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 (54) Title: PROTEIN PHOSPHATASES

(57) **Abrégé/Abstract:**

The invention provides human protein phosphatases (PP) and polynucleotides which identify and encode PP. 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 aberrant expression of PP.

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(54) Title: PROTEIN PHOSPHATASES

(57) Abstract: The invention provides human protein phosphatases (PP) and polynucleotides which identify and encode PP. 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 aberrant expression of PP.



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PROTEIN PHOSPHATASES

TECHNICAL FIELD

This invention relates to nucleic acid and amino acid sequences of protein phosphatases and to
5 the use of these sequences in the diagnosis, treatment, and prevention of immune system disorders,
neurological disorders, developmental disorders, and cell proliferative disorders, including cancer, and
in the assessment of the effects of exogenous compounds on the expression of nucleic acid and amino
acid sequences of protein phosphatases.

10 BACKGROUND OF THE INVENTION

Reversible protein phosphorylation is the ubiquitous strategy used to control many of the
intracellular events in eukaryotic cells. It is estimated that more than ten percent of proteins active in a
typical mammalian cell are phosphorylated. Kinases catalyze the transfer of high-energy phosphate
groups from adenosine triphosphate (ATP) to target proteins on the hydroxyamino acid residues serine,
15 threonine, or tyrosine. Phosphatases, in contrast, remove these phosphate groups. Extracellular signals
including hormones, neurotransmitters, and growth and differentiation factors can activate kinases,
which can occur as cell surface receptors or as the activator of the final effector protein, but can also
occur along the signal transduction pathway. Cascades of kinases occur, as well as kinases sensitive to
second messenger molecules. This system allows for the amplification of weak signals (low abundance
20 growth factor molecules, for example), as well as the synthesis of many weak signals into an all-or-
nothing response. Phosphatases, then, are essential in determining the extent of phosphorylation in the
cell and, together with kinases, regulate key cellular processes such as metabolic enzyme activity,
proliferation, cell growth and differentiation, cell adhesion, and cell cycle progression.

Protein phosphatases are generally characterized as either serine/threonine- or tyrosine-specific
25 based on their preferred phospho-amino acid substrate. However, some phosphatases (DSPs, for dual
specificity phosphatases) can act on phosphorylated tyrosine, serine, or threonine residues. The protein
serine/threonine phosphatases (PSPs) are important regulators of many cAMP-mediated hormone
responses in cells. Protein tyrosine phosphatases (PTPs) play a significant role in cell cycle and cell
signaling processes. Another family of phosphatases is the acid phosphatase or histidine acid
30 phosphatase (HAP) family whose members hydrolyze phosphate esters at acidic pH conditions.

PSPs are found in the cytosol, nucleus, and mitochondria and in association with cytoskeletal
and membranous structures in most tissues, especially the brain. Some PSPs require divalent cations,
such as Ca^{2+} or Mn^{2+} , for activity. PSPs play important roles in glycogen metabolism, muscle
contraction, protein synthesis, T cell function, neuronal activity, oocyte maturation, and hepatic

metabolism (reviewed in Cohen, P. (1989) *Annu. Rev. Biochem.* 58:453-508). PSPs can be separated into two classes. The PPP class includes PP1, PP2A, PP2B/calcineurin, PP4, PP5, PP6, and PP7. Members of this class are composed of a homologous catalytic subunit bearing a very highly conserved signature sequence, coupled with one or more regulatory subunits (PROSITE PDOC00115). Further interactions with scaffold and anchoring molecules determine the intracellular localization of PSPs and substrate specificity. The PPM class consists of several closely related isoforms of PP2C and is evolutionarily unrelated to the PPP class.

PP1 dephosphorylates many of the proteins phosphorylated by cyclic AMP-dependent protein kinase (PKA) and is an important regulator of many cAMP-mediated hormone responses in cells. A number of isoforms have been identified, with the alpha and beta forms being produced by alternative splicing of the same gene. Both ubiquitous and tissue-specific targeting proteins for PP1 have been identified. In the brain, inhibition of PP1 activity by the dopamine and adenosine 3',5'-monophosphate-regulated phosphoprotein of 32kDa (DARPP-32) is necessary for normal dopamine response in neostriatal neurons (reviewed in Price, N.E. and M.C. Mumby (1999) *Curr. Opin. Neurobiol.* 9:336-342). PP1, along with PP2A, has been shown to limit motility in microvascular endothelial cells, suggesting a role for PSPs in the inhibition of angiogenesis (Gabel, S. et al. (1999) *Otolaryngol. Head Neck Surg.* 121:463-468).

PP2A is the main serine/threonine phosphatase. The core PP2A enzyme consists of a single 36 kDa catalytic subunit (C) associated with a 65 kDa scaffold subunit (A), whose role is to recruit additional regulatory subunits (B). Three gene families encoding B subunits are known (PR55, PR61, and PR72), each of which contain multiple-isoforms, and additional families may exist (Millward, T.A et al. (1999) *Trends Biosci.* 24:186-191). These "B-type" subunits are cell type- and tissue-specific and determine the substrate specificity, enzymatic activity, and subcellular localization of the holoenzyme. The PR55 family is highly conserved and bears a conserved motif (PROSITE PDOC00785). PR55 increases PP2A activity toward mitogen-activated protein kinase (MAPK) and MAPK kinase (MEK). PP2A dephosphorylates the MAPK active site, inhibiting the cell's entry into mitosis. Several proteins can compete with PR55 for PP2A core enzyme binding, including the CKII kinase catalytic subunit, polyomavirus middle and small T antigens, and SV40 small t antigen. Viruses may use this mechanism to commandeer PP2A and stimulate progression of the cell through the cell cycle (Pallas, D.C. et al. (1992) *J. Virol.* 66:886-893). Altered MAP kinase expression is also implicated in a variety of disease conditions including cancer, inflammation, immune disorders, and disorders affecting growth and development. PP2A, in fact, can dephosphorylate and modulate the activities of more than 30 protein kinases *in vitro*, and other evidence suggests that the same is true *in vivo* for such kinases as PKB, PKC, the calmodulin-dependent kinases, ERK family MAP kinases,

cyclin-dependent kinases, and the I κ B kinases (reviewed in Millward et al., supra). PP2A is itself a substrate for CKI and CKII kinases, and can be stimulated by polycationic macromolecules. A PP2A-like phosphatase is necessary to maintain the G1 phase destruction of mammalian cyclins A and B (Bastians, H. et al. (1999) Mol. Biol. Cell 10:3927-3941). PP2A is a major activity in the brain and is
5 implicated in regulating neurofilament stability and normal neural function, particularly the phosphorylation of the microtubule-associated protein tau. Hyperphosphorylation of tau has been proposed to lead to the neuronal degeneration seen in Alzheimer's disease (reviewed in Price and Mumby, supra).

PP2B, or calcineurin, is a Ca²⁺-activated dimeric phosphatase and is particularly abundant in
10 the brain. It consists of catalytic and regulatory subunits, and is activated by the binding of the calcium/calmodulin complex. Calcineurin is the target of the immunosuppressant drugs cyclosporine and FK506. Along with other cellular factors, these drugs interact with calcineurin and inhibit phosphatase activity. In T cells, this blocks the calcium dependent activation of the NF-AT family of transcription factors, leading to immunosuppression. This family is widely distributed, and it is likely
15 that calcineurin regulates gene expression in other tissues as well. In neurons, calcineurin modulates functions which range from the inhibition of neurotransmitter release to desensitization of postsynaptic NMDA-receptor coupled calcium channels to long term memory (reviewed in Price and Mumby, supra).

Other members of the PPP class have recently been identified (Cohen, P.T. (1997) Trends
20 Biochem. Sci. 22:245-251). One of them, PP5, contains regulatory domains with tetratricopeptide repeats. It can be activated by polyunsaturated fatty acids and anionic phospholipids in vitro and appears to be involved in a number of signaling pathways, including those controlled by atrial natriuretic peptide or steroid hormones (reviewed in Andreeva, A.V. and M.A. Kutuzov (1999) Cell Signal. 11:555-562).

25 PP2C is a ~42kDa monomer with broad substrate specificity and is dependent on divalent cations (mainly Mn²⁺ or Mg²⁺) for its activity. PP2C proteins share a conserved N-terminal region with an invariant DGH motif, which contains an aspartate residue involved in cation binding (PROSITE PDOC00792). Targeting proteins and mechanisms regulating PP2C activity have not been identified. PP2C has been shown to inhibit the stress-responsive p38 and Jun kinase (JNK) pathways (Takekawa,
30 M. et al. (1998) EMBO J. 17:4744-4752).

In contrast to PSPs, tyrosine-specific phosphatases (PTPs) are generally monomeric proteins of very diverse size (from 20kDa to greater than 100kDa) and structure that function primarily in the transduction of signals across the plasma membrane. PTPs are categorized as either soluble phosphatases or transmembrane receptor proteins that contain a phosphatase domain. All PTPs share a

conserved catalytic domain of about 300 amino acids which contains the active site. The active site consensus sequence includes a cysteine residue which executes a nucleophilic attack on the phosphate moiety during catalysis (Neel, B.G. and N.K. Tonks (1997) *Curr. Opin. Cell Biol.* 9:193-204).

Receptor PTPs are made up of an N-terminal extracellular domain of variable length, a transmembrane region, and a cytoplasmic region that generally contains two copies of the catalytic domain. Although
5 only the first copy seems to have enzymatic activity, the second copy apparently affects the substrate specificity of the first. The extracellular domains of some receptor PTPs contain fibronectin-like repeats, immunoglobulin-like domains, MAM domains (an extracellular motif likely to have an adhesive function), or carbonic anhydrase-like domains (PROSITE PDOC 00323). This wide variety of
10 structural motifs accounts for the diversity in size and specificity of PTPs.

PTPs play important roles in biological processes such as cell adhesion, lymphocyte activation, and cell proliferation. PTPs μ and κ are involved in cell-cell contacts, perhaps regulating cadherin/catenin function. A number of PTPs affect cell spreading, focal adhesions, and cell motility, most of them via the integrin/tyrosine kinase signaling pathway (reviewed in Neel and Tonks, *supra*).
15 CD45 phosphatases regulate signal transduction and lymphocyte activation (Ledbetter, J.A. et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:8628-8632). Soluble PTPs containing Src-homology-2 domains have been identified (SHPs), suggesting that these molecules might interact with receptor tyrosine kinases. SHP-1 regulates cytokine receptor signaling by controlling the Janus family PTKs in hematopoietic cells, as well as signaling by the T-cell receptor and c-Kit (reviewed in Neel and Tonks,
20 *supra*). M-phase inducer phosphatase plays a key role in the induction of mitosis by dephosphorylating and activating the PTK CDC2, leading to cell division (Sadhu, K. et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:5139-5143). In addition, the genes encoding at least eight PTPs have been mapped to chromosomal regions that are translocated or rearranged in various neoplastic conditions, including lymphoma, small cell lung carcinoma, leukemia, adenocarcinoma, and neuroblastoma (reviewed in
25 Charbonneau, H. and N.K. Tonks (1992) *Annu. Rev. Cell Biol.* 8:463-493). The PTP enzyme active site comprises the consensus sequence of the MTM1 gene family. The MTM1 gene is responsible for X-linked recessive myotubular myopathy, a congenital muscle disorder that has been linked to Xq28 (Kioschis, P. et al., (1998) *Genomics* 54:256-266. Many PTKs are encoded by oncogenes, and it is well known that oncogenesis is often accompanied by increased tyrosine phosphorylation activity. It is
30 therefore possible that PTPs may serve to prevent or reverse cell transformation and the growth of various cancers by controlling the levels of tyrosine phosphorylation in cells. This is supported by studies showing that overexpression of PTP can suppress transformation in cells and that specific inhibition of PTP can enhance cell transformation (Charbonneau and Tonks, *supra*).

Dual specificity phosphatases (DSPs) are structurally more similar to the PTPs than the PSPs.

DSPs bear an extended PTP active site motif with an additional 7 amino acid residues. DSPs are primarily associated with cell proliferation and include the cell cycle regulators cdc25A, B, and C. The phosphatases DUSP1 and DUSP2 inactivate the MAPK family members ERK (extracellular signal-regulated kinase), JNK (c-Jun N-terminal kinase), and p38 on both tyrosine and threonine residues
5 (PROSITE PDOC 00323, *supra*). In the activated state, these kinases have been implicated in neuronal differentiation, proliferation, oncogenic transformation, platelet aggregation, and apoptosis. Thus, DSPs are necessary for proper regulation of these processes (Muda, M. et al. (1996) *J. Biol. Chem.* 271:27205-27208). The tumor suppressor PTEN is a DSP that also shows lipid phosphatase activity. It seems to negatively regulate interactions with the extracellular matrix and maintains sensitivity to
10 apoptosis. PTEN has been implicated in the prevention of angiogenesis (Giri, D. and M. Ittmann (1999) *Hum. Pathol.* 30:419-424) and abnormalities in its expression are associated with numerous cancers (reviewed in Tamura, M. et al. (1999) *J. Natl. Cancer Inst.* 91:1820-1828).

Histidine acid phosphatase (HAP; EXPASY EC 3.1.3.2), also known as acid phosphatase, hydrolyzes a wide spectrum of substrates including alkyl, aryl, and acyl orthophosphate monoesters and
15 phosphorylated proteins at low pH. HAPs share two regions of conserved sequences, each centered around a histidine residue which is involved in catalytic activity. Members of the HAP family include lysosomal acid phosphatase (LAP) and prostatic acid phosphatase (PAP), both sensitive to inhibition by L-tartrate (PROSITE PDOC00538).

LAP, an orthophosphoric monoester of the endosomal/lysosomal compartment is a
20 housekeeping gene whose enzymatic activity has been detected in all tissues examined (Geier, C. et al. (1989) *Eur. J. Biochem.* 183:611-616). LAP-deficient mice have progressive skeletal disorder and an increased disposition toward generalized seizures (Saftig, P. et al. (1997) *J. Biol. Chem.* 272:18628-18635). LAP-deficient patients were found to have the following clinical features: intermittent vomiting, hypotonia, lethargy, opisthotonos, terminal bleeding, seizures, and death in early
25 infancy (Online Mendelian Inheritance in Man (OMIM) *200950).

PAP, a prostate epithelium-specific differentiation antigen produced by the prostate gland, has been used to diagnose and stage prostate cancer. In prostate carcinomas, the enzymatic activity of PAP was shown to be decreased compared with normal or benign prostate hypertrophy cells (Foti, A.G. et al. (1977) *Cancer Res.* 37:4120-4124). Two forms of PAP have been identified, secreted and intracellular.
30 Mature secreted PAP is detected in the seminal fluid and is active as a glycosylated homodimer with a molecular weight of approximately 100-kilodalton. Intracellular PAP is found to exhibit endogenous phosphotyrosyl protein phosphatase activity and is involved in regulating prostate cell growth (Meng, T.C. and M.F. Lin (1998) *J. Biol. Chem.* 34:22096-22104).

Synaptojanin, a polyphosphoinositide phosphatase, dephosphorylates phosphoinositides at

positions 3, 4 and 5 of the inositol ring. Synaptojanin is a major presynaptic protein found at clathrin-coated endocytic intermediates in nerve terminals, and binds the clathrin coat-associated protein, EPS15, which is mediated by the C-terminal region of synaptojanin-170, which has 3 Asp-Pro-Phe amino acid repeats. Further, this 3 residue repeat had been found to be the binding site for the EH domains of EPS15 (Haffner, C. et al. (1997) FEBS Lett. 419:175-180). Additionally, synaptojanin may potentially regulate interactions of endocytic proteins with the plasma membrane, and be involved in synaptic vesicle recycling (Brodin, L. et al. (2000) Curr. Opin. Neurobiol. 10:312-320). Studies in mice with a targeted disruption in the synaptojanin 1 gene (Synj1) were shown to support coat formation of endocytic vesicles more effectively than was seen in wild-type mice, suggesting that Synj1 can act as a negative regulator of membrane-coat protein interactions. These findings provide genetic evidence for a crucial role of phosphoinositide metabolism in synaptic vesicle recycling (Cremona, O. et al. (1999) Cell 99:179-188).

The discovery of new protein phosphatases and the polynucleotides encoding them satisfies a need in the art by providing new compositions which are useful in the diagnosis, prevention, and treatment of immune system disorders, neurological disorders, developmental disorders, and cell proliferative disorders, including cancer, and in the assessment of the effects of exogenous compounds on the expression of nucleic acid and amino acid sequences of protein phosphatases.

SUMMARY OF THE INVENTION

The invention features purified polypeptides, protein phosphatases, referred to collectively as "PP" and individually as "PP-1," "PP-2," "PP-3," "PP-4," "PP-5," "PP-6," "PP-7," "PP-8," and "PP-9." In one aspect, the invention provides an isolated polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-9. In one alternative, the invention provides an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:1-9.

The invention further provides an isolated polynucleotide encoding a polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the

group consisting of SEQ ID NO:1-9, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-9. In one alternative, the polynucleotide encodes a polypeptide selected from the group consisting of SEQ ID NO:1-9. In another alternative, the polynucleotide is selected from the group consisting of SEQ ID NO:10-18.

5 Additionally, the invention provides a recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide encoding a polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, c) a biologically
10 active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-9. In one alternative, the invention provides a cell transformed with the recombinant polynucleotide. In another alternative, the invention provides a transgenic organism comprising the recombinant polynucleotide.

15 The invention also provides a method for producing a polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group
20 consisting of SEQ ID NO:1-9, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-9. The method comprises a) culturing a cell under conditions suitable for expression of the polypeptide, wherein said cell is transformed with a recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide encoding the polypeptide, and b) recovering the polypeptide so expressed.

25 Additionally, the invention provides an isolated antibody which specifically binds to a polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, c) a biologically active fragment of a polypeptide having an amino acid
30 sequence selected from the group consisting of SEQ ID NO:1-9, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-9.

 The invention further provides an isolated polynucleotide selected from the group consisting of a) a polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:10-18, b) a polynucleotide comprising a naturally occurring polynucleotide sequence at least 90%

identical to a polynucleotide sequence selected from the group consisting of SEQ ID NO:10-18, c) a polynucleotide complementary to the polynucleotide of a), d) a polynucleotide complementary to the polynucleotide of b), and e) an RNA equivalent of a)-d). In one alternative, the polynucleotide comprises at least 60 contiguous nucleotides.

5 Additionally, the invention provides a method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide selected from the group consisting of a) a polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:10-18, b) a polynucleotide comprising a naturally occurring polynucleotide sequence at least 90% identical to a polynucleotide sequence selected from the group consisting of SEQ ID NO:10-18, c) a
10 polynucleotide complementary to the polynucleotide of a), d) a polynucleotide complementary to the polynucleotide of b), and e) an RNA equivalent of a)-d). The method comprises a) hybridizing the sample with a probe comprising at least 20 contiguous nucleotides comprising a sequence complementary to said target polynucleotide in the sample, and which probe specifically hybridizes to said target polynucleotide, under conditions whereby a hybridization complex is formed between said
15 probe and said target polynucleotide or fragments thereof, and b) detecting the presence or absence of said hybridization complex, and optionally, if present, the amount thereof. In one alternative, the probe comprises at least 60 contiguous nucleotides.

The invention further provides a method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide selected from the group consisting of a) a
20 polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:10-18, b) a polynucleotide comprising a naturally occurring polynucleotide sequence at least 90% identical to a polynucleotide sequence selected from the group consisting of SEQ ID NO:10-18, c) a polynucleotide complementary to the polynucleotide of a), d) a polynucleotide complementary to the polynucleotide of b), and e) an RNA equivalent of a)-d). The method comprises a) amplifying said
25 target polynucleotide or fragment thereof using polymerase chain reaction amplification, and b) detecting the presence or absence of said amplified target polynucleotide or fragment thereof, and, optionally, if present, the amount thereof.

