression of CPSH, 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, encoding CPSH, under

conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with values from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained in this manner may be compared with values obtained from  
5 samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

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

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  
15 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.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding CPSH may involve the use of PCR. These oligomers may be chemically  
20 synthesized, generated enzymatically, or produced in vitro. Oligomers will preferably contain a fragment of a polynucleotide encoding CPSH, or a fragment of a polynucleotide complementary to the polynucleotide encoding CPSH, and will be employed under optimized conditions for identification of a specific gene or condition. Oligomers may also be employed under less stringent conditions for detection or quantitation of closely  
25 related DNA or RNA sequences.

Methods which may also be used to quantitate the expression of CPSH include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (See, e.g., Melby, P.C. et al. (1993) J. Immunol. Methods 159:235-244; and Duplaa, C. et al. (1993) Anal. Biochem. 229-236.)  
30 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, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used as targets in a microarray. The microarray can be used to monitor the expression level of large numbers of genes simultaneously and to identify genetic variants, mutations, and polymorphisms. This  
5 information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, and to develop and monitor the activities of therapeutic agents.

Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g., Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996)  
10 Proc. Natl. Acad. Sci. 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.)

In another embodiment of the invention, nucleic acid sequences encoding CPSH  
15 may be used to generate hybridization probes useful in 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, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries.  
20 (See, e.g., Price, C.M. (1993) Blood Rev. 7:127-134; and Trask, B.J. (1991) Trends Genet. 7:149-154.)

Fluorescent in situ hybridization (FISH) may be correlated with other physical chromosome mapping techniques and genetic map data. (See, e.g., Heinz-Ulrich, et al. (1995) in Meyers, R.A. (ed.) Molecular Biology and Biotechnology, VCH Publishers New  
25 York, NY, pp. 965-968.) Examples of genetic map data can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) site. Correlation between the location of the gene encoding CPSH on a physical chromosomal map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA associated with that disorder. The nucleotide sequences of the invention may be used to  
30 detect differences in gene sequences among normal, carrier, and 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 by physical mapping. This provides valuable information to  
5 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, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R.A. et al. (1988) Nature 336:577-580.) The nucleotide sequence of the  
10 subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc., among normal, carrier, or affected individuals.

In another embodiment of the invention, CPSH, 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  
15 may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between CPSH and the agent being tested may be measured.

Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the protein of interest. (See, e.g., Geysen, et al. (1984) PCT application WO84/03564.) In this method, large numbers of different  
20 small test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The test compounds are reacted with CPSH, or fragments thereof, and washed. Bound CPSH is then detected by methods well known in the art. Purified CPSH can also be coated directly onto plates for use in the aforementioned drug screening  
25 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 neutralizing antibodies capable of binding CPSH specifically compete with a test compound for binding CPSH. In this manner, antibodies can be used to detect the  
30 presence of any peptide which shares one or more antigenic determinants with CPSH.

In additional embodiments, the nucleotide sequences which encode CPSH may be used in 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  
5 included for the purpose of limiting the invention.

## EXAMPLES

### I. THP1T7T01 cDNA Library Construction

The THP1T7T01 library was constructed using RNA isolated from 50,000 cultured  
10 THP-1 cells, which was amplified using a proprietary T7 amplification method developed at Incyte. THP-1 (ATCC TIB 202) is a human promonocyte line derived from the peripheral blood of a 1-year-old Caucasian male with acute monocytic leukemia. Antisense RNA (aRNA) was generated using T7 RNA polymerase following the round of cDNA synthesis. aRNA was then random-primed to generate double-stranded cDNA,  
15 ligated to EcoR1 adaptors, and cloned non-directionally into the pINCY vector (Incyte). The resulting plasmids were subsequently transformed into DH5 $\alpha$ <sup>TM</sup> competent cells (Cat. #18258-012; GIBCO BRL).

### II. Isolation and Sequencing of cDNA Clones

20 Plasmid DNA was released from the cells and purified using the REAL Prep 96 plasmid kit (QIAGEN Inc). The recommended protocol was employed except for the following changes: 1) the bacteria were cultured in 1 ml of sterile Terrific Broth (GIBCO BRL) with carbenicillin at 25 mg/L and glycerol at 0.4%; 2) after inoculation, the cultures were incubated for 19 hours and then the cells were lysed with 0.3 ml of lysis buffer; and  
25 3) following isopropanol precipitation, the plasmid DNA pellets were resuspended in 0.1 ml of distilled water. The plasmid DNA samples were stored at 4°C.

The cDNAs were sequenced by the method of Sanger et al. (1975, J. Mol. Biol. 94:441f), using a Hamilton Micro Lab 2200 (Hamilton, Reno, NV) in combination with Peltier Thermal Cyclers (PTC200 from MJ Research, Watertown, MA) and Applied  
30 Biosystems 377 DNA Sequencing Systems and the reading frame was determined.