The invention further provides a composition comprising an effective amount of a polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from
30 the group consisting of SEQ ID NO:1-9, b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, and a pharmaceutically

acceptable excipient. In one embodiment, the composition comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1-9. The invention additionally provides a method of treating a disease or condition associated with decreased expression of functional PP, comprising administering to a patient in need of such treatment the composition.

5 The invention also provides a method for screening a compound for effectiveness as an agonist of a polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-9. The method comprises a) exposing a sample comprising the polypeptide to a compound, and b) detecting agonist activity in the sample. In one alternative, the invention provides a composition comprising an agonist compound identified by the method and a pharmaceutically acceptable excipient. In another alternative, the invention provides a method of treating a disease or condition associated with decreased expression of functional PP, comprising administering to a patient in need of such treatment the composition.

 Additionally, the invention provides a method for screening a compound for effectiveness as an antagonist of a polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-9. The method comprises a) exposing a sample comprising the polypeptide to a compound, and b) detecting antagonist activity in the sample. In one alternative, the invention provides a composition comprising an antagonist compound identified by the method and a pharmaceutically acceptable excipient. In another alternative, the invention provides a method of treating a disease or condition associated with overexpression of functional PP, comprising administering to a patient in need of such treatment the composition.

 The invention further provides a method of screening for a compound that specifically binds to a polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from

the group consisting of SEQ ID NO:1-9, c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-9. The method comprises a) combining the polypeptide with at least one test compound under suitable conditions, and b) detecting binding of the polypeptide to the test compound, thereby identifying a compound that specifically binds to the polypeptide.

The invention further provides a method of screening for a compound that modulates the activity of a polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-9. The method comprises a) combining the polypeptide with at least one test compound under conditions permissive for the activity of the polypeptide, b) assessing the activity of the polypeptide in the presence of the test compound, and c) comparing the activity of the polypeptide in the presence of the test compound with the activity of the polypeptide in the absence of the test compound, wherein a change in the activity of the polypeptide in the presence of the test compound is indicative of a compound that modulates the activity of the polypeptide.

The invention further provides a method for screening a compound for effectiveness in altering expression of a target polynucleotide, wherein said target polynucleotide comprises a sequence selected from the group consisting of SEQ ID NO:10-18, the method comprising a) exposing a sample comprising the target polynucleotide to a compound, and b) detecting altered expression of the target polynucleotide.

The invention further provides a method for assessing toxicity of a test compound, said method comprising a) treating a biological sample containing nucleic acids with the test compound; b) hybridizing the nucleic acids of the treated biological sample with a probe comprising at least 20 contiguous nucleotides of a polynucleotide selected from the group consisting of i) a polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:10-18, ii) a polynucleotide comprising a naturally occurring polynucleotide sequence at least 90% identical to a polynucleotide sequence selected from the group consisting of SEQ ID NO:10-18, iii) a polynucleotide having a sequence complementary to i), iv) a polynucleotide complementary to the polynucleotide of ii), and v) an RNA equivalent of i)-iv). Hybridization occurs under conditions whereby a specific hybridization complex is formed between said probe and a target polynucleotide in

the biological sample, said target polynucleotide selected from the group consisting of i) a polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:10-18, ii) a polynucleotide comprising a naturally occurring polynucleotide sequence at least 90% identical to a polynucleotide sequence selected from the group consisting of SEQ ID NO:10-18, iii) a polynucleotide complementary to the polynucleotide of i), iv) a polynucleotide complementary to the polynucleotide of ii), and v) an RNA equivalent of i)-iv). Alternatively, the target polynucleotide comprises a fragment of a polynucleotide sequence selected from the group consisting of i)-v) above; c) quantifying the amount of hybridization complex; and d) comparing the amount of hybridization complex in the treated biological sample with the amount of hybridization complex in an untreated biological sample, wherein a difference in the amount of hybridization complex in the treated biological sample is indicative of toxicity of the test compound.

BRIEF DESCRIPTION OF THE TABLES

Table 1 summarizes the nomenclature for the full length polynucleotide and polypeptide sequences of the present invention.

Table 2 shows the GenBank identification number and annotation of the nearest GenBank homolog for polypeptides of the invention. The probability score for the match between each polypeptide and its GenBank homolog is also shown.

Table 3 shows structural features of polypeptide sequences of the invention, including predicted motifs and domains, along with the methods, algorithms, and searchable databases used for analysis of the polypeptides.

Table 4 lists the cDNA and/or genomic DNA fragments which were used to assemble polynucleotide sequences of the invention, along with selected fragments of the polynucleotide sequences.

Table 5 shows the representative cDNA library for polynucleotides of the invention.

Table 6 provides an appendix which describes the tissues and vectors used for construction of the cDNA libraries shown in Table 5.

Table 7 shows the tools, programs, and algorithms used to analyze the polynucleotides and polypeptides of the invention, along with applicable descriptions, references, and threshold parameters.

DESCRIPTION OF THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular machines, materials and methods described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing

particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural reference unless the context clearly dictates otherwise. Thus, for example, a
5 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
10 any machines, materials, and methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred machines, materials and methods are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, protocols, reagents and vectors 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
15 not entitled to antedate such disclosure by virtue of prior invention.

DEFINITIONS

“PP” refers to the amino acid sequences of substantially purified PP obtained from any species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and human, and from any source, whether natural, synthetic, semi-synthetic, or recombinant.

20 The term “agonist” refers to a molecule which intensifies or mimics the biological activity of PP. Agonists may include proteins, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of PP either by directly interacting with PP or by acting on components of the biological pathway in which PP participates.

An “allelic variant” is an alternative form of the gene encoding PP. Allelic variants may result
25 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. A gene may have none, one, or many allelic variants of its naturally occurring form. 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
30 a given sequence.

“Altered” nucleic acid sequences encoding PP include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polypeptide the same as PP or a polypeptide with at least one functional characteristic of PP. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of

the polynucleotide encoding PP, and improper or unexpected hybridization to allelic variants, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding PP. 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 PP. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or immunological activity of PP is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid, and positively charged amino acids may include lysine and arginine. Amino acids with uncharged polar side chains having similar hydrophilicity values may include: asparagine and glutamine; and serine and threonine. Amino acids with uncharged side chains having similar hydrophilicity values may include: leucine, isoleucine, and valine; glycine and alanine; and phenylalanine and tyrosine.

The terms "amino acid" and "amino acid sequence" refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. Where "amino acid sequence" is recited to refer to a sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

"Amplification" 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.

The term "antagonist" refers to a molecule which inhibits or attenuates the biological activity of PP. Antagonists may include proteins such as antibodies, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of PP either by directly interacting with PP or by acting on components of the biological pathway in which PP participates.

The term "antibody" refers to intact immunoglobulin molecules as well as to fragments thereof, such as Fab, F(ab')₂, and Fv fragments, which are capable of binding an epitopic determinant. Antibodies that bind PP polypeptides can be prepared using 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 keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

The term "antigenic determinant" refers to that region 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 protein may induce the production of antibodies which bind specifically to antigenic determinants (particular 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.

5 The term “antisense” refers to any composition capable of base-pairing with the “sense” (coding) strand of a specific nucleic acid sequence. Antisense compositions may include DNA; RNA; peptide nucleic acid (PNA); oligonucleotides having modified backbone linkages such as phosphorothioates, methylphosphonates, or benzylphosphonates; oligonucleotides having modified sugar groups such as 2'-methoxyethyl sugars or 2'-methoxyethoxy sugars; or oligonucleotides having
10 modified bases such as 5-methyl cytosine, 2'-deoxyuracil, or 7-deaza-2'-deoxyguanosine. Antisense molecules may be produced by any method including chemical synthesis or transcription. Once introduced into a cell, the complementary antisense molecule base-pairs with a naturally occurring nucleic acid sequence produced by the cell to form duplexes which block either transcription or translation. The designation “negative” or “minus” can refer to the antisense strand, and the
15 designation “positive” or “plus” can refer to the sense strand of a reference DNA molecule.

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

20 “Complementary” describes the relationship between two single-stranded nucleic acid sequences that anneal by base-pairing. For example, 5'-AGT-3' pairs with its complement, 3'-TCA-5'.

A “composition comprising a given polynucleotide sequence” and a “composition comprising a given amino acid sequence” refer broadly to any composition containing the given polynucleotide or
25 amino acid sequence. The composition may comprise a dry formulation or an aqueous solution. Compositions comprising polynucleotide sequences encoding PP or fragments of PP may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts (e.g., NaCl), detergents (e.g., sodium dodecyl sulfate; SDS), and other
30 components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.).

“Consensus sequence” refers to a nucleic acid sequence which has been subjected to repeated DNA sequence analysis to resolve uncalled bases, extended using the XL-PCR kit (Applied Biosystems, Foster City CA) in the 5' and/or the 3' direction, and resequenced, or which has been assembled from one or more overlapping cDNA, EST, or genomic DNA fragments using a computer program for

fragment assembly, such as the GELVIEW fragment assembly system (GCG, Madison WI) or Phrap (University of Washington, Seattle WA). Some sequences have been both extended and assembled to produce the consensus sequence.

“Conservative amino acid substitutions” are those substitutions that are predicted to least
 5 interfere with the properties of the original protein, i.e., the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. The table below shows amino acids which may be substituted for an original amino acid in a protein and which are regarded as conservative amino acid substitutions.

	Original Residue	Conservative Substitution
10	Ala	Gly, Ser
	Arg	His, Lys
	Asn	Asp, Gln, His
	Asp	Asn, Glu
	Cys	Ala, Ser
15	Gln	Asn, Glu, His
	Glu	Asp, Gln, His
	Gly	Ala
	His	Asn, Arg, Gln, Glu
	Ile	Leu, Val
20	Leu	Ile, Val
	Lys	Arg, Gln, Glu
	Met	Leu, Ile
	Phe	His, Met, Leu, Trp, Tyr
	Ser	Cys, Thr
25	Thr	Ser, Val
	Trp	Phe, Tyr
	Tyr	His, Phe, Trp
	Val	Ile, Leu, Thr

30 Conservative amino acid substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a beta sheet or alpha helical conformation, (b) the charge or hydrophobicity of the molecule at the site of the substitution, and/or (c) the bulk of the side chain.

A “deletion” refers to a change in the amino acid or nucleotide sequence that results in the
 35 absence of one or more amino acid residues or nucleotides.

The term “derivative” refers to a chemically modified polynucleotide or polypeptide. Chemical modifications of a polynucleotide can include, for example, replacement of hydrogen by an alkyl, acyl, hydroxyl, 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
 40 by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

A "detectable label" refers to a reporter molecule or enzyme that is capable of generating a measurable signal and is covalently or noncovalently joined to a polynucleotide or polypeptide.

"Differential expression" refers to increased or upregulated; or decreased, downregulated, or absent gene or protein expression, determined by comparing at least two different samples. Such
5 comparisons may be carried out between, for example, a treated and an untreated sample, or a diseased and a normal sample.

A "fragment" is a unique portion of PP or the polynucleotide encoding PP which is identical in sequence to but shorter in length than the parent sequence. A fragment may comprise up to the entire length of the defined sequence, minus one nucleotide/amino acid residue. For example, a
10 fragment may comprise from 5 to 1000 contiguous nucleotides or amino acid residues. A fragment used as a probe, primer, antigen, therapeutic molecule, or for other purposes, may be at least 5, 10, 15, 16, 20, 25, 30, 40, 50, 60, 75, 100, 150, 250 or at least 500 contiguous nucleotides or amino acid residues in length. Fragments may be preferentially selected from certain regions of a molecule. For example, a polypeptide fragment may comprise a certain length of contiguous amino acids selected
15 from the first 250 or 500 amino acids (or first 25% or 50%) of a polypeptide as shown in a certain defined sequence. Clearly these lengths are exemplary, and any length that is supported by the specification, including the Sequence Listing, tables, and figures, may be encompassed by the present embodiments.

A fragment of SEQ ID NO:10-18 comprises a region of unique polynucleotide sequence that
20 specifically identifies SEQ ID NO:10-18, for example, as distinct from any other sequence in the genome from which the fragment was obtained. A fragment of SEQ ID NO:10-18 is useful, for example, in hybridization and amplification technologies and in analogous methods that distinguish SEQ ID NO:10-18 from related polynucleotide sequences. The precise length of a fragment of SEQ ID NO:10-18 and the region of SEQ ID NO:10-18 to which the fragment corresponds are routinely
25 determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

A fragment of SEQ ID NO:1-9 is encoded by a fragment of SEQ ID NO:10-18. A fragment of SEQ ID NO:1-9 comprises a region of unique amino acid sequence that specifically identifies SEQ ID NO:1-9. For example, a fragment of SEQ ID NO:1-9 is useful as an immunogenic peptide for the development of antibodies that specifically recognize SEQ ID NO:1-9. The precise length of a
30 fragment of SEQ ID NO:1-9 and the region of SEQ ID NO:1-9 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

A "full length" polynucleotide sequence is one containing at least a translation initiation codon (e.g., methionine) followed by an open reading frame and a translation termination codon. A "full

length” polynucleotide sequence encodes a “full length” polypeptide sequence.

“Homology” refers to sequence similarity or, interchangeably, sequence identity, between two or more polynucleotide sequences or two or more polypeptide sequences.

The terms “percent identity” and “% identity,” as applied to polynucleotide sequences, refer to
5 the percentage of residue matches between at least two polynucleotide sequences aligned using a
standardized algorithm. Such an algorithm may insert, in a standardized and reproducible way, gaps in
the sequences being compared in order to optimize alignment between two sequences, and therefore
achieve a more meaningful comparison of the two sequences.

Percent identity between polynucleotide sequences may be determined using the default
10 parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence
alignment program. This program is part of the LASERGENE software package, a suite of molecular
biological analysis programs (DNASTAR, Madison WI). CLUSTAL V is described in Higgins, D.G.
and P.M. Sharp (1989) CABIOS 5:151-153 and in Higgins, D.G. et al. (1992) CABIOS 8:189-191.
For pairwise alignments of polynucleotide sequences, the default parameters are set as follows:
15 Ktuple=2, gap penalty=5, window=4, and “diagonals saved”=4. The “weighted” residue weight table is
selected as the default. Percent identity is reported by CLUSTAL V as the “percent similarity” between
aligned polynucleotide sequences.

Alternatively, a suite of commonly used and freely available sequence comparison algorithms is
provided by the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search
20 Tool (BLAST) (Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410), which is available from several
sources, including the NCBI, Bethesda, MD, and on the Internet at
<http://www.ncbi.nlm.nih.gov/BLAST/>. The BLAST software suite includes various sequence analysis
programs including “blastn,” that is used to align a known polynucleotide sequence with other
polynucleotide sequences from a variety of databases. Also available is a tool called “BLAST 2
25 Sequences” that is used for direct pairwise comparison of two nucleotide sequences. “BLAST 2
Sequences” can be accessed and used interactively at <http://www.ncbi.nlm.nih.gov/gorf/bl2.html>. The
“BLAST 2 Sequences” tool can be used for both blastn and blastp (discussed below). BLAST
programs are commonly used with gap and other parameters set to default settings. For example, to
compare two nucleotide sequences, one may use blastn with the “BLAST 2 Sequences” tool Version
30 2.0.12 (April-21-2000) set at default parameters. Such default parameters may be, for example:

Matrix: BLOSUM62

Reward for match: 1

Penalty for mismatch: -2

Open Gap: 5 and Extension Gap: 2 penalties

Gap x drop-off: 50

Expect: 10

Word Size: 11

Filter: on

5 Percent identity may be measured over the length of an entire defined sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined sequence, for instance, a fragment of at least 20, at least 30, at least 40, at least 50, at least 70, at least 100, or at least 200 contiguous nucleotides. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences
10 shown herein, in the tables, figures, or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

Nucleic acid sequences that do not show a high degree of identity may nevertheless encode similar amino acid sequences due to the degeneracy of the genetic code. It is understood that changes in a nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid sequences
15 that all encode substantially the same protein.

The phrases “percent identity” and “% identity,” as applied to polypeptide sequences, refer to the percentage of residue matches between at least two polypeptide sequences aligned using a standardized algorithm. Methods of polypeptide sequence alignment are well-known. Some alignment methods take into account conservative amino acid substitutions. Such conservative substitutions,
20 explained in more detail above, generally preserve the charge and hydrophobicity at the site of substitution, thus preserving the structure (and therefore function) of the polypeptide.

Percent identity between polypeptide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program (described and referenced above). For pairwise alignments of polypeptide sequences using
25 CLUSTAL V, the default parameters are set as follows: Ktuple=1, gap penalty=3, window=5, and “diagonals saved”=5. The PAM250 matrix is selected as the default residue weight table. As with polynucleotide alignments, the percent identity is reported by CLUSTAL V as the “percent similarity” between aligned polypeptide sequence pairs.

Alternatively the NCBI BLAST software suite may be used. For example, for a pairwise
30 comparison of two polypeptide sequences, one may use the “BLAST 2 Sequences” tool Version 2.0.12 (April-21-2000) with blastp set at default parameters. Such default parameters may be, for example:

Matrix: BLOSUM62

Open Gap: 11 and Extension Gap: 1 penalties

Gap x drop-off: 50

Expect: 10

Word Size: 3

Filter: on

Percent identity may be measured over the length of an entire defined polypeptide sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polypeptide sequence, for instance, a fragment of at least 15, at least 20, at least 30, at least 40, at least 50, at least 70 or at least 150 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

“Human artificial chromosomes” (HACs) 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 chromosome replication, segregation and maintenance.

The term “humanized antibody” refers to an antibody molecule 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” refers to the process by which a polynucleotide strand anneals with a complementary strand through base pairing under defined hybridization conditions. Specific hybridization is an indication that two nucleic acid sequences share a high degree of complementarity. Specific hybridization complexes form under permissive annealing conditions and remain hybridized after the “washing” step(s). The washing step(s) is particularly important in determining the stringency of the hybridization process, with more stringent conditions allowing less non-specific binding, i.e., binding between pairs of nucleic acid strands that are not perfectly matched. Permissive conditions for annealing of nucleic acid sequences are routinely determinable by one of ordinary skill in the art and may be consistent among hybridization experiments, whereas wash conditions may be varied among experiments to achieve the desired stringency, and therefore hybridization specificity. Permissive annealing conditions occur, for example, at 68°C in the presence of about 6 x SSC, about 1% (w/v) SDS, and about 100 µg/ml sheared, denatured salmon sperm DNA.

Generally, stringency of hybridization is expressed, in part, with reference to the temperature under which the wash step is carried out. Such wash temperatures are typically selected to be about 5°C to 20°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. An equation for calculating T_m and conditions for nucleic acid hybridization are well known and can be found in Sambrook, J. et al. (1989) Molecular

Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; specifically see volume 2, chapter 9.

High stringency conditions for hybridization between polynucleotides of the present invention include wash conditions of 68°C in the presence of about 0.2 x SSC and about 0.1% SDS, for 1 hour. Alternatively, temperatures of about 65°C, 60°C, 55°C, or 42°C may be used. SSC concentration may be varied from about 0.1 to 2 x SSC, with SDS being present at about 0.1%. Typically, blocking reagents are used to block non-specific hybridization. Such blocking reagents include, for instance, sheared and denatured salmon sperm DNA at about 100-200 µg/ml. Organic solvent, such as formamide at a concentration of about 35-50% v/v, may also be used under particular circumstances, such as for RNA:DNA hybridizations. Useful variations on these wash conditions will be readily apparent to those of ordinary skill in the art. Hybridization, particularly under high stringency conditions, may be suggestive of evolutionary similarity between the nucleotides. Such similarity is strongly indicative of a similar role for the nucleotides and their encoded polypeptides.

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., C₀t or R₀t analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

The words "insertion" and "addition" refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively.

"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.

An "immunogenic fragment" is a polypeptide or oligopeptide fragment of PP which is capable of eliciting an immune response when introduced into a living organism, for example, a mammal. The term "immunogenic fragment" also includes any polypeptide or oligopeptide fragment of PP which is useful in any of the antibody production methods disclosed herein or known in the art.

The term "microarray" refers to an arrangement of a plurality of polynucleotides, polypeptides, or other chemical compounds on a substrate.

The terms "element" and "array element" refer to a polynucleotide, polypeptide, or other chemical compound having a unique and defined position on a microarray.

The term "modulate" refers to a change in the activity of PP. For example, modulation may

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

The phrases "nucleic acid" and "nucleic acid sequence" 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 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.

"Operably linked" refers to the situation in which a first nucleic acid sequence is placed in a functional relationship with a second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Operably linked DNA sequences may be in close proximity or contiguous and, where necessary to join two protein coding regions, in the same reading frame.

"Peptide nucleic acid" (PNA) 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 single stranded DNA or RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell.

"Post-translational modification" of an PP may involve lipidation, glycosylation, phosphorylation, acetylation, racemization, proteolytic cleavage, and other modifications known in the art. These processes may occur synthetically or biochemically. Biochemical modifications will vary by cell type depending on the enzymatic milieu of PP.

"Probe" refers to nucleic acid sequences encoding PP, their complements, or fragments thereof, which are used to detect identical, allelic or related nucleic acid sequences. Probes are isolated oligonucleotides or polynucleotides attached to a detectable label or reporter molecule. Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes. "Primers" are short nucleic acids, usually DNA oligonucleotides, which may be annealed to a target polynucleotide by complementary base-pairing. The primer may then be extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification (and identification) of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR).

Probes and primers as used in the present invention typically comprise at least 15 contiguous nucleotides of a known sequence. In order to enhance specificity, longer probes and primers may also be employed, such as probes and primers that comprise at least 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or at least 150 consecutive nucleotides of the disclosed nucleic acid sequences. Probes and primers may be considerably longer than these examples, and it is understood that any length supported by the specification, including the tables, figures, and Sequence Listing, may be used.

Methods for preparing and using probes and primers are described in the references, for example Sambrook, J. et al. (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; Ausubel, F.M. et al. (1987) Current Protocols in Molecular Biology, Greene Publ. Assoc. & Wiley-Intersciences, New York NY; Innis, M. et al. (1990) PCR Protocols, A Guide to Methods and Applications, Academic Press, San Diego CA. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge MA).

Oligonucleotides for use as primers are selected using software known in the art for such purpose. For example, OLIGO 4.06 software is useful for the selection of PCR primer pairs of up to 100 nucleotides each, and for the analysis of oligonucleotides and larger polynucleotides of up to 5,000 nucleotides from an input polynucleotide sequence of up to 32 kilobases. Similar primer selection programs have incorporated additional features for expanded capabilities. For example, the PrimOU primer selection program (available to the public from the Genome Center at University of Texas South West Medical Center, Dallas TX) is capable of choosing specific primers from megabase sequences and is thus useful for designing primers on a genome-wide scope. The Primer3 primer selection program (available to the public from the Whitehead Institute/MIT Center for Genome Research, Cambridge MA) allows the user to input a "mispriming library," in which sequences to avoid as primer binding sites are user-specified. Primer3 is useful, in particular, for the selection of oligonucleotides for microarrays. (The source code for the latter two primer selection programs may also be obtained from their respective sources and modified to meet the user's specific needs.) The PrimeGen program (available to the public from the UK Human Genome Mapping Project Resource Centre, Cambridge UK) designs primers based on multiple sequence alignments, thereby allowing selection of primers that hybridize to either the most conserved or least conserved regions of aligned nucleic acid sequences. Hence, this program is useful for identification of both unique and conserved oligonucleotides and polynucleotide fragments. The oligonucleotides and polynucleotide fragments identified by any of the above selection methods are useful in hybridization technologies, for example, as PCR or sequencing primers, microarray elements, or specific probes to identify fully or partially complementary polynucleotides in a sample of nucleic acids. Methods of oligonucleotide selection are not limited to those described above.

A "recombinant nucleic acid" is a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two or more otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques

such as those described in Sambrook, supra. The term recombinant includes nucleic acids that have been altered solely by addition, substitution, or deletion of a portion of the nucleic acid. Frequently, a recombinant nucleic acid may include a nucleic acid sequence operably linked to a promoter sequence. Such a recombinant nucleic acid may be part of a vector that is used, for example, to transform a cell.

5 Alternatively, such recombinant nucleic acids may be part of a viral vector, e.g., based on a vaccinia virus, that could be used to vaccinate a mammal wherein the recombinant nucleic acid is expressed, inducing a protective immunological response in the mammal.

A "regulatory element" refers to a nucleic acid sequence usually derived from untranslated regions of a gene and includes enhancers, promoters, introns, and 5' and 3' untranslated regions (UTRs).

10 Regulatory elements interact with host or viral proteins which control transcription, translation, or RNA stability.

"Reporter molecules" are chemical or biochemical moieties used for labeling a nucleic acid, amino acid, or antibody. Reporter molecules include radionuclides; enzymes; fluorescent, chemiluminescent, or chromogenic agents; substrates; cofactors; inhibitors; magnetic particles; and
15 other moieties known in the art.

An "RNA equivalent," in reference to a DNA sequence, is composed of the same linear sequence of nucleotides as the reference DNA sequence with the exception that all occurrences of the nitrogenous base thymine are replaced with uracil, and the sugar backbone is composed of ribose instead of deoxyribose.

20 The term "sample" is used in its broadest sense. A sample suspected of containing PP, nucleic acids encoding PP, or fragments thereof 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 substrate; a tissue; a tissue print; etc.

The terms "specific binding" and "specifically binding" refer to that interaction between a
25 protein or peptide and an agonist, an antibody, an antagonist, a small molecule, or any natural or synthetic binding composition. The 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 comprising the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will
30 reduce the amount of labeled A that binds to the antibody.

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

A "substitution" refers to the replacement of one or more amino acid residues or nucleotides by different amino acid residues or nucleotides, respectively.

"Substrate" refers to any suitable rigid or semi-rigid support including membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers, 5 microparticles and capillaries. The substrate can have a variety of surface forms, such as wells, trenches, pins, channels and pores, to which polynucleotides or polypeptides are bound.

A "transcript image" refers to the collective pattern of gene expression by a particular cell type or tissue under given conditions at a given time.

"Transformation" describes a process by which exogenous DNA is introduced into a recipient 10 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, bacteriophage or viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed cells" 15 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 "transgenic organism," as used herein, is any organism, including but not limited to animals and plants, in which one or more of the cells of the organism contains heterologous nucleic 20 acid introduced by way of human intervention, such as by transgenic techniques well known in the art. The nucleic acid is introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. The term genetic manipulation does not include classical cross-breeding, or in vitro fertilization, but rather is directed to the introduction of a recombinant DNA molecule. The 25 transgenic organisms contemplated in accordance with the present invention include bacteria, cyanobacteria, fungi, plants and animals. The isolated DNA of the present invention can be introduced into the host by methods known in the art, for example infection, transfection, transformation or transconjugation. Techniques for transferring the DNA of the present invention into such organisms are widely known and provided in references such as Sambrook et al. (1989), 30 supra.

A "variant" of a particular nucleic acid sequence is defined as a nucleic acid sequence having at least 40% sequence identity to the particular nucleic acid sequence over a certain length of one of the nucleic acid sequences using blastn with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of nucleic acids may show, for example, at least 50%, at least

60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% or greater sequence identity over a certain defined length. A variant may be described as, for example, an "allelic" (as defined above), "splice," "species," or "polymorphic" variant. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternative splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or lack domains that are present in the reference molecule. Species variants are polynucleotide sequences that vary from one species to another. The resulting polypeptides will generally have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs) in which the polynucleotide sequence varies by one nucleotide base. The presence of SNPs may be indicative of, for example, a certain population, a disease state, or a propensity for a disease state.

A "variant" of a particular polypeptide sequence is defined as a polypeptide sequence having at least 40% sequence identity to the particular polypeptide sequence over a certain length of one of the polypeptide sequences using blastp with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of polypeptides may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% or greater sequence identity over a certain defined length of one of the polypeptides.

THE INVENTION

The invention is based on the discovery of new human protein phosphatases (PP), the polynucleotides encoding PP, and the use of these compositions for the diagnosis, treatment, or prevention of immune system disorders, neurological disorders, developmental disorders, and cell proliferative disorders, including cancer.

Table 1 summarizes the nomenclature for the full length polynucleotide and polypeptide sequences of the invention. Each polynucleotide and its corresponding polypeptide are correlated to a single Incyte project identification number (Incyte Project ID). Each polypeptide sequence is denoted by both a polypeptide sequence identification number (Polypeptide SEQ ID NO:) and an Incyte polypeptide sequence number (Incyte Polypeptide ID) as shown. Each polynucleotide sequence is denoted by both a polynucleotide sequence identification number (Polynucleotide SEQ ID NO:) and an Incyte polynucleotide consensus sequence number (Incyte Polynucleotide ID) as shown.

Table 2 shows sequences with homology to the polypeptides of the invention as identified by

BLAST analysis against the GenBank protein (genpept) database. Columns 1 and 2 show the polypeptide sequence identification number (Polypeptide SEQ ID NO:) and the corresponding Incyte polypeptide sequence number (Incyte Polypeptide ID) for polypeptides of the invention. Column 3 shows the GenBank identification number (Genbank ID NO:) of the nearest GenBank homolog.

5 Column 4 shows the probability score for the match between each polypeptide and its GenBank homolog. Column 5 shows the annotation of the GenBank homolog.

Table 3 shows various structural features of the polypeptides of the invention. Columns 1 and 2 show the polypeptide sequence identification number (SEQ ID NO:) and the corresponding Incyte polypeptide sequence number (Incyte Polypeptide ID) for each polypeptide of the invention. Column 3
10 shows the number of amino acid residues in each polypeptide. Column 4 shows potential phosphorylation sites, and column 5 shows potential glycosylation sites, as determined by the MOTIFS program of the GCG sequence analysis software package (Genetics Computer Group, Madison WI). Column 6 shows amino acid residues comprising signature sequences, domains, and motifs. Column 7 shows analytical methods for protein structure/function analysis and in some cases, searchable
15 databases to which the analytical methods were applied.

Together, Tables 2 and 3 summarize the properties of each polypeptide of the invention, and these properties establish that the claimed polypeptides are protein phosphatases. For example, SEQ ID NO:1 is 38% identical to Arabidopsis thaliana protein phosphatase 2C (GenBank ID g1945140) as determined by the Basic Local Alignment Search Tool (BLAST). (See Table 2.) The BLAST
20 probability score is $1.1e-30$, which indicates the probability of obtaining the observed polypeptide sequence alignment by chance. SEQ ID NO:1 also contains a protein phosphatase 2C domain as determined by searching for statistically significant matches in the hidden Markov model (HMM)-based PFAM database of conserved protein family domains. (See Table 3.) Data from BLIMPS, MOTIFS, DOMO and PRODOM analyses provide further corroborative evidence that SEQ ID NO:1 is a protein
25 phosphatase 2C.

SEQ ID NO:3 is 90% identical to human tyrosine phosphatase (GenBank ID g292376) as determined by the Basic Local Alignment Search Tool (BLAST). (See Table 2.) The BLAST probability score is $7.3e-76$. SEQ ID NO:3 also contains protein phosphatase domains as determined by searching for statistically significant matches in the hidden Markov model (HMM)-based PFAM
30 database of conserved protein family domains. (See Table 3.) Data from BLIMPS, MOTIFS, and PROFILESCAN analyses provide further corroborative evidence that SEQ ID NO:3 is a protein phosphatase.

SEQ ID NO:4 is 64% identical to murine protein-tyrosine phosphatase (GenBank ID g2665458) as determined by the BLAST analysis. (See Table 2.) The BLAST probability score is

3.8e-143. SEQ ID NO:4 also contains an protein-tyrosine phosphatase domain as determined by searching for statistically significant matches in the hidden Markov model (HMM)-based PFAM database of conserved protein family domains. (See Table 3.) Data from BLIMPS, MOTIFS, and PROFILESCAN analyses provide further corroborative evidence that SEQ ID NO:4 is a protein-tyrosine phosphatase, note that “protein-tyrosine phosphatase” is a specific subfamily of the primary gene family, “protein phosphatases.”

SEQ ID NO:5 is 95% identical to rice serine threonine phosphatase (GenBank ID g5714762) as determined by the BLAST analysis. (See Table 2.) The BLAST probability score is 1.1e-163. SEQ ID NO:5 also contains a serine threonine protein phosphatase domain as determined by searching for statistically significant matches in the hidden Markov model (HMM)-based PFAM database of conserved protein family domains. (See Table 3.) Data from BLIMPS, MOTIFS, and PROFILESCAN analyses provide further corroborative evidence that SEQ ID NO:5 is a serine threonine phosphatase.

SEQ ID NO:7 is 98% identical to human striatum-enriched phosphatase (GenBank ID g957217) as determined by the BLAST analysis. (See Table 2.) The BLAST probability score is 1.4e-294. SEQ ID NO:7 also contains a protein tyrosine phosphatase domain as determined by searching for statistically significant matches in the hidden Markov model (HMM)-based PFAM database of conserved protein family domains. (See Table 3.) Data from BLIMPS, MOTIFS, and PROFILESCAN analyses provide further corroborative evidence that SEQ ID NO:7 is a protein tyrosine phosphatase.

SEQ ID NO:8 is 35% identical to Schizosaccharomyces pombe 4-nitrophenylphosphatase (GenBank ID g3451473) as determined by the BLAST analysis. (See Table 2.) The BLAST probability score is 7.0e-41. SEQ ID NO:8 also has homology to nitrophenylphosphatase domains in the DOMO and PRODOM databases. (See Table 3.)

SEQ ID NO:9 is 37% identical to human Fas-associated phosphatase-1 (GenBank ID g7542482) as determined by the BLAST analysis. (See Table 2.) The BLAST probability score is 4.1e-36. SEQ ID NO:9 also contains at least one PDZ domain, as indicated by HMMER, BLIMPS, and BLAST analyses. PDZ domains mediate protein-protein interactions in which Fas-associated phosphatase-1 participates (Irie, S. et al. (1999) FEBS Lett. 460:191-198). SEQ ID NO:2 and SEQ ID NO:6 were analyzed and annotated in a similar manner. The algorithms and parameters for the analysis of SEQ ID NO:1-9 are described in Table 7.

As shown in Table 4, the full length polynucleotide sequences of the present invention were assembled using cDNA sequences or coding (exon) sequences derived from genomic DNA, or any combination of these two types of sequences. Columns 1 and 2 list the polynucleotide sequence identification number (Polynucleotide SEQ ID NO:) and the corresponding Incyte polynucleotide consensus sequence number (Incyte Polynucleotide ID) for each polynucleotide of the invention.

Column 3 shows the length of each polynucleotide sequence in basepairs. Column 4 lists fragments of the polynucleotide sequences which are useful, for example, in hybridization or amplification technologies that identify SEQ ID NO:10-18 or that distinguish between SEQ ID NO:10-18 and related polynucleotide sequences. Column 5 shows identification numbers corresponding to cDNA sequences, coding sequences (exons) predicted from genomic DNA, and/or sequence assemblages comprised of both cDNA and genomic DNA. These sequences were used to assemble the full length polynucleotide sequences of the invention. Columns 6 and 7 of Table 4 show the nucleotide start (5') and stop (3') positions of the cDNA and/or genomic sequences in column 5 relative to their respective full length sequences.

10 The identification numbers in Column 5 of Table 4 may refer specifically, for example, to Incyte cDNAs along with their corresponding cDNA libraries. For example, 8103438J1 is the identification number of an Incyte cDNA sequence, and MIXDDIE02 is the cDNA library from which it is derived. Incyte cDNAs for which cDNA libraries are not indicated were derived from pooled cDNA libraries (e.g., 70528419V1). Alternatively, the identification numbers in column 5 may refer to
15 GenBank cDNAs or ESTs which contributed to the assembly of the full length polynucleotide sequences. Alternatively, the identification numbers in column 5 may refer to coding regions predicted by Genscan analysis of genomic DNA. The Genscan-predicted coding sequences may have been edited prior to assembly. (See Example IV.) Alternatively, the identification numbers in column 5 may refer to assemblages of both cDNA and Genscan-predicted exons brought together by an “exon stitching”
20 algorithm. (See Example V.) Alternatively, the identification numbers in column 5 may refer to assemblages of both cDNA and Genscan-predicted exons brought together by an “exon-stretching” algorithm. For example, FL7473604-g7329576_000027-g6692782 is the identification number of a “stretched” sequence, with 7473604 being the Incyte project identification number, g7329576 being the GenBank identification number of the human genomic sequence to which the “exon-stretching”
25 algorithm was applied, and g6692782 being the GenBank identification number of the nearest GenBank protein homolog. (See Example V.) In some cases, Incyte cDNA coverage redundant with the sequence coverage shown in column 5 was obtained to confirm the final consensus polynucleotide sequence, but the relevant Incyte cDNA identification numbers are not shown.

Table 5 shows the representative cDNA libraries for those full length polynucleotide sequences
30 which were assembled using Incyte cDNA sequences. The representative cDNA library is the Incyte cDNA library which is most frequently represented by the Incyte cDNA sequences which were used to assemble and confirm the above polynucleotide sequences. The tissues and vectors which were used to construct the cDNA libraries shown in Table 5 are described in Table 6.

The invention also encompasses PP variants. A preferred PP variant is one which has at least

about 80%, or alternatively at least about 90%, or even at least about 95% amino acid sequence identity to the PP amino acid sequence, and which contains at least one functional or structural characteristic of PP.

The invention also encompasses polynucleotides which encode PP. In a particular embodiment, 5 the invention encompasses a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:10-18, which encodes PP. The polynucleotide sequences of SEQ ID NO:10-18, as presented in the Sequence Listing, embrace the equivalent RNA sequences, wherein occurrences of the nitrogenous base thymine are replaced with uracil, and the sugar backbone is composed of ribose instead of deoxyribose.

10 The invention also encompasses a variant of a polynucleotide sequence encoding PP. In particular, such a variant polynucleotide sequence will have at least about 70%, or alternatively at least about 85%, or even at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding PP. A particular aspect of the invention encompasses a variant of a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:10-18 which has at least 15 about 70%, or alternatively at least about 85%, or even at least about 95% polynucleotide sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:10-18. Any one of the polynucleotide variants described above can encode an amino acid sequence which contains at least one functional or structural characteristic of PP.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic 20 code, a multitude of polynucleotide sequences encoding PP, some bearing minimal similarity to the polynucleotide sequences of any known and naturally occurring 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 25 occurring PP, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode PP and its variants are generally capable of hybridizing to the nucleotide sequence of the naturally occurring PP under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding PP or its derivatives possessing a substantially different codon usage, e.g., inclusion of non-naturally occurring 30 codons. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding PP and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from

the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode PP and PP 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
5 reagents well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding PP 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:10-18 and fragments thereof under various conditions of stringency. (See, e.g., Wahl, G.M. and
10 S.L. Berger (1987) *Methods Enzymol.* 152:399-407; Kimmel, A.R. (1987) *Methods Enzymol.* 152:507-511.) Hybridization conditions, including annealing and wash conditions, are described in "Definitions."

Methods for DNA sequencing are well known 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
15 DNA polymerase I, SEQUENASE (US Biochemical, Cleveland OH), Taq polymerase (Applied Biosystems), thermostable T7 polymerase (Amersham Pharmacia Biotech, Piscataway NJ), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE amplification system (Life Technologies, Gaithersburg MD). Preferably, sequence preparation is automated with machines such as the MICROLAB 2200 liquid transfer system (Hamilton, Reno NV),
20 PTC200 thermal cycler (MJ Research, Watertown MA) and ABI CATALYST 800 thermal cycler (Applied Biosystems). Sequencing is then carried out using either the ABI 373 or 377 DNA sequencing system (Applied Biosystems), the MEGABACE 1000 DNA sequencing system (Molecular Dynamics, Sunnyvale CA), or other systems known in the art. The resulting sequences are analyzed using a variety of algorithms which are well known in the art. (See, e.g., Ausubel, F.M. (1997) Short Protocols
25 in Molecular Biology, John Wiley & Sons, New York NY, unit 7.7; Meyers, R.A. (1995) Molecular Biology and Biotechnology, Wiley VCH, New York NY, pp. 856-853.)