### III. Similarity Searching of cDNA Clones and Their Deduced Proteins

The nucleotide sequences and/or amino acid sequences of the Sequence Listing were used to query sequences in the GenBank, SwissProt, BLOCKS, and Pima II databases. These databases, which contain previously identified and annotated sequences, were searched for regions of similarity using BLAST (Basic Local Alignment Search Tool). (See, e.g., Altschul, S.F. (1993) J. Mol. Evol 36:290-300; and Altschul et al. (1990) J. Mol. Biol. 215:403-410.)

BLAST produced alignments of both nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST was 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 could have been used when dealing with primary sequence patterns and secondary structure gap penalties. (See, e.g., Smith, T. et al. (1992) Protein Engineering 5:35-51.) The sequences disclosed in this application have lengths of at least 49 nucleotides and have no more than 12% uncalled bases (where N is recorded rather than A, C, G, or T).

The BLAST approach searched for matches between a query sequence and a database sequence. BLAST evaluated the statistical significance of any matches found, and reported only those matches that satisfy the user-selected threshold of significance. In this application, threshold was set at  $10^{-25}$  for nucleotides and  $10^{-8}$  for peptides.

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

Additionally, sequences identified from cDNA libraries may be analyzed to identify those gene sequences encoding conserved protein motifs using an appropriate analysis program, e.g., BLOCKS. BLOCKS is a weighted matrix analysis algorithm based on short amino acid segments, or blocks, compiled from the PROSITE database. (Bairoch, A. et al. (1997) Nucleic Acids Res. 25:217-221.) The BLOCKS algorithm is useful for classifying genes with unknown functions. (Henikoff S. And Henikoff G.J., Nucleic Acids Research (1991) 19:6565-6572.) Blocks, which are 3-60 amino acids in length, correspond to the most highly conserved regions of proteins. The BLOCKS

algorithm compares a query sequence with a weighted scoring matrix of blocks in the BLOCKS database. Blocks in the BLOCKS database are calibrated against protein sequences with known functions from the SWISS-PROT database to determine the stochastic distribution of matches. Similar databases such as PRINTS, a protein  
5 fingerprint database, are also searchable using the BLOCKS algorithm. (Attwood, T. K. et al. (1997) J. Chem. Inf. Comput. Sci. 37:417-424.) PRINTS is based on non-redundant sequences obtained from sources such as SWISS-PROT, GenBank, PIR, and NRL-3D.

The BLOCKS algorithm searches for matches between a query sequence and the BLOCKS or PRINTS database and evaluates the statistical significance of any matches  
10 found. Matches from a BLOCKS or PRINTS search can be evaluated on two levels, local similarity and global similarity. The degree of local similarity is measured by scores, and the extent of global similarity is measured by score ranking and probability values. A score of 1000 or greater for a BLOCKS match of highest ranking indicates that the match falls within the 0.5 percentile level of false positives when the matched block is calibrated  
15 against SWISS-PROT. Likewise, a probability value of less than  $1.0 \times 10^{-3}$  indicates that the match would occur by chance no more than one time in every 1000 searches. Only those matches with a cutoff score of 1000 or greater and a cutoff probability value of  $1.0 \times 10^{-3}$  or less are considered in the functional analyses of the protein sequences in the Sequence Listing.

20 Nucleic and amino acid sequences of the Sequence Listing may also be analyzed using PFAM. PFAM is a Hidden Markov Model (HMM) based protocol useful in protein family searching. HMM is a probabilistic approach which analyzes consensus primary structures of gene families. (See, e.g., Eddy, S.R. (1996) Cur. Opin. Str. Biol. 6:361-365.)

The PFAM database contains protein sequences of 527 protein families gathered  
25 from publicly available sources, e.g., SWISS-PROT and PROSITE. PFAM searches for well characterized protein domain families using two high-quality alignment routines, seed alignment and full alignment. (See, e.g., Sonnhammer, E.L.L. et al. (1997) Proteins 28:405-420.) The seed alignment utilizes the hmmls program, a program that searches for local matches, and a non-redundant set of the PFAM database. The full alignment utilizes  
30 the hmmfs program, a program that searches for multiple fragments in long sequences, e.g., repeats and motifs, and all sequences in the PFAM database. A result or score of 100 "bits" can signify that it is  $2^{100}$ -fold more likely that the sequence is a true match to the

model or comparison sequence. Cutoff scores which range from 10 to 50 bits are generally used for individual protein families using the SWISS-PROT sequences as model or comparison sequences.