The nucleic acid sequences encoding PP 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
30 PCR, uses universal and nested primers to amplify unknown sequence from genomic DNA within a cloning vector. (See, e.g., 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 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
5 performing PCR. Other methods which may be used to retrieve unknown sequences are known in the art. (See, e.g., Parker, J.D. et al. (1991) Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PROMOTERFINDER libraries (Clontech, Palo Alto CA) to walk genomic DNA. 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
10 OLIGO 4.06 primer analysis software (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 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
15 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 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
20 sequencing may employ flowable polymers for electrophoretic separation, four different nucleotide-specific, laser-stimulated fluorescent dyes, and a 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, Applied Biosystems), and the entire process from loading of samples to computer analysis and electronic data display may be computer
25 controlled. Capillary electrophoresis is especially preferable for sequencing small DNA 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 PP may be cloned in recombinant DNA molecules that direct expression of PP, or fragments or functional equivalents thereof, in appropriate host cells. Due to the inherent degeneracy of the genetic
30 code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and used to express PP.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter PP-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA

shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, 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.

5 The nucleotides of the present invention may be subjected to DNA shuffling techniques such as MOLECULARBREEDING (Maxygen Inc., Santa Clara CA; described in U.S. Patent Number 5,837,458; Chang, C.-C. et al. (1999) *Nat. Biotechnol.* 17:793-797; Christians, F.C. et al. (1999) *Nat. Biotechnol.* 17:259-264; and Crameri, A. et al. (1996) *Nat. Biotechnol.* 14:315-319) to alter or improve the biological properties of PP, such as its biological or enzymatic activity or its ability to
10 bind to other molecules or compounds. DNA shuffling is a process by which a library of gene variants is produced using PCR-mediated recombination of gene fragments. The library is then subjected to selection or screening procedures that identify those gene variants with the desired properties. These preferred variants may then be pooled and further subjected to recursive rounds of DNA shuffling and selection/screening. Thus, genetic diversity is created through "artificial"
15 breeding and rapid molecular evolution. For example, fragments of a single gene containing random point mutations may be recombined, screened, and then reshuffled until the desired properties are optimized. Alternatively, fragments of a given gene may be recombined with fragments of homologous genes in the same gene family, either from the same or different species, thereby maximizing the genetic diversity of multiple naturally occurring genes in a directed and controllable
20 manner.

In another embodiment, sequences encoding PP 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) *Nucleic Acids Symp. Ser.* 7:215-223; and Horn, T. et al. (1980) *Nucleic Acids Symp. Ser.* 7:225-232.) Alternatively, PP itself or a fragment thereof may be synthesized using chemical methods. For example, peptide synthesis
25 can be performed using various solution-phase or solid-phase techniques. (See, e.g., Creighton, T. (1984) Proteins, Structures and Molecular Properties, WH Freeman, New York NY, pp. 55-60; and Roberge, J.Y. et al. (1995) *Science* 269:202-204.) Automated synthesis may be achieved using the ABI 431A peptide synthesizer (Applied Biosystems). Additionally, the amino acid sequence of PP, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other
30 proteins, or any part thereof, to produce a variant polypeptide or a polypeptide having a sequence of a naturally occurring 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, supra, pp. 28-53.)

In order to express a biologically active PP, the nucleotide sequences encoding PP 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 PP. Such elements may vary in their strength and specificity. Specific initiation signals may also be used to achieve more efficient translation of sequences encoding PP. Such signals include the ATG initiation codon and adjacent sequences, e.g. the Kozak sequence. In cases where sequences encoding PP 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 PP 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; Ausubel, F.M. et al. (1995) 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 PP. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression 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. (See, e.g., Sambrook, supra; Ausubel, supra; Van Heeke, G. and S.M. Schuster (1989) *J. Biol. Chem.* 264:5503-5509; 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; Takamatsu, N. (1987) *EMBO J.* 6:307-311; The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196; Logan, J. and T. Shenk (1984) *Proc. Natl. Acad. Sci. USA* 81:3655-3659; and

Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355.) 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. (See, e.g., Di Nicola, M. et al. (1998) Cancer Gen. Ther. 5(6):350-356; Yu, M. et al. (1993) Proc. Natl. Acad. Sci. USA 90(13):6340-6344; Buller, R.M. et al. (1985) Nature 317(6040):813-815; McGregor, D.P. et al. (1994) Mol. Immunol. 31(3):219-226; and Verma, I.M. and N. Somia (1997) Nature 389:239-242.) The invention is not limited by the host cell employed.

In bacterial systems, a number of cloning and expression vectors may be selected depending upon the use intended for polynucleotide sequences encoding PP. For example, routine cloning, subcloning, and propagation of polynucleotide sequences encoding PP can be achieved using a multifunctional *E. coli* vector such as PBLUESCRIPT (Stratagene, La Jolla CA) or PSPORT1 plasmid (Life Technologies). Ligation of sequences encoding PP 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. Biol. Chem. 264:5503-5509.) When large quantities of PP are needed, e.g. for the production of antibodies, vectors which direct high level expression of PP may be used. For example, vectors containing the strong, inducible SP6 or T7 bacteriophage promoter may be used.

Yeast expression systems may be used for production of PP. A number of vectors containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH promoters, 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, 1995, *supra*; Bitter, G.A. et al. (1987) Methods Enzymol. 153:516-544; and Scorer, C.A. et al. (1994) Bio/Technology 12:181-184.)

Plant systems may also be used for expression of PP. Transcription of sequences encoding PP may be driven by 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 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., *The 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. In cases where an adenovirus is used as an expression vector, sequences encoding PP 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
5 infective virus which expresses PP in host cells. (See, e.g., Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. USA 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells. 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
10 DNA than can be contained in and expressed from a plasmid. HACs of about 6 kb to 10 Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355.)

For long term production of recombinant proteins in mammalian systems, stable expression of PP in cell lines is preferred. For example, sequences encoding PP can be transformed into cell lines
15 using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for 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
20 sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase and adenine phosphoribosyltransferase genes, for use in *tk* and *apr* cells, respectively. (See, e.g., Wigler, M. et al. (1977) Cell 11:223-232;
25 Lowy, I. et al. (1980) Cell 22:817-823.) Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate; *neo* confers resistance to the aminoglycosides neomycin and G-418; and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g., Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. USA 77:3567-3570; Colbere-Garapin, F. et al. (1981) J. Mol. Biol. 150:1-14.)
30 Additional selectable genes have been described, e.g., *trpB* and *hisD*, which alter cellular requirements for metabolites. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. USA 85:8047-8051.) Visible markers, e.g., anthocyanins, green fluorescent proteins (GFP; Clontech), β glucuronidase and its substrate β -glucuronide, or luciferase and its substrate luciferin may be used. These markers can be used not only to identify transformants, but also to quantify the amount of

transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, C.A. (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
5 sequence encoding PP is inserted within a marker gene sequence, transformed cells containing sequences encoding PP can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding PP 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.

10 In general, host cells that contain the nucleic acid sequence encoding PP and that express PP 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.

15 Immunological methods for detecting and measuring the expression of PP 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 PP is preferred, but a competitive binding assay
20 may be 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, Sect. IV; Coligan, J.E. et al. (1997) *Current Protocols in Immunology*, Greene Pub. Associates and Wiley-Interscience, New York NY; and Pound, J.D. (1998) *Immunochemical Protocols*, Humana Press, Totowa NJ.)

A wide variety of labels and conjugation techniques are known by those skilled in the art and
25 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 PP include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding PP, 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
30 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 Amersham Pharmacia Biotech, Promega (Madison WI), and US Biochemical. 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 PP may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or 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 PP may be designed to contain signal sequences which direct secretion of PP through a prokaryotic or eukaryotic cell membrane.

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" or "pro" 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 characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38) are available from the American Type Culture Collection (ATCC, Manassas VA) 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 acid sequences encoding PP 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 PP protein containing a heterologous moiety that can be recognized by a commercially available antibody may facilitate the screening of peptide libraries for inhibitors of PP activity. Heterologous protein and peptide moieties may also facilitate 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, 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 PP encoding sequence and the heterologous protein sequence, so that PP may be cleaved away from the heterologous moiety following purification. Methods for fusion protein expression and purification are discussed in Ausubel (1995, *supra*, 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 PP may be achieved in vitro

using the TNT rabbit reticulocyte lysate or wheat germ extract system (Promega). 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, for example, ³⁵S-methionine.

5 PP of the present invention or fragments thereof may be used to screen for compounds that specifically bind to PP. At least one and up to a plurality of test compounds may be screened for specific binding to PP. Examples of test compounds include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

In one embodiment, the compound thus identified is closely related to the natural ligand of
10 PP, e.g., a ligand or fragment thereof, a natural substrate, a structural or functional mimetic, or a natural binding partner. (See, e.g., Coligan, J.E. et al. (1991) Current Protocols in Immunology 1(2): Chapter 5.) Similarly, the compound can be closely related to the natural receptor to which PP binds, or to at least a fragment of the receptor, e.g., the ligand binding site. In either case, the compound can be rationally designed using known techniques. In one embodiment, screening for these compounds
15 involves producing appropriate cells which express PP, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, Drosophila, or E. coli. Cells expressing PP or cell membrane fractions which contain PP are then contacted with a test compound and binding, stimulation, or inhibition of activity of either PP or the compound is analyzed.

An assay may simply test binding of a test compound to the polypeptide, wherein binding is
20 detected by a fluorophore, radioisotope, enzyme conjugate, or other detectable label. For example, the assay may comprise the steps of combining at least one test compound with PP, either in solution or affixed to a solid support, and detecting the binding of PP to the compound. Alternatively, the assay may detect or measure binding of a test compound in the presence of a labeled competitor. Additionally, the assay may be carried out using cell-free preparations, chemical libraries, or natural
25 product mixtures, and the test compound(s) may be free in solution or affixed to a solid support.

PP of the present invention or fragments thereof may be used to screen for compounds that modulate the activity of PP. Such compounds may include agonists, antagonists, or partial or inverse agonists. In one embodiment, an assay is performed under conditions permissive for PP activity, wherein PP is combined with at least one test compound, and the activity of PP in the presence of a test
30 compound is compared with the activity of PP in the absence of the test compound. A change in the activity of PP in the presence of the test compound is indicative of a compound that modulates the activity of PP. Alternatively, a test compound is combined with an in vitro or cell-free system comprising PP under conditions suitable for PP activity, and the assay is performed. In either of these assays, a test compound which modulates the activity of PP may do so indirectly and need not come in

direct contact with the test compound. At least one and up to a plurality of test compounds may be screened.

In another embodiment, polynucleotides encoding PP or their mammalian homologs may be “knocked out” in an animal model system using homologous recombination in embryonic stem (ES) cells. Such techniques are well known in the art and are useful for the generation of animal models of human disease. (See, e.g., U.S. Patent Number 5,175,383 and U.S. Patent Number 5,767,337.) For example, mouse ES cells, such as the mouse 129/SvJ cell line, are derived from the early mouse embryo and grown in culture. The ES cells are transformed with a vector containing the gene of interest disrupted by a marker gene, e.g., the neomycin phosphotransferase gene (neo; Capecchi, M.R. (1989) Science 244:1288-1292). The vector integrates into the corresponding region of the host genome by homologous recombination. Alternatively, homologous recombination takes place using the Cre-loxP system to knockout a gene of interest in a tissue- or developmental stage-specific manner (Marth, J.D. (1996) Clin. Invest. 97:1999-2002; Wagner, K.U. et al. (1997) Nucleic Acids Res. 25:4323-4330). Transformed ES cells are identified and microinjected into mouse cell blastocysts such as those from the C57BL/6 mouse strain. The blastocysts are surgically transferred to pseudopregnant dams, and the resulting chimeric progeny are genotyped and bred to produce heterozygous or homozygous strains. Transgenic animals thus generated may be tested with potential therapeutic or toxic agents.

Polynucleotides encoding PP may also be manipulated in vitro in ES cells derived from human blastocysts. Human ES cells have the potential to differentiate into at least eight separate cell lineages including endoderm, mesoderm, and ectodermal cell types. These cell lineages differentiate into, for example, neural cells, hematopoietic lineages, and cardiomyocytes (Thomson, J.A. et al. (1998) Science 282:1145-1147).

Polynucleotides encoding PP can also be used to create “knockin” humanized animals (pigs) or transgenic animals (mice or rats) to model human disease. With knockin technology, a region of a polynucleotide encoding PP is injected into animal ES cells, and the injected sequence integrates into the animal cell genome. Transformed cells are injected into blastulae, and the blastulae are implanted as described above. Transgenic progeny or inbred lines are studied and treated with potential pharmaceutical agents to obtain information on treatment of a human disease. Alternatively, a mammal inbred to overexpress PP, e.g., by secreting PP in its milk, may also serve as a convenient source of that protein (Janne, J. et al. (1998) Biotechnol. Annu. Rev. 4:55-74).

THERAPEUTICS

Chemical and structural similarity, e.g., in the context of sequences and motifs, exists between regions of PP and protein phosphatases. In addition, the expression of PP is closely associated with cardiovascular tissue and brain tissue; therefore, PP appears to play a role in immune

system disorders, neurological disorders, developmental disorders and cell proliferative disorders, including cancer. In the treatment of disorders associated with increased PP expression or activity, it is desirable to decrease the expression or activity of PP. In the treatment of disorders associated with decreased PP expression or activity, it is desirable to increase the expression or activity of PP.

5 Therefore, in one embodiment, PP or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of PP. Examples of such disorders include, but are not limited to, an immune system disorder, such as acquired immunodeficiency syndrome (AIDS), X-linked agammaglobinemia of Bruton, common variable immunodeficiency (CVI), DiGeorge's syndrome (thymic hypoplasia), thymic dysplasia, 10 isolated IgA deficiency, severe combined immunodeficiency disease (SCID), immunodeficiency with thrombocytopenia and eczema (Wiskott-Aldrich syndrome), Chediak-Higashi syndrome, chronic granulomatous diseases, hereditary angioneurotic edema, immunodeficiency associated with Cushing's disease, Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, 15 autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, 20 myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma; a 25 neurological disorder, such as epilepsy, ischemic cerebrovascular disease, stroke, cerebral neoplasms, Alzheimer's disease, Pick's disease, Huntington's disease, dementia, Parkinson's disease and other extrapyramidal disorders, amyotrophic lateral sclerosis and other motor neuron disorders, progressive neural muscular atrophy, retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases, bacterial and viral meningitis, brain abscess, subdural empyema, epidural 30 abscess, suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous system disease, prion diseases including kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome, fatal familial insomnia, nutritional and metabolic diseases of the nervous system, neurofibromatosis, tuberous sclerosis, cerebelloretinal hemangioblastomatosis, encephalotrigeminal syndrome, mental retardation and other developmental disorders of the central nervous system including

Down syndrome, cerebral palsy, neuroskeletal disorders, autonomic nervous system disorders, cranial nerve disorders, spinal cord diseases, muscular dystrophy and other neuromuscular disorders, peripheral nervous system disorders, dermatomyositis and polymyositis, inherited, metabolic, endocrine, and toxic myopathies, myasthenia gravis, periodic paralysis, mental disorders including mood, anxiety, and schizophrenic disorders, seasonal affective disorder (SAD), akathisia, amnesia, catatonia, diabetic neuropathy, tardive dyskinesia, dystonias, paranoid psychoses, postherpetic neuralgia, Tourette's disorder, progressive supranuclear palsy, corticobasal degeneration, and familial frontotemporal dementia; a developmental disorder, such as renal tubular acidosis, anemia, Cushing's syndrome, achondroplastic dwarfism, Duchenne and Becker muscular dystrophy, epilepsy, gonadal dysgenesis, WAGR syndrome (Wilms' tumor, aniridia, genitourinary abnormalities, and mental retardation), Smith-Magenis syndrome, myelodysplastic syndrome, hereditary mucoepithelial dysplasia, hereditary keratodermas, hereditary neuropathies such as Charcot-Marie-Tooth disease and neurofibromatosis, hypothyroidism, hydrocephalus, seizure disorders such as Sydenham's chorea and cerebral palsy, spina bifida, anencephaly, craniorachischisis, congenital glaucoma, cataract, and sensorineural hearing loss; and a cell proliferative disorder, such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including 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.

In another embodiment, a vector capable of expressing PP or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of PP including, but not limited to, those described above.

In a further embodiment, a composition comprising a substantially purified PP in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of PP including, but not limited to, those provided above.

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

In a further embodiment, an antagonist of PP may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of PP. Examples of such disorders include, but are not limited to, those immune system disorders, neurological disorders, developmental

disorders, and cell proliferative disorders, including cancer, described above. In one aspect, an antibody which specifically binds PP may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissues which express PP.

In an additional embodiment, a vector expressing the complement of the polynucleotide
5 encoding PP may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of PP 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 combination therapy may be made by
10 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.

An antagonist of PP may be produced using methods which are generally known in the art. In
15 particular, purified PP may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind PP. Antibodies to PP 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 single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit dimer formation) are generally
20 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 PP 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 include, but are not limited to, Freund's, mineral gels such as
25 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 antibodies to PP have an amino acid sequence consisting of at least about 5 amino acids, and generally will consist of at
30 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. Short stretches of PP amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to PP may be prepared using any technique which provides for the

production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-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. USA* 80:2026-2030; and
5 Cole, S.P. et al. (1984) *Mol. Cell Biol.* 62:109-120.)

In addition, techniques developed for the production of "chimeric antibodies," such 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. USA* 81:6851-6855; Neuberger, M.S. et al. (1984) *Nature* 312:604-608; and Takeda,
10 S. et al. (1985) *Nature* 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce PP-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. USA* 88:10134-10137.)

15 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. USA* 86:3833-3837; Winter, G. et al. (1991) *Nature* 349:293-299.)

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

25 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 PP and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two
30 non-interfering PP epitopes is generally used, but a competitive binding assay may also be employed (Pound, *supra*).

Various methods such as Scatchard analysis in conjunction with radioimmunoassay techniques may be used to assess the affinity of antibodies for PP. Affinity is expressed as an association constant, K_a , which is defined as the molar concentration of PP-antibody complex divided by the molar

concentrations of free antigen and free antibody under equilibrium conditions. The K_a determined for a preparation of polyclonal antibodies, which are heterogeneous in their affinities for multiple PP epitopes, represents the average affinity, or avidity, of the antibodies for PP. The K_a determined for a preparation of monoclonal antibodies, which are monospecific for a particular PP epitope, represents a true measure of affinity. High-affinity antibody preparations with K_a ranging from about 10^9 to 10^{12} L/mole are preferred for use in immunoassays in which the PP-antibody complex must withstand rigorous manipulations. Low-affinity antibody preparations with K_a ranging from about 10^6 to 10^7 L/mole are preferred for use in immunopurification and similar procedures which ultimately require dissociation of PP, preferably in active form, from the antibody (Catty, D. (1988) Antibodies, Volume I: A Practical Approach, IRL Press, Washington DC; Liddell, J.E. and A. Cryer (1991) A Practical Guide to Monoclonal Antibodies, John Wiley & Sons, New York NY).

The titer and avidity of polyclonal antibody preparations may be further evaluated to determine the quality and suitability of such preparations for certain downstream applications. For example, a polyclonal antibody preparation containing at least 1-2 mg specific antibody/ml, preferably 5-10 mg specific antibody/ml, is generally employed in procedures requiring precipitation of PP-antibody complexes. Procedures for evaluating antibody specificity, titer, and avidity, and guidelines for antibody quality and usage in various applications, are generally available. (See, e.g., Catty, supra, and Coligan et al. supra.)

In another embodiment of the invention, the polynucleotides encoding PP, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, modifications of gene expression can be achieved by designing complementary sequences or antisense molecules (DNA, RNA, PNA, or modified oligonucleotides) to the coding or regulatory regions of the gene encoding PP. Such technology is well known in the art, and antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions of sequences encoding PP. (See, e.g., Agrawal, S., ed. (1996) Antisense Therapeutics, Humana Press Inc., Totawa NJ.)

In therapeutic use, any gene delivery system suitable for introduction of the antisense sequences into appropriate target cells can be used. Antisense sequences can be delivered intracellularly in the form of an expression plasmid which, upon transcription, produces a sequence complementary to at least a portion of the cellular sequence encoding the target protein. (See, e.g., Slater, J.E. et al. (1998) *J. Allergy Clin. Immunol.* 102(3):469-475; and Scanlon, K.J. et al. (1995) 9(13):1288-1296.) Antisense sequences can also be introduced intracellularly through the use of viral vectors, such as retrovirus and adeno-associated virus vectors. (See, e.g., Miller, A.D. (1990) *Blood* 76:271; Ausubel, supra; Uckert, W. and W. Walther (1994) *Pharmacol. Ther.* 63(3):323-347.) Other gene delivery mechanisms include liposome-derived systems, artificial viral envelopes, and other

systems known in the art. (See, e.g., Rossi, J.J. (1995) Br. Med. Bull. 51(1):217-225; Boado, R.J. et al. (1998) J. Pharm. Sci. 87(11):1308-1315; and Morris, M.C. et al. (1997) Nucleic Acids Res. 25(14):2730-2736.)

In another embodiment of the invention, polynucleotides encoding PP may be used for somatic or germline gene therapy. Gene therapy may be performed to (i) correct a genetic deficiency (e.g., in the cases of severe combined immunodeficiency (SCID)-X1 disease characterized by X-linked inheritance (Cavazzana-Calvo, M. et al. (2000) Science 288:669-672), severe combined immunodeficiency syndrome associated with an inherited adenosine deaminase (ADA) deficiency (Blaese, R.M. et al. (1995) Science 270:475-480; Bordignon, C. et al. (1995) Science 270:470-475), cystic fibrosis (Zabner, J. et al. (1993) Cell 75:207-216; Crystal, R.G. et al. (1995) Hum. Gene Therapy 6:643-666; Crystal, R.G. et al. (1995) Hum. Gene Therapy 6:667-703), thalassamias, familial hypercholesterolemia, and hemophilia resulting from Factor VIII or Factor IX deficiencies (Crystal, R.G. (1995) Science 270:404-410; Verma, I.M. and N. Somia (1997) Nature 389:239-242)), (ii) express a conditionally lethal gene product (e.g., in the case of cancers which result from unregulated cell proliferation), or (iii) express a protein which affords protection against intracellular parasites (e.g., against human retroviruses, such as human immunodeficiency virus (HIV) (Baltimore, D. (1988) Nature 335:395-396; Poeschla, E. et al. (1996) Proc. Natl. Acad. Sci. USA. 93:11395-11399), hepatitis B or C virus (HBV, HCV); fungal parasites, such as Candida albicans and Paracoccidioides brasiliensis; and protozoan parasites such as Plasmodium falciparum and Trypanosoma cruzi). In the case where a genetic deficiency in PP expression or regulation causes disease, the expression of PP from an appropriate population of transduced cells may alleviate the clinical manifestations caused by the genetic deficiency.

In a further embodiment of the invention, diseases or disorders caused by deficiencies in PP are treated by constructing mammalian expression vectors encoding PP and introducing these vectors by mechanical means into PP-deficient cells. Mechanical transfer technologies for use with cells in vivo or ex vitro include (i) direct DNA microinjection into individual cells, (ii) ballistic gold particle delivery, (iii) liposome-mediated transfection, (iv) receptor-mediated gene transfer, and (v) the use of DNA transposons (Morgan, R.A. and W.F. Anderson (1993) Annu. Rev. Biochem. 62:191-217; Ivics, Z. (1997) Cell 91:501-510; Boulay, J-L. and H. Récipon (1998) Curr. Opin. Biotechnol. 9:445-450).

Expression vectors that may be effective for the expression of PP include, but are not limited to, the PCDNA 3.1, EPITAG, PRCCMV2, PREP, PVAX vectors (Invitrogen, Carlsbad CA), PCMV-SCRIPT, PCMV-TAG, PEGSH/PERV (Stratagene, La Jolla CA), and PTET-OFF, PTET-ON, PTRE2, PTRE2-LUC, PTK-HYG (Clontech, Palo Alto CA). PP may be expressed using (i) a constitutively active promoter, (e.g., from cytomegalovirus (CMV), Rous sarcoma virus (RSV),

SV40 virus, thymidine kinase (TK), or β -actin genes), (ii) an inducible promoter (e.g., the tetracycline-regulated promoter (Gossen, M. and H. Bujard (1992) Proc. Natl. Acad. Sci. USA 89:5547-5551; Gossen, M. et al. (1995) Science 268:1766-1769; Rossi, F.M.V. and H.M. Blau (1998) Curr. Opin. Biotechnol. 9:451-456), commercially available in the T-REX plasmid (Invitrogen)); the
5 ecdysone-inducible promoter (available in the plasmids PVGRXR and PIND; Invitrogen); the FK506/rapamycin inducible promoter; or the RU486/mifepristone inducible promoter (Rossi, F.M.V. and Blau, H.M. supra)), or (iii) a tissue-specific promoter or the native promoter of the endogenous gene encoding PP from a normal individual.

Commercially available liposome transformation kits (e.g., the PERFECT LIPID
10 TRANSFECTION KIT, available from Invitrogen) allow one with ordinary skill in the art to deliver polynucleotides to target cells in culture and require minimal effort to optimize experimental parameters. In the alternative, transformation is performed using the calcium phosphate method (Graham, F.L. and A.J. Eb (1973) Virology 52:456-467), or by electroporation (Neumann, E. et al. (1982) EMBO J. 1:841-845). The introduction of DNA to primary cells requires modification of these
15 standardized mammalian transfection protocols.

In another embodiment of the invention, diseases or disorders caused by genetic defects with respect to PP expression are treated by constructing a retrovirus vector consisting of (i) the polynucleotide encoding PP under the control of an independent promoter or the retrovirus long terminal repeat (LTR) promoter, (ii) appropriate RNA packaging signals, and (iii) a Rev-responsive element
20 (RRE) along with additional retrovirus *cis*-acting RNA sequences and coding sequences required for efficient vector propagation. Retrovirus vectors (e.g., PFB and PFBNEO) are commercially available (Stratagene) and are based on published data (Riviere, I. et al. (1995) Proc. Natl. Acad. Sci. USA 92:6733-6737), incorporated by reference herein. The vector is propagated in an appropriate vector producing cell line (VPCL) that expresses an envelope gene with a tropism for receptors on the target
25 cells or a promiscuous envelope protein such as VSVg (Armentano, D. et al. (1987) J. Virol. 61:1647-1650; Bender, M.A. et al. (1987) J. Virol. 61:1639-1646; Adam, M.A. and A.D. Miller (1988) J. Virol. 62:3802-3806; Dull, T. et al. (1998) J. Virol. 72:8463-8471; Zufferey, R. et al. (1998) J. Virol. 72:9873-9880). U.S. Patent Number 5,910,434 to Rigg ("Method for obtaining retrovirus packaging cell lines producing high transducing efficiency retroviral supernatant") discloses a method for
30 obtaining retrovirus packaging cell lines and is hereby incorporated by reference. Propagation of retrovirus vectors, transduction of a population of cells (e.g., CD4⁺ T-cells), and the return of transduced cells to a patient are procedures well known to persons skilled in the art of gene therapy and have been well documented (Ranga, U. et al. (1997) J. Virol. 71:7020-7029; Bauer, G. et al. (1997) Blood 89:2259-2267; Bonyhadi, M.L. (1997) J. Virol. 71:4707-4716; Ranga, U. et al. (1998) Proc.

Natl. Acad. Sci. USA 95:1201-1206; Su, L. (1997) Blood 89:2283-2290).

In the alternative, an adenovirus-based gene therapy delivery system is used to deliver polynucleotides encoding PP to cells which have one or more genetic abnormalities with respect to the expression of PP. The construction and packaging of adenovirus-based vectors are well known to those with ordinary skill in the art. Replication defective adenovirus vectors have proven to be versatile for importing genes encoding immunoregulatory proteins into intact islets in the pancreas (Csete, M.E. et al. (1995) Transplantation 27:263-268). Potentially useful adenoviral vectors are described in U.S. Patent Number 5,707,618 to Armentano ("Adenovirus vectors for gene therapy"), hereby incorporated by reference. For adenoviral vectors, see also Antinozzi, P.A. et al. (1999) Annu. Rev. Nutr. 19:511-544 and Verma, I.M. and N. Somia (1997) Nature 18:389:239-242, both incorporated by reference herein.

In another alternative, a herpes-based, gene therapy delivery system is used to deliver polynucleotides encoding PP to target cells which have one or more genetic abnormalities with respect to the expression of PP. The use of herpes simplex virus (HSV)-based vectors may be especially valuable for introducing PP to cells of the central nervous system, for which HSV has a tropism. The construction and packaging of herpes-based vectors are well known to those with ordinary skill in the art. A replication-competent herpes simplex virus (HSV) type 1-based vector has been used to deliver a reporter gene to the eyes of primates (Liu, X. et al. (1999) Exp. Eye Res. 169:385-395). The construction of a HSV-1 virus vector has also been disclosed in detail in U.S. Patent Number 5,804,413 to DeLuca ("Herpes simplex virus strains for gene transfer"), which is hereby incorporated by reference. U.S. Patent Number 5,804,413 teaches the use of recombinant HSV d92 which consists of a genome containing at least one exogenous gene to be transferred to a cell under the control of the appropriate promoter for purposes including human gene therapy. Also taught by this patent are the construction and use of recombinant HSV strains deleted for ICP4, ICP27 and ICP22. For HSV vectors, see also Goins, W.F. et al. (1999) J. Virol. 73:519-532 and Xu, H. et al. (1994) Dev. Biol. 163:152-161, hereby incorporated by reference. The manipulation of cloned herpesvirus sequences, the generation of recombinant virus following the transfection of multiple plasmids containing different segments of the large herpesvirus genomes, the growth and propagation of herpesvirus, and the infection of cells with herpesvirus are techniques well known to those of ordinary skill in the art.

In another alternative, an alphavirus (positive, single-stranded RNA virus) vector is used to deliver polynucleotides encoding PP to target cells. The biology of the prototypic alphavirus, Semliki Forest Virus (SFV), has been studied extensively and gene transfer vectors have been based on the SFV genome (Garoff, H. and K.-J. Li (1998) Curr. Opin. Biotechnol. 9:464-469). During alphavirus RNA replication, a subgenomic RNA is generated that normally encodes the viral capsid proteins. This

subgenomic RNA replicates to higher levels than the full length genomic RNA, resulting in the overproduction of capsid proteins relative to the viral proteins with enzymatic activity (e.g., protease and polymerase). Similarly, inserting the coding sequence for PP into the alphavirus genome in place of the capsid-coding region results in the production of a large number of PP-coding RNAs and the synthesis of high levels of PP in vector transduced cells. While alphavirus infection is typically associated with cell lysis within a few days, the ability to establish a persistent infection in hamster normal kidney cells (BHK-21) with a variant of Sindbis virus (SIN) indicates that the lytic replication of alphaviruses can be altered to suit the needs of the gene therapy application (Dryga, S.A. et al. (1997) *Virology* 228:74-83). The wide host range of alphaviruses will allow the introduction of PP into a variety of cell types. The specific transduction of a subset of cells in a population may require the sorting of cells prior to transduction. The methods of manipulating infectious cDNA clones of alphaviruses, performing alphavirus cDNA and RNA transfections, and performing alphavirus infections, are well known to those with ordinary skill in the art.

Oligonucleotides derived from the transcription initiation site, e.g., between about positions -10 and +10 from the start site, may also be employed to inhibit gene expression. 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 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, 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.

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 encoding PP.

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 evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Complementary ribonucleic acid molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis.

Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding PP. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize complementary RNA, constitutively or inducibly, can be introduced into cell lines, cells, or tissues.

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

An additional embodiment of the invention encompasses a method for screening for a compound which is effective in altering expression of a polynucleotide encoding PP. Compounds which may be effective in altering expression of a specific polynucleotide may include, but are not limited to, oligonucleotides, antisense oligonucleotides, triple helix-forming oligonucleotides, transcription factors and other polypeptide transcriptional regulators, and non-macromolecular chemical entities which are capable of interacting with specific polynucleotide sequences. Effective compounds may alter polynucleotide expression by acting as either inhibitors or promoters of polynucleotide expression. Thus, in the treatment of disorders associated with increased PP expression or activity, a compound which specifically inhibits expression of the polynucleotide encoding PP may be therapeutically useful, and in the treatment of disorders associated with decreased PP expression or activity, a compound which specifically promotes expression of the polynucleotide encoding PP may be therapeutically useful.

At least one, and up to a plurality, of test compounds may be screened for effectiveness in altering expression of a specific polynucleotide. A test compound may be obtained by any method commonly known in the art, including chemical modification of a compound known to be effective in altering polynucleotide expression; selection from an existing, commercially-available or proprietary library of naturally-occurring or non-natural chemical compounds; rational design of a compound based on chemical and/or structural properties of the target polynucleotide; and selection from a library of chemical compounds created combinatorially or randomly. A sample comprising a polynucleotide encoding PP is exposed to at least one test compound thus obtained. The sample may

comprise, for example, an intact or permeabilized cell, or an in vitro cell-free or reconstituted biochemical system. Alterations in the expression of a polynucleotide encoding PP are assayed by any method commonly known in the art. Typically, the expression of a specific nucleotide is detected by hybridization with a probe having a nucleotide sequence complementary to the sequence of the polynucleotide encoding PP. The amount of hybridization may be quantified, thus forming the basis for a comparison of the expression of the polynucleotide both with and without exposure to one or more test compounds. Detection of a change in the expression of a polynucleotide exposed to a test compound indicates that the test compound is effective in altering the expression of the polynucleotide. A screen for a compound effective in altering expression of a specific polynucleotide can be carried out, for example, using a Schizosaccharomyces pombe gene expression system (Atkins, D. et al. (1999) U.S. Patent No. 5,932,435; Arndt, G.M. et al. (2000) Nucleic Acids Res. 28:E15) or a human cell line such as HeLa cell (Clarke, M.L. et al. (2000) Biochem. Biophys. Res. Commun. 268:8-13). A particular embodiment of the present invention involves screening a combinatorial library of oligonucleotides (such as deoxyribonucleotides, ribonucleotides, peptide nucleic acids, and modified oligonucleotides) for antisense activity against a specific polynucleotide sequence (Bruice, T.W. et al. (1997) U.S. Patent No. 5,686,242; Bruice, T.W. et al. (2000) U.S. Patent No. 6,022,691).

Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or 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) Nat. Biotechnol. 15:462-466.)

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

An additional embodiment of the invention relates to the administration of a composition which generally comprises an active ingredient formulated with a pharmaceutically acceptable excipient. Excipients may include, for example, sugars, starches, celluloses, gums, and proteins. Various formulations are commonly known and are thoroughly discussed in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing, Easton PA). Such compositions may consist of PP, antibodies to PP, and mimetics, agonists, antagonists, or inhibitors of PP.

The 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, pulmonary, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical,

sublingual, or rectal means.

Compositions for pulmonary administration may be prepared in liquid or dry powder form. These compositions are generally aerosolized immediately prior to inhalation by the patient. In the case of small molecules (e.g. traditional low molecular weight organic drugs), aerosol delivery of fast-acting formulations is well-known in the art. In the case of macromolecules (e.g. larger peptides and proteins), recent developments in the field of pulmonary delivery via the alveolar region of the lung have enabled the practical delivery of drugs such as insulin to blood circulation (see, e.g., Patton, J.S. et al., U.S. Patent No. 5,997,848). Pulmonary delivery has the advantage of administration without needle injection, and obviates the need for potentially toxic penetration enhancers.

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

Specialized forms of compositions may be prepared for direct intracellular delivery of macromolecules comprising PP or fragments thereof. For example, liposome preparations containing a cell-impermeable macromolecule may promote cell fusion and intracellular delivery of the macromolecule. Alternatively, PP or a fragment thereof may be joined to a short cationic N-terminal portion from the HIV Tat-1 protein. Fusion proteins thus generated have been found to transduce into the cells of all tissues, including the brain, in a mouse model system (Schwarze, S.R. et al. (1999) Science 285:1569-1572).

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, monkeys, or pigs. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example PP or fragments thereof, antibodies of PP, and agonists, antagonists or inhibitors of PP, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating the ED₅₀ (the dose therapeutically effective in 50% of the population) or LD₅₀ (the dose lethal to 50% of the population) statistics. The dose ratio of toxic to therapeutic effects is the therapeutic index, which can be expressed as the LD₅₀/ED₅₀ ratio. Compositions which exhibit large 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 ED₅₀ 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 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 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.

10 Normal dosage amounts may vary from about 0.1 μg to 100,000 μg , 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 formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, 15 conditions, locations, etc.

DIAGNOSTICS

In another embodiment, antibodies which specifically bind PP may be used for the diagnosis of disorders characterized by expression of PP, or in assays to monitor patients being treated with PP or agonists, antagonists, or inhibitors of PP. Antibodies useful for diagnostic purposes may be prepared in the same manner as described above for therapeutics. Diagnostic assays for PP include methods which 20 utilize the antibody and a label to detect PP 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.

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

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

5 In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding PP or closely related molecules may be used to identify nucleic acid sequences which encode PP. 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 will determine whether the probe
10 identifies only naturally occurring sequences encoding PP, allelic variants, or related sequences.

Probes may also be used for the detection of related sequences, and may have at least 50% sequence identity to any of the PP 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:10-18 or from genomic sequences including promoters, enhancers, and introns of the PP gene.

15 Means for producing specific hybridization probes for DNAs encoding PP include the cloning of polynucleotide sequences encoding PP or PP 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 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,
20 by radionuclides such as ^{32}P or ^{35}S , or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding PP may be used for the diagnosis of disorders associated with expression of PP. Examples of such disorders include, but are not limited to, an immune system disorder, such as acquired immunodeficiency syndrome (AIDS), X-linked agammaglobinemia of
25 Bruton, common variable immunodeficiency (CVI), DiGeorge's syndrome (thymic hypoplasia), thymic dysplasia, isolated IgA deficiency, severe combined immunodeficiency disease (SCID), immunodeficiency with thrombocytopenia and eczema (Wiskott-Aldrich syndrome), Chediak-Higashi syndrome, chronic granulomatous diseases, hereditary angioneurotic edema, immunodeficiency associated with Cushing's disease, Addison's disease, adult respiratory distress syndrome, allergies,
30 ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's

syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic
5 sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma; a neurological disorder, such as epilepsy, ischemic cerebrovascular disease, stroke, cerebral neoplasms, Alzheimer's disease, Pick's disease, Huntington's disease, dementia, Parkinson's disease and other extrapyramidal disorders, amyotrophic lateral sclerosis and
10 other motor neuron disorders, progressive neural muscular atrophy, retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases, bacterial and viral meningitis, brain abscess, subdural empyema, epidural abscess, suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous system disease, prion diseases including kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome, fatal familial insomnia, nutritional and
15 metabolic diseases of the nervous system, neurofibromatosis, tuberous sclerosis, cerebelloretinal hemangioblastomatosis, encephalotrigeminal syndrome, mental retardation and other developmental disorders of the central nervous system including Down syndrome, cerebral palsy, neuroskeletal disorders, autonomic nervous system disorders, cranial nerve disorders, spinal cord diseases, muscular dystrophy and other neuromuscular disorders, peripheral nervous system disorders, dermatomyositis
20 and polymyositis, inherited, metabolic, endocrine, and toxic myopathies, myasthenia gravis, periodic paralysis, mental disorders including mood, anxiety, and schizophrenic disorders, seasonal affective disorder (SAD), akathisia, amnesia, catatonia, diabetic neuropathy, tardive dyskinesia, dystonias, paranoid psychoses, postherpetic neuralgia, Tourette's disorder, progressive supranuclear palsy, corticobasal degeneration, and familial frontotemporal dementia; a developmental disorder, such as
25 renal tubular acidosis, anemia, Cushing's syndrome, achondroplastic dwarfism, Duchenne and Becker muscular dystrophy, epilepsy, gonadal dysgenesis, WAGR syndrome (Wilms' tumor, aniridia, genitourinary abnormalities, and mental retardation), Smith-Magenis syndrome, myelodysplastic syndrome, hereditary mucoepithelial dysplasia, hereditary keratodermas, hereditary neuropathies such as Charcot-Marie-Tooth disease and neurofibromatosis, hypothyroidism, hydrocephalus, seizure
30 disorders such as Sydenham's chorea and cerebral palsy, spina bifida, anencephaly, craniorachischisis, congenital glaucoma, cataract, and sensorineural hearing loss; and a cell proliferative disorder, such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including 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. The polynucleotide sequences encoding PP may be used in Southern or northern
5 analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and multiformat ELISA-like assays; and in microarrays utilizing fluids or tissues from patients to detect altered PP expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding PP may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences
10 encoding PP 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 quantified 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 PP in the sample indicates the presence of
15 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 PP, a normal or standard profile for expression is established. This may be accomplished by combining body
20 fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding PP, 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 samples from
25 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 subject. The results obtained from
30 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 an abnormal amount of transcript (either under- or overexpressed) in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual

clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding PP may involve the use of PCR. These oligomers may be chemically synthesized, generated enzymatically, or produced in vitro. Oligomers will preferably contain a fragment of a polynucleotide encoding PP, or a fragment of a polynucleotide complementary to the polynucleotide encoding PP, 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 quantification of closely related DNA or RNA sequences.