Two other algorithms, SIGPEPT and TM, both based on the HMM algorithm  
5 described above (see, e.g., Eddy, supra; and Sonnhammer, supra), identify potential signal sequences and transmembrane domains, respectively. SIGPEPT was created using protein sequences having signal sequence annotations derived from SWISS-PROT. It contains about 1413 non-redundant signal sequences ranging in length from 14 to 36 amino acid residues. TM was created similarly using transmembrane domain annotations. It contains  
10 about 453 non-redundant transmembrane sequences encompassing 1579 transmembrane domain segments. Suitable HMM models were constructed using the above sequences and were refined with known SWISS-PROT signal peptide sequences or transmembrane domain sequences until a high correlation coefficient, a measurement of the correctness of the analysis, was obtained. Using the protein sequences from the SWISS-PROT database  
15 as a test set, a cutoff score of 11 bits, as determined above, correlated with 91-94% true-positives and about 4.1% false-positives, yielding a correlation coefficient of about 0.87-0.90 for SIGPEPT. A score of 11 bits for TM will typically give the following results: 75% true positives; 1.72% false positives; and a correlation coefficient of 0.76. Each search evaluates the statistical significance of any matches found and reports only those  
20 matches that score at least 11 bits.

#### 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  
25 membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, supra, ch. 7; and Ausubel, supra, ch. 4 and 16.)

Analogous computer techniques applying BLAST are used to search for identical or related molecules in nucleotide databases such as GenBank or LIFESEQ™ database (Incyte Pharmaceuticals). This analysis is much faster than multiple membrane-based  
30 hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or similar.

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

% sequence identity x % 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  
5 will be exact within a 1% to 2% error, and, with a product score of 70, the match will be exact. Similar molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules.

The results of Northern analysis are reported as a list of libraries in which the transcript encoding CPSH occurs. Abundance and percent abundance are also reported.  
10 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.

**V. Extension of CPSH Encoding Polynucleotides**

15 The nucleic acid sequence of Incyte Clone 2045605 was used to design oligonucleotide primers for extending a partial nucleotide sequence to full length. One primer was synthesized to initiate extension of an antisense polynucleotide, and the other was synthesized to initiate extension of a sense polynucleotide. Primers were used to facilitate the extension of the known sequence "outward" generating amplicons containing  
20 new unknown nucleotide sequence for the region of interest. The initial primers were designed from the cDNA using OLIGO™ 4.06 (National Biosciences, Plymouth, MN), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin  
25 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.

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

components of the kit, with the following parameters:

|    |         |                                                       |
|----|---------|-------------------------------------------------------|
|    | Step 1  | 94° C for 1 min (initial denaturation)                |
|    | Step 2  | 65° C for 1 min                                       |
|    | Step 3  | 68° C for 6 min                                       |
| 5  | Step 4  | 94° C for 15 sec                                      |
|    | Step 5  | 65° C for 1 min                                       |
|    | Step 6  | 68° C for 7 min                                       |
|    | Step 7  | Repeat steps 4 through 6 for an additional 15 cycles  |
|    | Step 8  | 94° C for 15 sec                                      |
| 10 | Step 9  | 65° C for 1 min                                       |
|    | Step 10 | 68° C for 7:15 min                                    |
|    | Step 11 | Repeat steps 8 through 10 for an additional 12 cycles |
|    | Step 12 | 72° C for 8 min                                       |
| 15 | Step 13 | 4° C (and holding)                                    |

A 5  $\mu$ l to 10  $\mu$ l aliquot of the reaction mixture was analyzed by electrophoresis on a low concentration (about 0.6% to 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.), and trimmed of  
20 overhangs using Klenow enzyme to facilitate religation and cloning.

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

For PCR amplification, 18  $\mu$ l of concentrated PCR reaction mix (3.3x) containing  
35 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                                     |
| 5 | Step 4 | 72° C for 90 sec                                     |
|   | Step 5 | Repeat steps 2 through 4 for an additional 29 cycles |
|   | Step 6 | 72° C for 180 sec                                    |
|   | Step 7 | 4° C (and holding)                                   |

10 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 is used to obtain 5' regulatory sequences using the procedure above, oligonucleotides designed for 5' extension, and an appropriate genomic library.

## VI. Labeling and Use of Individual Hybridization Probes

Hybridization probes derived from SEQ ID NO:2 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 software (National Biosciences) and labeled by combining 50 pmol of each oligomer, 250  $\mu$ Ci of [ $\gamma$ -<sup>32</sup>P] adenosine triphosphate (Amersham, Chicago, IL), and T4 polynucleotide kinase (DuPont NEN®, Boston, MA). The labeled oligonucleotides are substantially purified using a Sephadex™ G-25 superfine size exclusion dextran bead column (Pharmacia & Upjohn, Kalamazoo, MI). An aliquot containing 10<sup>7</sup> 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, Boston, MA).

30 The DNA from each digest is fractionated on a 0.7% 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 to film for several hours, hybridization patterns are compared visually.

## VII. Microarrays

5 A chemical coupling procedure and an ink jet device can be used to synthesize array elements on the surface of a substrate. (See, e.g., Baldeschweiler, supra.) An array analogous to a dot or slot blot may also be used to arrange and link elements to the surface of a substrate using thermal, UV, chemical, or mechanical bonding procedures. A typical array may be produced by hand or using available methods and machines and contain any  
10 appropriate number of elements. After hybridization, nonhybridized probes are removed and a scanner used to determine the levels and patterns of fluorescence. The degree of complementarity and the relative abundance of each probe which hybridizes to an element on the microarray may be assessed through analysis of the scanned images.

Full-length cDNAs, Expressed Sequence Tags (ESTs), or fragments thereof may  
15 comprise the elements of the microarray. Fragments suitable for hybridization can be selected using software well known in the art such as LASERGENE™. Full-length cDNAs, ESTs, or fragments thereof corresponding to one of the nucleotide sequences of the present invention, or selected at random from a cDNA library relevant to the present invention, are arranged on an appropriate substrate, e.g., a glass slide. The cDNA is fixed  
20 to the slide using, e.g., UV cross-linking followed by thermal and chemical treatments and subsequent drying. (See, e.g., Schena, M. et al. (1995) Science 270:467-470; and Shalon, D. et al. (1996) Genome Res. 6:639-645.) Fluorescent probes are prepared and used for hybridization to the elements on the substrate. The substrate is analyzed by procedures described above.

25

## VIII. Complementary Polynucleotides

Sequences complementary to the CPSH-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring CPSH. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially  
30 the same procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using OLIGO™ 4.06 software and the coding sequence of CPSH. 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 CPSH-encoding transcript.

## 5 IX. Expression of CPSH

Expression and purification of CPSH is achieved using bacterial or virus-based expression systems. For expression of CPSH in bacteria, cDNA is subcloned into an appropriate vector containing an antibiotic resistance gene and an inducible promoter that directs high levels of cDNA transcription. Examples of such promoters include, but are  
10 not limited to, the *trp-lac (tac)* hybrid promoter and the T5 or T7 bacteriophage promoter in conjunction with the *lac* operator regulatory element. Recombinant vectors are transformed into suitable bacterial hosts, e.g., BL21(DE3). Antibiotic resistant bacteria express CPSH upon induction with isopropyl beta-D-thiogalactopyranoside (IPTG). Expression of CPSH in eukaryotic cells is achieved by infecting insect or mammalian cell  
15 lines with recombinant Autographica californica nuclear polyhedrosis virus (AcMNPV), commonly known as baculovirus. The nonessential polyhedrin gene of baculovirus is replaced with cDNA encoding CPSH by either homologous recombination or bacterial-mediated transposition involving transfer plasmid intermediates. Viral infectivity is maintained and the strong polyhedrin promoter drives high levels of cDNA transcription.  
20 Recombinant baculovirus is used to infect Spodoptera frugiperda (Sf9) insect cells in most cases, or human hepatocytes, in some cases. Infection of the latter requires additional genetic modifications to baculovirus. (See Engelhard, E. K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945.)

In most expression systems, CPSH is synthesized as a fusion protein with, e.g.,  
25 glutathione S-transferase (GST) or a peptide epitope tag, such as FLAG or 6-His, permitting rapid, single-step, affinity-based purification of recombinant fusion protein from crude cell lysates. GST, a 26-kilodalton enzyme from Schistosoma japonicum, enables the purification of fusion proteins on immobilized glutathione under conditions that maintain protein activity and antigenicity (Pharmacia, Piscataway, NJ). Following  
30 purification, the GST moiety can be proteolytically cleaved from CPSH at specifically engineered sites. FLAG, an 8-amino acid peptide, enables immunoaffinity purification using commercially available monoclonal and polyclonal anti-FLAG antibodies (Eastman

Kodak, Rochester, NY). 6-His, a stretch of six consecutive histidine residues, enables purification on metal-chelate resins (QIAGEN Inc, Chatsworth, CA). Methods for protein expression and purification are discussed in Ausubel, F. M. et al. (1995 and periodic supplements) Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY, ch 10, 16. Purified CPSH obtained by these methods can be used directly in the following activity assay.

#### **X. Demonstration of CPSH Activity**

CPSH activity is determined by a coupled assay procedure, wherein purified CPSH catalyzes the production of carbamoyl phosphate. The enzyme ornithine transcarbamoylase then catalyzes the reaction between carbamoyl phosphate and ornithine, producing citrulline, which absorbs at 464 nm. The reaction mixtures contain 50 mM HEPES/NaOH, pH 7.6, 100 mM KCl, 10 mM ATP, 20 mM magnesium acetate, 20 mM NaHCO<sub>3</sub>, 10 mM glutamine (or 300 mM NH<sub>4</sub>Cl), 5 mM ornithine, and 2 units of ornithine transcarbamoylase in a final volume of 250  $\mu$ l. The reaction is initiated by the addition of an appropriate amount of CPSH (e.g., 2-10  $\mu$ g). The reaction is incubated for 10 minutes at 37 °C and terminated by the addition of 1 ml of acid reagent (3.7g antipyrine, 2.5 g ferric ammonium sulfate in 500 ml H<sub>2</sub>O, 250 ml concentrated H<sub>2</sub>SO<sub>4</sub> and 250 ml 85% H<sub>3</sub>PO<sub>4</sub>). 500  $\mu$ l of 0.4% diacetylmonoxime is added, and the samples are incubated for 15 minutes in a boiling water bath. Citrulline concentration is determined by reading the absorbance at 464 nm. The concentration of citrulline is proportional to the activity of CPSH in the sample. (Rubino, S.D. et al. (1986) J. Biol. Chem. 261:11320-11327.)