In a particular aspect, oligonucleotide primers derived from the polynucleotide sequences encoding PP may be used to detect single nucleotide polymorphisms (SNPs). SNPs are substitutions, insertions and deletions that are a frequent cause of inherited or acquired genetic disease in humans. Methods of SNP detection include, but are not limited to, single-stranded conformation polymorphism (SSCP) and fluorescent SSCP (fSSCP) methods. In SSCP, oligonucleotide primers derived from the polynucleotide sequences encoding PP are used to amplify DNA using the polymerase chain reaction (PCR). The DNA may be derived, for example, from diseased or normal tissue, biopsy samples, bodily fluids, and the like. SNPs in the DNA cause differences in the secondary and tertiary structures of PCR products in single-stranded form, and these differences are detectable using gel electrophoresis in non-denaturing gels. In fSSCP, the oligonucleotide primers are fluorescently labeled, which allows detection of the amplimers in high-throughput equipment such as DNA sequencing machines. Additionally, sequence database analysis methods, termed *in silico* SNP (isSNP), are capable of identifying polymorphisms by comparing the sequence of individual overlapping DNA fragments which assemble into a common consensus sequence. These computer-based methods filter out sequence variations due to laboratory preparation of DNA and sequencing errors using statistical models and automated analyses of DNA sequence chromatograms. In the alternative, SNPs may be detected and characterized by mass spectrometry using, for example, the high throughput MASSARRAY system (Sequenom, Inc., San Diego CA).

Methods which may also be used to quantify the expression of PP 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; Duplaa, C. et al. (1993) *Anal. Biochem.* 212:229-236.) The speed of quantitation of multiple samples may be accelerated by running the assay in a high-throughput format where the oligomer or polynucleotide 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 elements on a microarray. The microarray can be used in transcript imaging techniques which monitor the relative expression levels of large numbers of genes simultaneously as described below. The microarray may also be used to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, to monitor progression/regression of disease as a function of gene expression, and to develop and monitor the activities of therapeutic agents in the treatment of disease. In particular, this information may be used to develop a pharmacogenomic profile of a patient in order to select the most appropriate and effective treatment regimen for that patient. For example, therapeutic agents which are highly effective and display the fewest side effects may be selected for a patient based on his/her pharmacogenomic profile.

In another embodiment, PP, fragments of PP, or antibodies specific for PP may be used as elements on a microarray. The microarray may be used to monitor or measure protein-protein interactions, drug-target interactions, and gene expression profiles, as described above.

A particular embodiment relates to the use of the polynucleotides of the present invention to generate a transcript image of a tissue or cell type. A transcript image represents the global pattern of gene expression by a particular tissue or cell type. Global gene expression patterns are analyzed by quantifying the number of expressed genes and their relative abundance under given conditions and at a given time. (See Seilhamer et al., "Comparative Gene Transcript Analysis," U.S. Patent Number 5,840,484, expressly incorporated by reference herein.) Thus a transcript image may be generated by hybridizing the polynucleotides of the present invention or their complements to the totality of transcripts or reverse transcripts of a particular tissue or cell type. In one embodiment, the hybridization takes place in high-throughput format, wherein the polynucleotides of the present invention or their complements comprise a subset of a plurality of elements on a microarray. The resultant transcript image would provide a profile of gene activity.

Transcript images may be generated using transcripts isolated from tissues, cell lines, biopsies, or other biological samples. The transcript image may thus reflect gene expression in vivo, as in the case of a tissue or biopsy sample, or in vitro, as in the case of a cell line.

Transcript images which profile the expression of the polynucleotides of the present invention may also be used in conjunction with in vitro model systems and preclinical evaluation of pharmaceuticals, as well as toxicological testing of industrial and naturally-occurring environmental compounds. All compounds induce characteristic gene expression patterns, frequently termed molecular fingerprints or toxicant signatures, which are indicative of mechanisms of action and toxicity

(Nuwaysir, E.F. et al. (1999) *Mol. Carcinog.* 24:153-159; Steiner, S. and N.L. Anderson (2000) *Toxicol. Lett.* 112-113:467-471, expressly incorporated by reference herein). If a test compound has a signature similar to that of a compound with known toxicity, it is likely to share those toxic properties. These fingerprints or signatures are most useful and refined when they contain expression information
5 from a large number of genes and gene families. Ideally, a genome-wide measurement of expression provides the highest quality signature. Even genes whose expression is not altered by any tested compounds are important as well, as the levels of expression of these genes are used to normalize the rest of the expression data. The normalization procedure is useful for comparison of expression data after treatment with different compounds. While the assignment of gene function to elements of a
10 toxicant signature aids in interpretation of toxicity mechanisms, knowledge of gene function is not necessary for the statistical matching of signatures which leads to prediction of toxicity. (See, for example, Press Release 00-02 from the National Institute of Environmental Health Sciences, released February 29, 2000, available at <http://www.niehs.nih.gov/oc/news/toxchip.htm>.) Therefore, it is important and desirable in toxicological screening using toxicant signatures to include all expressed
15 gene sequences.

In one embodiment, the toxicity of a test compound is assessed by treating a biological sample containing nucleic acids with the test compound. Nucleic acids that are expressed in the treated biological sample are hybridized with one or more probes specific to the polynucleotides of the present invention, so that transcript levels corresponding to the polynucleotides of the present
20 invention may be quantified. The transcript levels in the treated biological sample are compared with levels in an untreated biological sample. Differences in the transcript levels between the two samples are indicative of a toxic response caused by the test compound in the treated sample.

Another particular embodiment relates to the use of the polypeptide sequences of the present invention to analyze the proteome of a tissue or cell type. The term proteome refers to the global
25 pattern of protein expression in a particular tissue or cell type. Each protein component of a proteome can be subjected individually to further analysis. Proteome expression patterns, or profiles, are analyzed by quantifying the number of expressed proteins and their relative abundance under given conditions and at a given time. A profile of a cell's proteome may thus be generated by separating and analyzing the polypeptides of a particular tissue or cell type. In one embodiment, the separation is
30 achieved using two-dimensional gel electrophoresis, in which proteins from a sample are separated by isoelectric focusing in the first dimension, and then according to molecular weight by sodium dodecyl sulfate slab gel electrophoresis in the second dimension (Steiner and Anderson, *supra*). The proteins are visualized in the gel as discrete and uniquely positioned spots, typically by staining the gel with an agent such as Coomassie Blue or silver or fluorescent stains. The optical density of each protein spot is

generally proportional to the level of the protein in the sample. The optical densities of equivalently positioned protein spots from different samples, for example, from biological samples either treated or untreated with a test compound or therapeutic agent, are compared to identify any changes in protein spot density related to the treatment. The proteins in the spots are partially sequenced using, for
5 example, standard methods employing chemical or enzymatic cleavage followed by mass spectrometry. The identity of the protein in a spot may be determined by comparing its partial sequence, preferably of at least 5 contiguous amino acid residues, to the polypeptide sequences of the present invention. In some cases, further sequence data may be obtained for definitive protein identification.

A proteomic profile may also be generated using antibodies specific for PP to quantify the
10 levels of PP expression. In one embodiment, the antibodies are used as elements on a microarray, and protein expression levels are quantified by exposing the microarray to the sample and detecting the levels of protein bound to each array element (Lueking, A. et al. (1999) *Anal. Biochem.* 270:103-111; Mendozze, L.G. et al. (1999) *Biotechniques* 27:778-788). Detection may be performed by a variety of methods known in the art, for example, by reacting the proteins in the sample with a thiol- or amino-
15 reactive fluorescent compound and detecting the amount of fluorescence bound at each array element.

Toxicant signatures at the proteome level are also useful for toxicological screening, and should be analyzed in parallel with toxicant signatures at the transcript level. There is a poor correlation between transcript and protein abundances for some proteins in some tissues (Anderson, N.L. and J. Seilhamer (1997) *Electrophoresis* 18:533-537), so proteome toxicant signatures may be useful in the
20 analysis of compounds which do not significantly affect the transcript image, but which alter the proteomic profile. In addition, the analysis of transcripts in body fluids is difficult, due to rapid degradation of mRNA, so proteomic profiling may be more reliable and informative in such cases.

In another embodiment, the toxicity of a test compound is assessed by treating a biological sample containing proteins with the test compound. Proteins that are expressed in the treated biological
25 sample are separated so that the amount of each protein can be quantified. The amount of each protein is compared to the amount of the corresponding protein in an untreated biological sample. A difference in the amount of protein between the two samples is indicative of a toxic response to the test compound in the treated sample. Individual proteins are identified by sequencing the amino acid residues of the individual proteins and comparing these partial sequences to the polypeptides of the present invention.

30 In another embodiment, the toxicity of a test compound is assessed by treating a biological sample containing proteins with the test compound. Proteins from the biological sample are incubated with antibodies specific to the polypeptides of the present invention. The amount of protein recognized by the antibodies is quantified. The amount of protein in the treated biological sample is compared with the amount in an untreated biological sample. A difference in the amount of protein between the two

samples is indicative of a toxic response to the test compound in the treated sample.

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) Proc. Natl. Acad. Sci. USA 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. 5 (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. USA 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.) Various types of microarrays are well known and thoroughly described in DNA Microarrays: A Practical Approach, M. Schena, ed. (1999) Oxford University Press, London, hereby expressly incorporated by reference.

In another embodiment of the invention, nucleic acid sequences encoding PP may be used to 10 generate hybridization probes useful in mapping the naturally occurring genomic sequence. Either coding or noncoding sequences may be used, and in some instances, noncoding sequences may be preferable over coding sequences. For example, conservation of a coding sequence among members of a multi-gene family may potentially cause undesired cross hybridization during chromosomal mapping. The sequences may be mapped to a particular chromosome, to a specific region of a 15 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. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355; Price, C.M. (1993) Blood Rev. 7:127-134; and Trask, B.J. (1991) Trends Genet. 7:149-154.) Once mapped, the nucleic acid sequences of the invention may be used to develop genetic 20 linkage maps, for example, which correlate the inheritance of a disease state with the inheritance of a particular chromosome region or restriction fragment length polymorphism (RFLP). (See, for example, Lander, E.S. and D. Botstein (1986) Proc. Natl. Acad. Sci. USA 83:7353-7357.)

Fluorescent in situ hybridization (FISH) may be correlated with other physical and genetic map data. (See, e.g., Heinz-Ulrich, et al. (1995) in Meyers, supra, pp. 965-968.) Examples of genetic map 25 data can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) World Wide Web site. Correlation between the location of the gene encoding PP on a physical map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA associated with that disorder and thus may further positional cloning efforts.

In situ hybridization of chromosomal preparations and physical mapping techniques, such as 30 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 exact chromosomal locus is not known. This information is valuable to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the gene or genes responsible for a disease or syndrome have 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 instant invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc.,
5 among normal, carrier, or affected individuals.

In another embodiment of the invention, PP, its catalytic or immunogenic fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes
10 between PP 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 small test compounds are synthesized on a solid substrate. The test compounds are reacted with PP, or fragments thereof, and
15 washed. Bound PP is then detected by methods well known in the art. Purified PP can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

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

In additional embodiments, the nucleotide sequences which encode PP 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
25 properties as the triplet genetic code and specific base pair interactions.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

30 The disclosures of all patents, applications and publications, mentioned above and below, in particular U.S. Serial Number 60/212,447, U.S. Serial Number 60/213,746, U.S. Serial Number 60/215,210, U.S. Serial Number 60/216,529, U.S. Serial Number 60/218,080, and U.S. Serial Number 60/220,117, are expressly incorporated by reference herein.

EXAMPLES

I. Construction of cDNA Libraries

Incyte cDNAs were derived from cDNA libraries described in the LIFESEQ GOLD database (Incyte Genomics, Palo Alto CA) and shown in Table 4, column 5. Some tissues were homogenized and lysed in guanidinium isothiocyanate, while others were homogenized and lysed in phenol or in a suitable mixture of denaturants, such as TRIZOL (Life Technologies), a monophasic solution of phenol and guanidine isothiocyanate. The resulting lysates were centrifuged over CsCl cushions or extracted with chloroform. RNA was precipitated from the lysates with either isopropanol or sodium acetate and ethanol, or by other routine methods.

Phenol extraction and precipitation of RNA were repeated as necessary to increase RNA purity. In some cases, RNA was treated with DNase. For most libraries, poly(A)+ RNA was isolated using oligo d(T)-coupled paramagnetic particles (Promega), OLIGOTEX latex particles (QIAGEN, Chatsworth CA), or an OLIGOTEX mRNA purification kit (QIAGEN). Alternatively, RNA was isolated directly from tissue lysates using other RNA isolation kits, e.g., the POLY(A)PURE mRNA purification kit (Ambion, Austin TX).

In some cases, Stratagene was provided with RNA and constructed the corresponding cDNA libraries. Otherwise, cDNA was synthesized and cDNA libraries were constructed with the UNIZAP vector system (Stratagene) or SUPERSCRIPt plasmid system (Life Technologies), using the recommended procedures or similar methods known in the art. (See, e.g., Ausubel, 1997, *supra*, units 5.1-6.6.) Reverse transcription was initiated using oligo d(T) or random primers. Synthetic oligonucleotide adapters were ligated to double stranded cDNA, and the cDNA was digested with the appropriate restriction enzyme or enzymes. For most libraries, the cDNA was size-selected (300-1000 bp) using SEPHACRYL S1000, SEPHAROSE CL2B, or SEPHAROSE CL4B column chromatography (Amersham Pharmacia Biotech) or preparative agarose gel electrophoresis. cDNAs were ligated into compatible restriction enzyme sites of the polylinker of a suitable plasmid, e.g., PBLUESCRIPt plasmid (Stratagene), PSPORT1 plasmid (Life Technologies), PCDNA2.1 plasmid (Invitrogen, Carlsbad CA), PBK-CMV plasmid (Stratagene), or pINCY (Incyte Genomics, Palo Alto CA), or derivatives thereof. Recombinant plasmids were transformed into competent *E. coli* cells including XL1-Blue, XL1-BlueMRF, or SOLR from Stratagene or DH5 α , DH10B, or Electromax DH10B from Life Technologies.

II. Isolation of cDNA Clones

Plasmids obtained as described in Example I were recovered from host cells by *in vivo* excision using the UNIZAP vector system (Stratagene) or by cell lysis. Plasmids were purified using at least one of the following: a Magic or WIZARD Minipreps DNA purification system (Promega); an AGTC

Miniprep purification kit (Edge Biosystems, Gaithersburg MD); and QIAWELL 8 Plasmid, QIAWELL 8 Plus Plasmid, QIAWELL 8 Ultra Plasmid purification systems or the R.E.A.L. PREP 96 plasmid purification kit from QIAGEN. Following precipitation, plasmids were resuspended in 0.1 ml of distilled water and stored, with or without lyophilization, at 4°C.

5 Alternatively, plasmid DNA was amplified from host cell lysates using direct link PCR in a high-throughput format (Rao, V.B. (1994) Anal. Biochem. 216:1-14). Host cell lysis and thermal cycling steps were carried out in a single reaction mixture. Samples were processed and stored in 384-well plates, and the concentration of amplified plasmid DNA was quantified fluorometrically using PICOGREEN dye (Molecular Probes, Eugene OR) and a FLUOROSKAN II fluorescence scanner
10 (Labsystems Oy, Helsinki, Finland).

III. Sequencing and Analysis

Incyte cDNA recovered in plasmids as described in Example II were sequenced as follows. Sequencing reactions were processed using standard methods or high-throughput instrumentation such as the ABI CATALYST 800 (Applied Biosystems) thermal cycler or the PTC-200 thermal cycler
15 (MJ Research) in conjunction with the HYDRA microdispenser (Robbins Scientific) or the MICROLAB 2200 (Hamilton) liquid transfer system. cDNA sequencing reactions were prepared using reagents provided by Amersham Pharmacia Biotech or supplied in ABI sequencing kits such as the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Applied Biosystems). Electrophoretic separation of cDNA sequencing reactions and detection of labeled polynucleotides were
20 carried out using the MEGABACE 1000 DNA sequencing system (Molecular Dynamics); the ABI PRISM 373 or 377 sequencing system (Applied Biosystems) in conjunction with standard ABI protocols and base calling software; or other sequence analysis systems known in the art. Reading frames within the cDNA sequences were identified using standard methods (reviewed in Ausubel, 1997, supra, unit 7.7). Some of the cDNA sequences were selected for extension using the techniques
25 disclosed in Example VIII.

The polynucleotide sequences derived from Incyte cDNAs were validated by removing vector, linker, and poly(A) sequences and by masking ambiguous bases, using algorithms and programs based on BLAST, dynamic programming, and dinucleotide nearest neighbor analysis. The Incyte cDNA sequences or translations thereof were then queried against a selection of public databases such as the
30 GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases, and BLOCKS, PRINTS, DOMO, PRODOM, and hidden Markov model (HMM)-based protein family databases such as PFAM. (HMM is a probabilistic approach which analyzes consensus primary structures of gene families. See, for example, Eddy, S.R. (1996) Curr. Opin. Struct. Biol. 6:361-365.) The queries were performed using programs based on BLAST, FASTA, BLIMPS, and HMMER. The Incyte cDNA

sequences were assembled to produce full length polynucleotide sequences. Alternatively, GenBank cDNAs, GenBank ESTs, stitched sequences, stretched sequences, or Genscan-predicted coding sequences (see Examples IV and V) were used to extend Incyte cDNA assemblages to full length. Assembly was performed using programs based on Phred, Phrap, and Consed, and cDNA assemblages were screened for open reading frames using programs based on GeneMark, BLAST, and FASTA. The full length polynucleotide sequences were translated to derive the corresponding full length polypeptide sequences. Alternatively, a polypeptide of the invention may begin at any of the methionine residues of the full length translated polypeptide. Full length polypeptide sequences were subsequently analyzed by querying against databases such as the GenBank protein databases (genpept), SwissProt, BLOCKS, PRINTS, DOMO, PRODOM, Prosite, and hidden Markov model (HMM)-based protein family databases such as PFAM. Full length polynucleotide sequences are also analyzed using MACDNASIS PRO software (Hitachi Software Engineering, South San Francisco CA) and LASERGENE software (DNASTAR). Polynucleotide and polypeptide sequence alignments are generated using default parameters specified by the CLUSTAL algorithm as incorporated into the MEGALIGN multisequence alignment program (DNASTAR), which also calculates the percent identity between aligned sequences.

Table 7 summarizes the tools, programs, and algorithms used for the analysis and assembly of Incyte cDNA and full length sequences and provides applicable descriptions, references, and threshold parameters. The first column of Table 7 shows the tools, programs, and algorithms used, the second column provides brief descriptions thereof, the third column presents appropriate references, all of which are incorporated by reference herein in their entirety, and the fourth column presents, where applicable, the scores, probability values, and other parameters used to evaluate the strength of a match between two sequences (the higher the score or the lower the probability value, the greater the identity between two sequences).

The programs described above for the assembly and analysis of full length polynucleotide and polypeptide sequences were also used to identify polynucleotide sequence fragments from SEQ ID NO:10-18. Fragments from about 20 to about 4000 nucleotides which are useful in hybridization and amplification technologies are described in Table 4, column 4.

IV. Identification and Editing of Coding Sequences from Genomic DNA

Putative protein phosphatases were initially identified by running the Genscan gene identification program against public genomic sequence databases (e.g., gbpri and gbhtg). Genscan is a general-purpose gene identification program which analyzes genomic DNA sequences from a variety of organisms (See Burge, C. and S. Karlin (1997) *J. Mol. Biol.* 268:78-94, and Burge, C. and S. Karlin (1998) *Curr. Opin. Struct. Biol.* 8:346-354). The program concatenates predicted exons to form an

assembled cDNA sequence extending from a methionine to a stop codon. The output of Genscan is a FASTA database of polynucleotide and polypeptide sequences. The maximum range of sequence for Genscan to analyze at once was set to 30 kb. To determine which of these Genscan predicted cDNA sequences encode protein phosphatases, the encoded polypeptides were analyzed by querying against PFAM models for protein phosphatases. Potential protein phosphatases were also identified by 5
homology to Incyte cDNA sequences that had been annotated as protein phosphatases. These selected Genscan-predicted sequences were then compared by BLAST analysis to the genpept and gbpr public databases. Where necessary, the Genscan-predicted sequences were then edited by comparison to the top BLAST hit from genpept to correct errors in the sequence predicted by Genscan, such as extra or 10
omitted exons. BLAST analysis was also used to find any Incyte cDNA or public cDNA coverage of the Genscan-predicted sequences, thus providing evidence for transcription. When Incyte cDNA coverage was available, this information was used to correct or confirm the Genscan predicted sequence. Full length polynucleotide sequences were obtained by assembling Genscan-predicted coding sequences with Incyte cDNA sequences and/or public cDNA sequences using the assembly process 15
described in Example III. Alternatively, full length polynucleotide sequences were derived entirely from edited or unedited Genscan-predicted coding sequences.

V. Assembly of Genomic Sequence Data with cDNA Sequence Data

"Stitched" Sequences

Partial cDNA sequences were extended with exons predicted by the Genscan gene identification 20
program described in Example IV. Partial cDNAs assembled as described in Example III were mapped to genomic DNA and parsed into clusters containing related cDNAs and Genscan exon predictions from one or more genomic sequences. Each cluster was analyzed using an algorithm based on graph theory and dynamic programming to integrate cDNA and genomic information, generating possible splice variants that were subsequently confirmed, edited, or extended to create a full length sequence. 25
Sequence intervals in which the entire length of the interval was present on more than one sequence in the cluster were identified, and intervals thus identified were considered to be equivalent by transitivity. For example, if an interval was present on a cDNA and two genomic sequences, then all three intervals were considered to be equivalent. This process allows unrelated but consecutive genomic sequences to be brought together, bridged by cDNA sequence. Intervals thus identified were then "stitched" together 30
by the stitching algorithm in the order that they appear along their parent sequences to generate the longest possible sequence, as well as sequence variants. Linkages between intervals which proceed along one type of parent sequence (cDNA to cDNA or genomic sequence to genomic sequence) were given preference over linkages which change parent type (cDNA to genomic sequence). The resultant stitched sequences were translated and compared by BLAST analysis to the genpept and gbpr public

databases. Incorrect exons predicted by Genscan were corrected by comparison to the top BLAST hit from genpept. Sequences were further extended with additional cDNA sequences, or by inspection of genomic DNA, when necessary.

“Stretched” Sequences

5 Partial DNA sequences were extended to full length with an algorithm based on BLAST analysis. First, partial cDNAs assembled as described in Example III were queried against public databases such as the GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases using the BLAST program. The nearest GenBank protein homolog was then compared by BLAST analysis to either Incyte cDNA sequences or GenScan exon predicted sequences described in Example IV. A
10 chimeric protein was generated by using the resultant high-scoring segment pairs (HSPs) to map the translated sequences onto the GenBank protein homolog. Insertions or deletions may occur in the chimeric protein with respect to the original GenBank protein homolog. The GenBank protein homolog, the chimeric protein, or both were used as probes to search for homologous genomic sequences from the public human genome databases. Partial DNA sequences were therefore “stretched” or extended by the
15 addition of homologous genomic sequences. The resultant stretched sequences were examined to determine whether it contained a complete gene.

VI. Chromosomal Mapping of PP Encoding Polynucleotides

The sequences which were used to assemble SEQ ID NO:10-18 were compared with sequences from the Incyte LIFESEQ database and public domain databases using BLAST and other
20 implementations of the Smith-Waterman algorithm. Sequences from these databases that matched SEQ ID NO:10-18 were assembled into clusters of contiguous and overlapping sequences using assembly algorithms such as Phrap (Table 7). Radiation hybrid and genetic mapping data available from public resources such as the Stanford Human Genome Center (SHGC), Whitehead Institute for Genome Research (WIGR), and Généthon were used to determine if any of the clustered sequences had been
25 previously mapped. Inclusion of a mapped sequence in a cluster resulted in the assignment of all sequences of that cluster, including its particular SEQ ID NO:, to that map location.

Map locations are represented by ranges, or intervals, of human chromosomes. The map position of an interval, in centiMorgans, is measured relative to the terminus of the chromosome’s p-arm. (The centiMorgan (cM) is a unit of measurement based on recombination frequencies between
30 chromosomal markers. On average, 1 cM is roughly equivalent to 1 megabase (Mb) of DNA in humans, although this can vary widely due to hot and cold spots of recombination.) The cM distances are based on genetic markers mapped by Généthon which provide boundaries for radiation hybrid markers whose sequences were included in each of the clusters. Human genome maps and other resources available to the public, such as the NCBI “GeneMap’99” World Wide Web site

(<http://www.ncbi.nlm.nih.gov/genemap/>), can be employed to determine if previously identified disease genes map within or in proximity to the intervals indicated above.

VII. Analysis of Polynucleotide Expression

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, *supra*, ch. 7; Ausubel (1995) *supra*, ch. 4 and 16.)

Analogous computer techniques applying BLAST were used to search for identical or related molecules in cDNA databases such as GenBank or LIFESEQ (Incyte Genomics). This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or similar. The basis of the search is the product score, which is defined as:

$$\frac{\text{BLAST Score} \times \text{Percent Identity}}{5 \times \text{minimum} \{ \text{length}(\text{Seq. 1}), \text{length}(\text{Seq. 2}) \}}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. The product score is a normalized value between 0 and 100, and is calculated as follows: the BLAST score is multiplied by the percent nucleotide identity and the product is divided by (5 times the length of the shorter of the two sequences). The BLAST score is calculated by assigning a score of +5 for every base that matches in a high-scoring segment pair (HSP), and -4 for every mismatch. Two sequences may share more than one HSP (separated by gaps). If there is more than one HSP, then the pair with the highest BLAST score is used to calculate the product score. The product score represents a balance between fractional overlap and quality in a BLAST alignment. For example, a product score of 100 is produced only for 100% identity over the entire length of the shorter of the two sequences being compared. A product score of 70 is produced either by 100% identity and 70% overlap at one end, or by 88% identity and 100% overlap at the other. A product score of 50 is

hemic and immune system; liver; musculoskeletal system; nervous system; pancreas; respiratory system; sense organs; skin; stomatognathic system; unclassified/mixed; or urinary tract. The number of libraries in each category is counted and divided by the total number of libraries across all categories. Similarly, each human tissue is classified into one of the following disease/condition categories: cancer, cell line, developmental, inflammation, neurological, trauma, cardiovascular, pooled, and other, and the number of libraries in each category is counted and divided by the total number of libraries across all categories. The resulting percentages reflect the tissue- and disease-specific expression of cDNA encoding PP. cDNA sequences and cDNA library/tissue information are found in the LIFESEQ GOLD database (Incyte Genomics, Palo Alto CA).

10 VIII. Extension of PP Encoding Polynucleotides

Full length polynucleotide sequences were also produced by extension of an appropriate fragment of the full length molecule using oligonucleotide primers designed from this fragment. One primer was synthesized to initiate 5' extension of the known fragment, and the other primer was synthesized to initiate 3' extension of the known fragment. The initial primers were designed using OLIGO 4.06 software (National Biosciences), 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 structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries were used to extend the sequence. If more than one extension was necessary or desired, additional or nested sets of primers were designed.

High fidelity amplification was obtained by PCR using methods well known in the art. PCR was performed in 96-well plates using the PTC-200 thermal cycler (MJ Research, Inc.). The reaction mix contained DNA template, 200 nmol of each primer, reaction buffer containing Mg²⁺, (NH₄)₂SO₄, and 2-mercaptoethanol, Taq DNA polymerase (Amersham Pharmacia Biotech), ELONGASE enzyme (Life Technologies), and Pfu DNA polymerase (Stratagene), with the following parameters for primer pair PCI A and PCI B: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C. In the alternative, the parameters for primer pair T7 and SK+ were as follows: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 57°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C.

The concentration of DNA in each well was determined by dispensing 100 µl PICOGREEN quantitation reagent (0.25% (v/v) PICOGREEN; Molecular Probes, Eugene OR) dissolved in 1X TE and 0.5 µl of undiluted PCR product into each well of an opaque fluorimeter plate (Corning Costar, Acton MA), allowing the DNA to bind to the reagent. The plate was scanned in a Fluoroskan II

(Labsystems Oy, Helsinki, Finland) to measure the fluorescence of the sample and to quantify the concentration of DNA. A 5 μ l to 10 μ l aliquot of the reaction mixture was analyzed by electrophoresis on a 1 % agarose gel to determine which reactions were successful in extending the sequence.

The extended nucleotides were desalted and concentrated, transferred to 384-well plates, 5 digested with CviJI cholera virus endonuclease (Molecular Biology Research, Madison WI), and sonicated or sheared prior to religation into pUC 18 vector (Amersham Pharmacia Biotech). For shotgun sequencing, the digested nucleotides were separated on low concentration (0.6 to 0.8%) agarose gels, fragments were excised, and agar digested with Agar ACE (Promega). Extended clones were 10 religated using T4 ligase (New England Biolabs, Beverly MA) into pUC 18 vector (Amersham Pharmacia Biotech), treated with Pfu DNA polymerase (Stratagene) to fill-in restriction site overhangs, and transfected into competent *E. coli* cells. Transformed cells were selected on antibiotic-containing media, and individual colonies were picked and cultured overnight at 37°C in 384-well plates in LB/2x carb liquid media.

The cells were lysed, and DNA was amplified by PCR using Taq DNA polymerase (Amersham 15 Pharmacia Biotech) and Pfu DNA polymerase (Stratagene) with the following parameters: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 72°C, 2 min; Step 5: steps 2, 3, and 4 repeated 29 times; Step 6: 72°C, 5 min; Step 7: storage at 4°C. DNA was quantified by PICOGREEN reagent (Molecular Probes) as described above. Samples with low DNA recoveries were reamplified using the same conditions as described above. Samples were diluted with 20% dimethylsulfoxide (1:2, 20 v/v), and sequenced using DYENAMIC energy transfer sequencing primers and the DYENAMIC DIRECT kit (Amersham Pharmacia Biotech) or the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Applied Biosystems).

In like manner, full length polynucleotide sequences are verified using the above procedure or are used to obtain 5' regulatory sequences using the above procedure along with oligonucleotides 25 designed for such extension, and an appropriate genomic library.

IX. Labeling and Use of Individual Hybridization Probes

Hybridization probes derived from SEQ ID NO:10-18 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. 30 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 μ Ci of [γ -³²P] adenosine triphosphate (Amersham Pharmacia Biotech), 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 (Amersham Pharmacia Biotech). An aliquot containing 10⁷ counts per

minute of the labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA digested with one of the following endonucleases: Ase I, Bgl II, Eco RI, Pst I, Xba I, or Pvu II (DuPont NEN).

The DNA from each digest is fractionated on a 0.7% 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 conditions of up to, for example, 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. Hybridization patterns are visualized using autoradiography or an alternative imaging means and compared.

10 X. Microarrays

The linkage or synthesis of array elements upon a microarray can be achieved utilizing photolithography, piezoelectric printing (ink-jet printing, See, e.g., Baldeschweiler, *supra.*), mechanical microspotting technologies, and derivatives thereof. The substrate in each of the aforementioned technologies should be uniform and solid with a non-porous surface (Schena (1999), *supra.*). Suggested substrates include silicon, silica, glass slides, glass chips, and silicon wafers. Alternatively, a procedure 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 using available methods and machines well known to those of ordinary skill in the art and may contain any appropriate number of elements. (See, e.g., Schena, M. et al. (1995) *Science* 270:467-470; Shalon, D. et al. (1996) *Genome Res.* 6:639-645; Marshall, A. and J. Hodgson (1998) *Nat. Biotechnol.* 16:27-31.)

Full length cDNAs, Expressed Sequence Tags (ESTs), or fragments or oligomers thereof may comprise the elements of the microarray. Fragments or oligomers suitable for hybridization can be selected using software well known in the art such as LASERGENE software (DNASTAR). The array elements are hybridized with polynucleotides in a biological sample. The polynucleotides in the biological sample are conjugated to a fluorescent label or other molecular tag for ease of detection. After hybridization, nonhybridized nucleotides from the biological sample are removed, and a fluorescence scanner is used to detect hybridization at each array element. Alternatively, laser desorption and mass spectrometry may be used for detection of hybridization. The degree of complementarity and the relative abundance of each polynucleotide which hybridizes to an element on the microarray may be assessed. In one embodiment, microarray preparation and usage is described in detail below.

Tissue or Cell Sample Preparation

Total RNA is isolated from tissue samples using the guanidinium thiocyanate method and

poly(A)⁺ RNA is purified using the oligo-(dT) cellulose method. Each poly(A)⁺ RNA sample is reverse transcribed using MMLV reverse-transcriptase, 0.05 pg/ μ l oligo-(dT) primer (21mer), 1X first strand buffer, 0.03 units/ μ l RNase inhibitor, 500 μ M dATP, 500 μ M dGTP, 500 μ M dTTP, 40 μ M dCTP, 40 μ M dCTP-Cy3 (BDS) or dCTP-Cy5 (Amersham Pharmacia Biotech). The reverse transcription reaction is performed in a 25 ml volume containing 200 ng poly(A)⁺ RNA with GEMBRIGHT kits (Incyte). Specific control poly(A)⁺ RNAs are synthesized by *in vitro* transcription from non-coding yeast genomic DNA. After incubation at 37° C for 2 hr, each reaction sample (one with Cy3 and another with Cy5 labeling) is treated with 2.5 ml of 0.5M sodium hydroxide and incubated for 20 minutes at 85° C to stop the reaction and degrade the RNA. Samples are purified using two successive CHROMA SPIN 30 gel filtration spin columns (CLONTECH Laboratories, Inc. (CLONTECH), Palo Alto CA) and after combining, both reaction samples are ethanol precipitated using 1 ml of glycogen (1 mg/ml), 60 ml sodium acetate, and 300 ml of 100% ethanol. The sample is then dried to completion using a SpeedVAC (Savant Instruments Inc., Holbrook NY) and resuspended in 14 μ l 5X SSC/0.2% SDS.

15 Microarray Preparation

Sequences of the present invention are used to generate array elements. Each array element is amplified from bacterial cells containing vectors with cloned cDNA inserts. PCR amplification uses primers complementary to the vector sequences flanking the cDNA insert. Array elements are amplified in thirty cycles of PCR from an initial quantity of 1-2 ng to a final quantity greater than 5 μ g. Amplified array elements are then purified using SEPHACRYL-400 (Amersham Pharmacia Biotech).

Purified array elements are immobilized on polymer-coated glass slides. Glass microscope slides (Corning) are cleaned by ultrasound in 0.1% SDS and acetone, with extensive distilled water washes between and after treatments. Glass slides are etched in 4% hydrofluoric acid (VWR Scientific Products Corporation (VWR), West Chester PA), washed extensively in distilled water, and coated with 0.05% aminopropyl silane (Sigma) in 95% ethanol. Coated slides are cured in a 110° C oven.

Array elements are applied to the coated glass substrate using a procedure described in US Patent No. 5,807,522, incorporated herein by reference. 1 μ l of the array element DNA, at an average concentration of 100 ng/ μ l, is loaded into the open capillary printing element by a high-speed robotic apparatus. The apparatus then deposits about 5 nl of array element sample per slide.

Microarrays are UV-crosslinked using a STRATALINKER UV-crosslinker (Stratagene). Microarrays are washed at room temperature once in 0.2% SDS and three times in distilled water. Non-specific binding sites are blocked by incubation of microarrays in 0.2% casein in phosphate buffered saline (PBS) (Tropix, Inc., Bedford MA) for 30 minutes at 60° C followed by washes in

0.2% SDS and distilled water as before.

Hybridization

Hybridization reactions contain 9 μ l of sample mixture consisting of 0.2 μ g each of Cy3 and Cy5 labeled cDNA synthesis products in 5X SSC, 0.2% SDS hybridization buffer. The sample
5 mixture is heated to 65° C for 5 minutes and is aliquoted onto the microarray surface and covered with an 1.8 cm² coverslip. The arrays are transferred to a waterproof chamber having a cavity just slightly larger than a microscope slide. The chamber is kept at 100% humidity internally by the addition of 140 μ l of 5X SSC in a corner of the chamber. The chamber containing the arrays is incubated for about 6.5 hours at 60° C. The arrays are washed for 10 min at 45° C in a first wash buffer (1X SSC,
10 0.1% SDS), three times for 10 minutes each at 45° C in a second wash buffer (0.1X SSC), and dried.

Detection

Reporter-labeled hybridization complexes are detected with a microscope equipped with an Innova 70 mixed gas 10 W laser (Coherent, Inc., Santa Clara CA) capable of generating spectral lines at 488 nm for excitation of Cy3 and at 632 nm for excitation of Cy5. The excitation laser light is
15 focused on the array using a 20X microscope objective (Nikon, Inc., Melville NY). The slide containing the array is placed on a computer-controlled X-Y stage on the microscope and raster-scanned past the objective. The 1.8 cm x 1.8 cm array used in the present example is scanned with a resolution of 20 micrometers.

In two separate scans, a mixed gas multiline laser excites the two fluorophores sequentially.
20 Emitted light is split, based on wavelength, into two photomultiplier tube detectors (PMT R1477, Hamamatsu Photonics Systems, Bridgewater NJ) corresponding to the two fluorophores. Appropriate filters positioned between the array and the photomultiplier tubes are used to filter the signals. The emission maxima of the fluorophores used are 565 nm for Cy3 and 650 nm for Cy5. Each array is typically scanned twice, one scan per fluorophore using the appropriate filters at the laser source,
25 although the apparatus is capable of recording the spectra from both fluorophores simultaneously.