#### **XI. Functional Assays**

CPSH function is assessed by expressing the sequences encoding CPSH at physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a mammalian expression vector containing a strong promoter that drives high levels of cDNA expression. Vectors of choice include pCMV SPORT™ (Life Technologies, Gaithersburg, MD) and pCR™ 3.1 (Invitrogen, Carlsbad, CA, both of which contain the cytomegalovirus promoter. 5-10  $\mu$ g of recombinant vector are transiently transfected into a human cell line, preferably of endothelial or hematopoietic origin, using either liposome formulations or electroporation. 1-2  $\mu$ g of an additional plasmid containing sequences

encoding a marker protein are co-transfected. Expression of a marker protein provides a means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., Green Fluorescent Protein (GFP) (Clontech, Palo Alto, CA), CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an automated, laser optics-based technique, is used to identify transfected cells expressing GFP or CD64-GFP, and to evaluate properties, for example, their apoptotic state. FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell death. These events include changes in nuclear DNA content as measured by staining of DNA with propidium iodide; changes in cell size and granularity as measured by forward light scatter and 90 degree side light scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in expression of cell surface and intracellular proteins as measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow cytometry are discussed in Ormerod, M. G. (1994) Flow Cytometry, Oxford, New York, NY.

The influence of CPSH on gene expression can be assessed using highly purified populations of cells transfected with sequences encoding CPSH and either CD64 or CD64-GFP. CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated from nontransfected cells using magnetic beads coated with either human IgG or antibody against CD64 (DYNAL, Lake Success, NY). mRNA can be purified from the cells using methods well known by those of skill in the art. Expression of mRNA encoding CPSH and other genes of interest can be analyzed by Northern analysis or microarray techniques.

## **XII. Production of CPSH Specific Antibodies**

CPSH substantially purified using polyacrylamide gel electrophoresis (PAGE)(see, e.g., Harrington, M.G. (1990) *Methods Enzymol.* 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols.

Alternatively, the CPSH amino acid sequence is analyzed using LASERGENE™

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. Methods for selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well described in the art. (See, e.g., Ausubel  
5 supra, ch. 11.)

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

#### 15 **XIII. Purification of Naturally Occurring CPSH Using Specific Antibodies**

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

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

#### **XIV. Identification of Molecules Which Interact with CPSH**

CPSH, or biologically active fragments thereof, are labeled with <sup>125</sup>I Bolton-Hunter reagent. (See, e.g., Bolton et al. (1973) Biochem. J. 133:529.) Candidate  
30 molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled CPSH, washed, and any wells with labeled CPSH complex are assayed. Data obtained using different concentrations of CPSH are used to calculate values for the

number, affinity, and association of CPSH with the candidate molecules.

Various modifications and variations of the described methods and systems 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  
5 specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited 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.

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PCT/US99/11161

## SEQUENCE LISTING

<110> INYCTE PHARMACEUTICALS, INC.  
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 CORLEY, Neil C.  
 LAL, Preeti

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|     |     |     |     | 110 |     |     |     |     | 115 |     |     |     |     | 120 |  |
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ggtgaccttt cagttttcct atggaacaaa agaaacttcg tatggcctgt ccttcttcaa 660
accacggccc ggaaacttcg aaagaaattt ctctgtaaac ttatataaag ttactggaca 720
gttcccttgg agctcactgc gggagacgga cagaggaatg tcagctgagt acagttttcc 780
catatggaag accagccaca ctgtcaagtg ggaaggcgta tggcgagAAC tgggctgcct 840
ctcaaggacg gcgtcatttg ctgttcgaaa agaaagcgga cattcactga aatcatctct 900
ttcgcacgcc atggtcatcg attctcgga tttctccatc ttaccaagga gaggtgcttt 960
gctgaaagtt aaccaggaac tggcaggcta cactggcggg gatgtgagct tcatcaaaga 1020
agattttgaa cttcagttga acaagcaact catatttgat tcagtttttt cagcgtcttt 1080
ctggggcgga atgttggtac ccattggtga taagccgtca agcattgctg ataggtttta 1140
ccttggggga cccacaagcg tccgcggatt cagcatgcac agcatcgggc cacagagcga 1200
aggagactac ctaggtggag aagcgtactg ggccggcggc ctgcacctct acacccatt 1260
acctttccgg ccaggccagg gtggcttttg agaacttttc cgaacacact tctttctcaa 1320
cgcaggaaac ctctgcaacc tcaactatgg ggagggcccc aaagctcata ttcgtaagct 1380
ggctgagtgc atccgctggt cgtacggggc cgggattgtc ctcaggcttg gcaacatcgc 1440
tcggttgga ctttaattact gcgtcccat gggagtacag acaggcgaca ggatatgtga 1500
tggcgtccag tttggagctg ggataagggt cctgtagccg acacccctac aggagaagct 1560
ctgggactgg ggcagcagca aggcgcccat gccacacacc gtctctcgag gaaacgcggt 1620
tcagcgattc tttgactgcg gaccctgtgg gaaaccccg caataaatgt taaagacaca 1680
ctcaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1740
aaaaaaaaaa aaaaggggcg gccgccgact agtgacctct tcgccc 1786
```

What is claimed is:

1. A substantially purified polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1.  
5
2. A substantially purified variant having at least 90% amino acid sequence identity to the sequence of claim 1.
3. An isolated and purified polynucleotide encoding the polypeptide of claim  
10 1.
4. An isolated and purified polynucleotide variant having at least 90% polynucleotide sequence identity to the polynucleotide of claim 3.
- 15 5. An isolated and purified polynucleotide which hybridizes under stringent conditions to the polynucleotide of claim 3.
6. An isolated and purified polynucleotide which is complementary to the polynucleotide of claim 3.  
20
7. An isolated and purified polynucleotide comprising the polynucleotide sequence of SEQ ID NO:2 or a fragment of SEQ ID NO:2.
8. An isolated and purified polynucleotide variant having at least 90%  
25 polynucleotide sequence identity to the polynucleotide of claim 7.
9. An isolated and purified polynucleotide having a sequence complementary to the polynucleotide of claim 7.
- 30 10. An expression vector comprising at least a fragment of the polynucleotide of claim 3.

11. A host cell comprising the expression vector of claim 10.
12. A method for producing a polypeptide comprising a sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1, the method comprising the steps of:
- 5 (a) culturing the host cell of claim 11 under conditions suitable for the expression of the polypeptide; and
- (b) recovering the polypeptide from the host cell culture.
13. A pharmaceutical composition comprising the polypeptide of claim 1 in
- 10 conjunction with a suitable pharmaceutical carrier.
14. A purified antibody which specifically binds to the polypeptide of claim 1.
15. A purified agonist of the polypeptide of claim 1.
- 15 16. A purified antagonist of the polypeptide of claim 1.
17. A method for treating or preventing cancer, the method comprising administering to a subject in need of such treatment an effective amount of the antagonist
- 20 of claim 16.
18. A method for treating or preventing a metabolic disorder, the method comprising administering to a subject in need of such treatment an effective amount of the pharmaceutical composition of claim 13.
19. A method for detecting a polynucleotide encoding a polypeptide
- 25 comprising the amino acid sequence of SEQ ID NO:1 in a biological sample containing nucleic acids, the method comprising the steps of:
- (a) hybridizing the polynucleotide of claim 6 to at least one of the nucleic acids of the biological sample, thereby forming a hybridization complex; and
- 30 (b) detecting the hybridization complex, wherein the presence of the hybridization complex correlates with the presence of a polynucleotide encoding the polypeptide in the biological sample.

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20. The method of claim 19 wherein the nucleic acids of the biological sample are amplified by the polymerase chain reaction prior to the hybridizing step.

|                                                                           |                         |     |     |     |     |
|---------------------------------------------------------------------------|-------------------------|-----|-----|-----|-----|
| 10                                                                        | 19                      | 28  | 37  | 46  | 55  |
| G TCC AGG GGG TTT GTG GGA GTT GCC TTG ACC TGC AGC TCC GCC ACC GCG GAC CCG |                         |     |     |     |     |
| 64                                                                        | 73                      | 82  | 91  | 100 | 109 |
| CCT TCT GCC CTC AGC AGC AGA CGC TCT GTC CCG CCC GGG CAG CTC TGC GAG GCA   |                         |     |     |     |     |
| 118                                                                       | 127                     | 136 | 145 | 154 | 163 |
| GCG GCT GGA GAG GGA ACC ATG GGG ACT GTG CAC GCC CGG AGT TTG GAG CCT CTT   |                         |     |     |     |     |
|                                                                           | M G T V H A R S L E P L |     |     |     |     |
| 172                                                                       | 181                     | 190 | 199 | 208 | 217 |
| CCA TCA AGT GGA CCT GAT TTT GGA GGA TTA GGA GAA GCT GAA TTT GTT GAA       |                         |     |     |     |     |
| P S S G G P D F G G L G E A E F V E                                       |                         |     |     |     |     |
| 226                                                                       | 235                     | 244 | 253 | 262 | 271 |
| GTT GAG CCT GAA GCT AAA CAG GAA ATT CTT GAA AAC AAA GAT GTG GTT GTT CAA   |                         |     |     |     |     |
| V E P E A K Q E I L E N K D V V Q                                         |                         |     |     |     |     |
| 280                                                                       | 289                     | 298 | 307 | 316 | 325 |
| CAT GTT CAT TTT GAT GGA CTT GGA AGG ACT AAA GAT GAT ATC ATC ATT TGT GAA   |                         |     |     |     |     |
| H V H F D G G L G R T K D D I I I C E                                     |                         |     |     |     |     |
| 334                                                                       | 343                     | 352 | 361 | 370 | 379 |
| ATT GGA GAT GTT TTC AAG GCC AAA AAC CTA ATT GAG GTA ATG CGG AAA TCT CAT   |                         |     |     |     |     |
| I G D V F K A K N L I E V M R K S H                                       |                         |     |     |     |     |

FIGURE 1A

|                                                                         |     |     |     |     |     |
|-------------------------------------------------------------------------|-----|-----|-----|-----|-----|
| 388                                                                     | 397 | 406 | 415 | 424 | 433 |
| GAA GCC CGT GAA AAA TTG CTC CGT CTT GGA ATT TTT AGA CAA GTG GAT GTT TTG |     |     |     |     |     |
| E A R E K L L L R L L G I F R Q V D V L                                 |     |     |     |     |     |
| 442                                                                     | 451 | 460 | 469 | 478 | 487 |
| ATT GAC ACA TGT CAA GGT GAT GAC GCA CTT CCA AAT GGG TTA GAC GTT ACC TTT |     |     |     |     |     |
| I D T C Q G G D D A L P N G L D V T F                                   |     |     |     |     |     |
| 496                                                                     | 505 | 514 | 523 | 532 | 541 |
| GAA GTA ACT GAA TTG AGG AGA TTA ACG GGC AGT TAT AAC ACC ATG GTT GGA AAC |     |     |     |     |     |
| E V T E L R R L T T G S Y N T M V G N                                   |     |     |     |     |     |
| 550                                                                     | 559 | 568 | 577 | 586 | 595 |
| AAT GAA GGC AGT ATG GTA CTT GGC CTC AAG CTT CCT AAT CTT GGT CGT GCA     |     |     |     |     |     |
| N E G S M V L G L K L P N L L G R A                                     |     |     |     |     |     |
| 604                                                                     | 613 | 622 | 631 | 640 | 649 |
| GAA AAG GTG ACC TTT CAG TTT TCC TAT GGA ACA AAA GAA ACT TCG TAT GGC CTG |     |     |     |     |     |
| E K V T F Q Q F S Y G T K E T S Y G L                                   |     |     |     |     |     |
| 658                                                                     | 667 | 676 | 685 | 694 | 703 |
| TCC TTC TTC AAA CCA CGG CCC GGA AAC TTC GAA AGA AAT TTC GTA AAC TTA     |     |     |     |     |     |
| S F F K P R P G N F E R N F S V N L                                     |     |     |     |     |     |
| 712                                                                     | 721 | 730 | 739 | 748 | 757 |
| TAT AAA GTT ACT GGA CAG TTC CCT TGG AGC TCA CTG CGG GAG ACG GAC AGA GGA |     |     |     |     |     |
| Y K V T G Q Q F P W S S L R E T D R G                                   |     |     |     |     |     |

FIGURE 1B

|                                                                         |      |      |      |      |      |
|-------------------------------------------------------------------------|------|------|------|------|------|
| 766                                                                     | 775  | 784  | 793  | 802  | 811  |
| ATG TCA GCT GAG TAC AGT TTT CCC ATA TGG AAG ACC AGC CAC ACT GTC AAG TGG |      |      |      |      |      |
| M S A E Y S F P I W K T S H T V K W                                     |      |      |      |      |      |
| 820                                                                     | 829  | 838  | 847  | 856  | 865  |
| GAA GGC GTA TGG CGA GAA CTG GGC TGC CTC TCA AGG ACG GCG TCA TTT GCT GTT |      |      |      |      |      |
| E G V W R E L G C L S R T A S F A V                                     |      |      |      |      |      |
| 874                                                                     | 883  | 892  | 901  | 910  | 919  |
| CGA AAA GAA AGC GGA CAT TCA CTG AAA TCA TCT CTT TCG CAC GCC ATG GTC ATC |      |      |      |      |      |
| R K E S G H S L K S S L S H A M V I                                     |      |      |      |      |      |
| 928                                                                     | 937  | 946  | 955  | 964  | 973  |
| GAT TCT CGG AAT TCT TCC ATC TTA CCA AGG AGA GGT GCT TTG CTG AAA GTT AAC |      |      |      |      |      |
| D S R N S S I L P R R G A L L K V N                                     |      |      |      |      |      |
| 982                                                                     | 991  | 1000 | 1009 | 1018 | 1027 |
| CAG GAA CTG GCA GGC TAC ACT GGC GGG GAT GTG AGC TTC ATC AAA GAA GAT TTT |      |      |      |      |      |
| Q E L A G Y T G G D V S F I K E D F                                     |      |      |      |      |      |
| 1036                                                                    | 1045 | 1054 | 1063 | 1072 | 1081 |
| GAA CTT CAG TTG AAC AAG CAA CTC ATA TTT GAT TCA GTT TTT TCA GCG TCT TTC |      |      |      |      |      |
| E L Q L N K Q L I F D S V F S A S F                                     |      |      |      |      |      |
| 1090                                                                    | 1099 | 1108 | 1117 | 1126 | 1135 |
| TGG GGC GGA ATG TTG GTA CCC ATT GGT GAT AAG CCG TCA AGC ATT GCT GAT AGG |      |      |      |      |      |
| W G G M L V P I G D K P S S I A D R                                     |      |      |      |      |      |

FIGURE 1C

|                                                                         |      |      |      |      |      |
|-------------------------------------------------------------------------|------|------|------|------|------|
| 1144                                                                    | 1153 | 1162 | 1171 | 1180 | 1189 |
| TTT TAC CTT GGG GGA CCC ACA AGC GTC CGC GGA TTC AGC ATG CAC AGC ATC GGG |      |      |      |      |      |
| F Y L G G P T S V R G F S M H S I G                                     |      |      |      |      |      |
| 1198                                                                    | 1207 | 1216 | 1225 | 1234 | 1243 |
| CCA CAG AGC GAA GGA GAC TAC CTA GGT GGA GAA GCG TAC TGG GCC GGC GGC CTG |      |      |      |      |      |
| P Q S E G D Y L G G E A Y W A G G L                                     |      |      |      |      |      |
| 1252                                                                    | 1261 | 1270 | 1279 | 1288 | 1297 |
| CAC CTC TAC ACC CCA TTA CCT TTC CGG CCA GGC CAG GGT GGC TTT GGA GAA CTT |      |      |      |      |      |
| H L Y T P L P F R P P G Q G G F G E L                                   |      |      |      |      |      |
| 1306                                                                    | 1315 | 1324 | 1333 | 1342 | 1351 |
| TTC CGA ACA CAC TTC TTT CTC AAC GCA GGA AAC CTC TGC AAC CTC AAC TAT GGG |      |      |      |      |      |
| F R T H F F L N A G N L C N L N Y G                                     |      |      |      |      |      |
| 1360                                                                    | 1369 | 1378 | 1387 | 1396 | 1405 |
| GAG GGC CCC AAA GCT CAT ATT CGT AAG CTG GCT GAG TGC ATC CGC TGG TCG TAC |      |      |      |      |      |
| E G P K A H I R K L A E C I R W S Y                                     |      |      |      |      |      |
| 1414                                                                    | 1423 | 1432 | 1441 | 1450 | 1459 |
| GGG GCC GGG ATT GTC CTC AGG CTT GGC AAC ATC GCT CGG TTG GAA CTT AAT TAC |      |      |      |      |      |
| G A G I V L R L G N I A R L E L N Y                                     |      |      |      |      |      |
| 1468                                                                    | 1477 | 1486 | 1495 | 1504 | 1513 |
| TGC GTC CCC ATG GGA GTA CAG ACA GGC GAC AGG ATA TGT GAT GGC GTC CAG TTT |      |      |      |      |      |
| C V P M G V Q T G D R I C D G V Q F                                     |      |      |      |      |      |

FIGURE 1D

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|                                                                         |      |      |      |      |      |
|-------------------------------------------------------------------------|------|------|------|------|------|
| 1522                                                                    | 1531 | 1540 | 1549 | 1558 | 1567 |
| GGA GCT GGG ATA AGG TTC CTG TAG CCG ACA CCC CTA CAG GAG AAG CTC TGG GAC |      |      |      |      |      |
| G A G I R F L                                                           |      |      |      |      |      |
| 1576                                                                    | 1585 | 1594 | 1603 | 1612 | 1621 |
| TGG GGC AGC AGC AAG GCG CCC ATG CCA CAC ACC GTC TCT CGA GGA AAC GCG GTT |      |      |      |      |      |
| 1630                                                                    | 1639 | 1648 | 1657 | 1666 | 1675 |
| CAG CGA TTC TTT GAC TGC GGA CCC TGT GGG AAA CCC CGT CAA TAA ATG TTA AAG |      |      |      |      |      |
| 1684                                                                    | 1693 | 1702 | 1711 | 1720 | 1729 |
| ACA CAC TCA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA         |      |      |      |      |      |
| 1738                                                                    | 1747 | 1756 | 1765 | 1774 | 1783 |
| AAA AAA AAA AAA AAA AAA AAA AGG GGC GGC CGC CGA CTA GTG ACC TCT TCG     |      |      |      |      |      |
| CCC                                                                     |      |      |      |      |      |

FIGURE 1E