The sensitivity of the scans is typically calibrated using the signal intensity generated by a cDNA control species added to the sample mixture at a known concentration. A specific location on the array contains a complementary DNA sequence, allowing the intensity of the signal at that location to be correlated with a weight ratio of hybridizing species of 1:100,000. When two samples
30 from different sources (e.g., representing test and control cells), each labeled with a different fluorophore, are hybridized to a single array for the purpose of identifying genes that are differentially expressed, the calibration is done by labeling samples of the calibrating cDNA with the two fluorophores and adding identical amounts of each to the hybridization mixture.

The output of the photomultiplier tube is digitized using a 12-bit RTI-835H analog-to-digital
35 (A/D) conversion board (Analog Devices, Inc., Norwood MA) installed in an IBM-compatible PC

computer. The digitized data are displayed as an image where the signal intensity is mapped using a linear 20-color transformation to a pseudocolor scale ranging from blue (low signal) to red (high signal). The data is also analyzed quantitatively. Where two different fluorophores are excited and measured simultaneously, the data are first corrected for optical crosstalk (due to overlapping
5 emission spectra) between the fluorophores using each fluorophore's emission spectrum.

A grid is superimposed over the fluorescence signal image such that the signal from each spot is centered in each element of the grid. The fluorescence signal within each element is then integrated to obtain a numerical value corresponding to the average intensity of the signal. The software used for signal analysis is the GEMTOOLS gene expression analysis program (Incyte).

10 **XI. Complementary Polynucleotides**

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

XII. Expression of PP

20 Expression and purification of PP is achieved using bacterial or virus-based expression systems. For expression of PP 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 not limited to, the *trp-lac (tac)* hybrid promoter and the T5 or T7 bacteriophage promoter in conjunction with the *lac* operator regulatory element.
25 Recombinant vectors are transformed into suitable bacterial hosts, e.g., BL21(DE3). Antibiotic resistant bacteria express PP upon induction with isopropyl beta-D-thiogalactopyranoside (IPTG). Expression of PP in eukaryotic cells is achieved by infecting insect or mammalian cell lines with recombinant Autographica californica nuclear polyhedrosis virus (AcMNPV), commonly known as baculovirus. The nonessential polyhedrin gene of baculovirus is replaced with cDNA encoding PP by
30 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. 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, PP is synthesized as a fusion protein with, e.g., 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 (Amersham Pharmacia Biotech). Following purification, the GST moiety can be proteolytically cleaved from PP at specifically engineered sites. FLAG, an 8-amino acid peptide, enables immunoaffinity purification using commercially available monoclonal and polyclonal anti-FLAG antibodies (Eastman Kodak). 6-His, a stretch of six consecutive histidine residues, enables purification on metal-chelate resins (QIAGEN). Methods for protein expression and purification are discussed in Ausubel (1995, supra, ch. 10 and 16). Purified PP obtained by these methods can be used directly in the assays shown in Examples XVI, XVII, XVIII, and XIX, where applicable.

XIII. Functional Assays

PP function is assessed by expressing the sequences encoding PP 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) and PCR3.1 (Invitrogen, Carlsbad CA), both of which contain the cytomegalovirus promoter. 5-10 μg of recombinant vector are transiently transfected into a human cell line, for example, an endothelial or hematopoietic cell line, using either liposome formulations or electroporation. 1-2 μg 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), 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 the apoptotic state of the cells and other cellular properties. 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 PP on gene expression can be assessed using highly purified populations of cells transfected with sequences encoding PP 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 PP and other genes of interest can be analyzed by northern analysis or microarray techniques.

10 **XIV. Production of PP Specific Antibodies**

PP 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 PP amino acid sequence is analyzed using LASERGENE software (DNASTAR) 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, 1995, *supra*, ch. 11.)

Typically, oligopeptides of about 15 residues in length are synthesized using an ABI 431A peptide synthesizer (Applied Biosystems) using FMOC chemistry and coupled to KLH (Sigma-Aldrich, St. Louis MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase immunogenicity. (See, e.g., Ausubel, 1995, *supra*.) Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for antipeptide and anti-PP activity by, for example, binding the peptide or PP to a substrate, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG.

25 **XV. Purification of Naturally Occurring PP Using Specific Antibodies**

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

Media containing PP are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of PP (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/PP binding (e.g., a

buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and PP is collected.

XVI. Identification of Molecules Which Interact with PP

PP, or biologically active fragments thereof, are labeled with ^{125}I Bolton-Hunter reagent. (See, 5 e.g., Bolton A.E. and W.M. Hunter (1973) *Biochem. J.* 133:529-539.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled PP, washed, and any wells with labeled PP complex are assayed. Data obtained using different concentrations of PP are used to calculate values for the number, affinity, and association of PP with the candidate molecules.

Alternatively, molecules interacting with PP are analyzed using the yeast two-hybrid system 10 as described in Fields, S. and O. Song (1989) *Nature* 340:245-246, or using commercially available kits based on the two-hybrid system, such as the MATCHMAKER system (Clontech).

PP may also be used in the PATHCALLING process (CuraGen Corp., New Haven CT) which employs the yeast two-hybrid system in a high-throughput manner to determine all interactions between the proteins encoded by two large libraries of genes (Nandabalan, K. et al. (2000) U.S. Patent No. 15 6,057,101).

XVII. Demonstration of PP Activity

PP activity is measured by the hydrolysis of para-nitrophenyl phosphate (PNPP). PP is incubated together with PNPP in HEPES buffer pH 7.5, in the presence of 0.1% β -mercaptoethanol at 37°C for 60 min. The reaction is stopped by the addition of 6 ml of 10 N NaOH (Diamond, R.H. et al. 20 (1994) *Mol. Cell. Biol.* 14:3752-62). Alternatively, acid phosphatase activity of PP is demonstrated by incubating PP-containing extract with 100 μl of 10 mM PNPP in 0.1 M sodium citrate, pH 4.5, and 50 μl of 40 mM NaCl at 37°C for 20 min. The reaction is stopped by the addition of 0.5 ml of 0.4 M glycine/NaOH, pH 10.4 (Saftig, P. et al. (1997) *J. Biol. Chem.* 272:18628-18635). The increase in light absorbance at 410 nm resulting from the hydrolysis of PNPP is measured using a 25 spectrophotometer. The increase in light absorbance is proportional to the activity of PP in the assay.

In the alternative, PP activity is determined by measuring the amount of phosphate removed from a phosphorylated protein substrate. Reactions are performed with 2 or 4 nM enzyme in a final volume of 30 μl containing 60 mM Tris, pH 7.6, 1 mM EDTA, 1 mM EGTA, 0.1% β -mercaptoethanol and 10 μM substrate, ^{32}P -labeled on serine/threonine or tyrosine, as appropriate. Reactions are initiated 30 with substrate and incubated at 30° C for 10-15 min. Reactions are quenched with 450 μl of 4% (w/v) activated charcoal in 0.6 M HCl, 90 mM $\text{Na}_4\text{P}_2\text{O}_7$, and 2 mM NaH_2PO_4 , then centrifuged at 12,000 $\times g$ for 5 min. Acid-soluble ^{32}P i is quantified by liquid scintillation counting (Sinclair, C. et al. (1999) *J. Biol. Chem.* 274:23666-23672).

XVIII. Identification of PP Inhibitors

Compounds to be tested are arrayed in the wells of a 384-well plate in varying concentrations along with an appropriate buffer and substrate, as described in the assays in Example XVII. PP activity is measured for each well and the ability of each compound to inhibit PP activity can be determined, as well as the dose-response kinetics. This assay could also be used to identify molecules
5 which enhance PP activity.

XIX. Identification of PP Substrates

A PP "substrate-trapping" assay takes advantage of the increased substrate affinity that may be conferred by certain mutations in the PTP signature sequence. PP bearing these mutations form a stable complex with their substrate; this complex may be isolated biochemically. Site-directed
10 mutagenesis of invariant residues in the PTP signature sequence in a clone encoding the catalytic domain of PP is performed using a method standard in the art or a commercial kit, such as the MUTA-GENE kit from BIO-RAD. For expression of PP mutants in Escherichia coli, DNA fragments containing the mutation are exchanged with the corresponding wild-type sequence in an expression vector bearing the sequence encoding PP or a glutathione S-transferase (GST)-PP fusion protein. PP
15 mutants are expressed in E. coli and purified by chromatography.

The expression vector is transfected into COS1 or 293 cells via calcium phosphate-mediated transfection with 20 μ g of CsCl-purified DNA per 10-cm dish of cells or 8 μ g per 6-cm dish. Forty-eight hours after transfection, cells are stimulated with 100 ng/ml epidermal growth factor to increase tyrosine phosphorylation in cells, as the tyrosine kinase EGFR is abundant in COS cells. Cells are
20 lysed in 50 mM Tris·HCl, pH 7.5/5 mM EDTA/150 mM NaCl/1% Triton X-100/5 mM iodoacetic acid/10 mM sodium phosphate/10 mM NaF/5 μ g/ml leupeptin/5 μ g/ml aprotinin/1 mM benzamidine (1 ml per 10-cm dish, 0.5 ml per 6-cm dish). PP is immunoprecipitated from lysates with an appropriate antibody. GST-PP fusion proteins are precipitated with glutathione-Sepharose, 4 μ g of mAb or 10 μ l of beads respectively per mg of cell lysate. Complexes can be visualized by PAGE or further purified
25 to identify substrate molecules (Flint, A.J. et al. (1997) Proc. Natl. Acad. Sci. USA 94:1680-1685).

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 certain embodiments, it should be
30 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.

Table 1

Incyte Project ID	Polypeptide SEQ ID NO:	Incyte Polypeptide ID	Polynucleotide SEQ ID NO:	Incyte Polynucleotide ID
8124196	1	8124196CD1	10	8124196CB1
7473604	2	7473604CD1	11	7473604CB1
1437588	3	1437588CD1	12	1437588CB1
7476861	4	7476861CD1	13	7476861CB1
5320695	5	5320695CD1	14	5320695CB1
8116710	6	8116710CD1	15	8116710CB1
5370008	7	5370008CD1	16	5370008CB1
3016191	8	3016191CD1	17	3016191CB1
7476860	9	7476860CD1	18	7476860CB1

Table 2

Polypeptide SEQ ID NO:	Incyte Polypeptide ID	GenBank ID NO:	Probability score	GenBank Homolog
1	8124196CD1	g1945140	1.1e-30	ABI2 protein phosphatase 2C [Arabidopsis thaliana] Leung, J. et al. (1997) The Arabidopsis ABSCISIC ACID-INSENSITIVE (ABI2) and ABI1 genes encode homologous protein phosphatases 2C involved in abscisic acid signal transduction. Plant Cell 9:759-771.
2	7473604CD1	g6692782	6.7e-34	Protein phosphatase [Homo sapiens]. Nakamura, K. et al. (1999) Molecular cloning and characterization of a novel dual-specificity protein phosphatase possibly involved in spermatogenesis, Biochem. J. 344:819-825.
3	1437588CD1	g292376	4e-81	Protein tyrosine phosphatase [Homo sapiens]. Rohan, P.J. et al. (1993) PAC-1: a mitogen-induced nuclear protein tyrosine phosphatase. Science 259:1763-1766.
4	7476861CD1	g2665458	1e-155	Protein-tyrosine-phosphatase [Mus musculus]. Ohsugi, M. et al. (1997) Molecular cloning and characterization of a novel cytoplasmic protein-tyrosine phosphatase that is specifically expressed in spermatocytes. J. Biol. Chem. 272:33092-33099.
5	5320695CD1	g5714762	1e-178	Serine/threonine protein phosphatase PP2A-4 catalytic subunit [Oryza sativa subsp. indica]
6	8116710CD1	g452192	3.6e-32	Protein tyrosine phosphatase (PTP-BAS, type 2) [Homo sapiens]. Maekawa, K. et al. (1994) FEBS Lett 10: 337:200-206)
7	5370008CD1	g957217	1.4e-294	Striatum-enriched phosphatase [Homo sapiens]. Li, X. et al. (1995) Genomics 28:442-449.
8	3016191CD1	g3451473	7.0e-41	4-nitrophenylphosphatase [Schizosaccharomyces pombe].
9	7476860CD1	g7542482	4.1e-36	Fas-associated phosphatase-1 [Homo sapiens].

Table 3

SEQ ID NO:	Incyte Polypeptide ID	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Domains and Motifs	Analytical Methods and Databases
1	8124196CD1	372	S41 S48 T140 S205 S263 S24 S80 T280 S368 T35	N338 N358	PROTEIN PHOSPHATASE 2C MAGNESIUM HYDROLASE MANGANESE MULTIGENE FAMILY PP2C ISOFORM PD001101:E153-A276, V274-N338, G96-E235, N255-V342 PROTEIN PHOSPHATASE 2C DM00377 P49597 108-431:Y122-Q315, A322-N354 Protein phosphatase 2C motif Pp2c:Y122-G130 Protein phosphatase 2C PP2C:R104-S339 Protein phosphatase 2C p BL01032:Y122-G131, N155-S172, S186-L195, S205-V244, H253-D266, T335-V344 Transmembrane domain: V152-R174	BLAST-PRODOM BLAST-DOMO MOTIFS HMMEP-PFAM BLIMPS-BLOCKS
2	7473604CD1	405	Y27, T91, T97, S136, T172, T231, T258, S280, S316, T351, S356, S362		Dual specificity phosphatase, catalytic domain, DSPc: E50-K195 VH1-TYPE DUAL SPECIFICITY PHOSPHATASE: DM03823 P28562 169-314: E53-M197	HMMEP-TRANSMEMBRANE HMMEP-PFAM BLAST-DOMO
3	1437588CD1	200	S161	N90, N183	Dual specificity phosphatase, catalytic domain, DSPc:G58-Q196 Tyrosine phosphatase domain: V141-I154 Protein tyrosine phosphatase: PR00700D: R138-A156 Tyrosine specific protein phosphatase: BL00383E: V141-A151 Tyrosine specific protein phosphatases active site (tyr_phosphatase.prf): A120-I180 VH1-TYPE DUAL SPECIFICITY PHOSPHATASE: DM03823 P28562 169-314: Q56-L198	HMMEP-PFAM MOTIFS BLIMPS-BLOCKS BLIMPS-BLOCKS PROFILESCAN BLAST-DOMO

Table 3 (cont.)

SEQ ID NO:	Incyte Polypeptide ID	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Domains and Motifs	Analytical Methods and Databases
4	7476861CD1	420	S120 S182 S197 S20 S234 S272 S3 S312 S34 S51 S57 S76 S95 S96 T115 T235 T39 T84 Y195	N18 N210 N246 N376	<p>Tyrosine specific protein phosphatases active site tyr_phosphatase.prf:A340-M388 transmembrane domain transmem_domain:V362-F379</p> <p>Protein-tyrosine phosphatase Y_phosphatase:N183-E411</p> <p>Tyr_Phosphatase V351-F363</p> <p>Tyrosine specific protein phosphatases proteins BL00383A: K186-V200, K206-I214, D238-S248, Q315-P327, V351-G361, R389-F404</p> <p>Protein tyrosine phosphatase signature PR00700A: D207-I214, Y225-E245, H311-A328, P348-V366, F379-G394, M395-C405</p> <p>PROTEIN-TYROSINE-PHOSPHATASE DM00089 A54971 2204-2475:E167-L416 DM00089 P23468 1623-1906:M164-L413 DM00089 P26045 632-904:I159-V412 DM00089 P34138 13-350:D207-L413</p> <p>PROTEIN TYROSINE PHOSPHATASE, NONRECEPTOR TYPE 20 EC 3.1.3.48 PHOSPHOTYROSINE PHOSPHATASE PTPASE HYDROLASE PD097276: M1-N179</p> <p>HYDROLASE PHOSPHATASE PROTEIN PROTEIN TYROSINE PRECURSOR SIGNAL TYROSINE TRANSMEMBRANE GLYCOPROTEIN RECEPTOR PD000167:N183-E411</p> <p>HYDROLASE PHOSPHATASE PROTEIN PROTEIN TYROSINE PRECURSOR SIGNAL TRANSMEMBRANE GLYCOPROTEIN RECEPTOR PD000155:T326-K415, L286-S393</p>	<p>PROFILESSCAN</p> <p>HMMER</p> <p>HMMER-PFAM</p> <p>MOTIFS</p> <p>BLIMPS-BLOCKS</p> <p>BLIMPS-PRINTS</p> <p>BLAST-DOMO</p> <p>BLAST-PRODOM</p> <p>BLAST-PRODOM</p> <p>BLAST-PRODOM</p>

Table 3 (cont.)

SEQ ID NO:	Incyte Polypeptide ID	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Domains and Motifs	Analytical Methods and Databases
5	5320695CD1	313	S183 S205 S216 S278 T149 T305 Y156 Y84	N233	Ser/Thr protein phosphatase: L14-R298 Serine/threonine specific protein phosphatases proteins BL00125A:P55-V91, BL00125B:S97-G142, BL00125C:A161-P207, BL00125A:G221-N275 Serine/threonine phosphatase family signature PR00114A:P55-T82, PR00114B:Y84-Y111, PR00114C:I117-Y141, PR00114D:D152-I178, PR00114E:L181-D208, PR00114F:N237-E257, PR00114G:K259-N275 Serine/threonine specific protein phosphatases signature: V98-N143 PHOSPHATASE SERINE/THREONINE HYDROLASE IRON MANGANESE SUBUNIT MULTIGENE FAMILY PD000252:L14-R299 SIMILAR TO SERINE/THREONINE PHOSPHATASE PD112269:E29-H113 PHOSPHOPROTEIN PHOSPHATASE DM00133 S52659 11-307:L14-T308 DM00133 Q07098 4-300:G12-T308 DM00133 P23636 20-316:G12-T308 DM00133 P48577 4-300:L14-R307 Ser_Thr_Phosphatase: L118-E123	HMMER-PFAM BLIMPS_BLOCKS BLIMPS-PRINTS PROFILESCAN BLAST-PRODOM BLAST-PRODOM BLAST-DOMO MOTIFS

Table 3 (cont.)

SEQ ID NO:	Incyte Polypeptide ID	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Domains and Motifs	Analytical Methods and Databases
6	8116710CD1	622	S124 S189 S296 S320 S375 S382 S395 S436 S490 S510 S544 S605 S73 T211 T252 T406 T536 T538 T604 T613 Y182		Band 4.1 family domain: BL00660D:F272-P295 BAND 4.1 PROTEIN FAMILY PR00935A:L49-V61, PR00935C:L147-F167, PR00935D:E214-A230 CYTOSKELETON STRUCTURAL PHOSPHATASE HYDROLASE PROTEIN TYROSINE PHOSPHORYLATION MOESIN TYROSINE BAND: PD000961:V18-Y234 BAND 4 DM00609 A54971 562-990: D14-K379 DM00609 P31976 1-405: W84-R367, K336-S382, D25-A88 DM00609 P26038 1-405: P106-R367, D25-L76 DM00609 P35241 1-406: W84-R367, D25-E87	BLIMPS-BLOCKS BLIMPS-PRINTS BLAST-PRODOM BLAST-DOMO
7	5370008CD1	541	S160 S221 S244 S249 S28 S4 S417 S420 S434 S529 T144 T168 T209 T318 T355 T375 T59	N222 N91	Transmembrane Domain: L122-L142 Transmembrane Domain: I468-L492 Protein-tyrosine phosphatase: L298-L530 Tyrosine specific protein phosphatases proteins BL00383A:K301-V315, BL00383B:S327-I335, BL00383C:D358-T368, BL00383D:H429-P441, BL00383E:V470-G480, BL00383F:R508-F523, Protein tyrosine phosphatase signature PR00700A:S328-I335, PR00700B:Y345-Q365, PR00700C:R425-D442, PR00700D:P467-T485, PR00700E:V498-G513, PR00700F:M514-V524 Tyrosine specific protein phosphatases active site: L447-R508 Tyr_Phosphatase: V470-F482	HMMER HMMER HMMER-PFAM BLIMPS-BLOCKS BLIMPS-PRINTS PROFILES SCAN MOTIFS

Table 3 (cont.)

SEQ ID NO:	Incyte Polypeptide ID	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Domains and Motifs	Analytical Methods and Databases
7 cont.					<p>PROTEIN TYROSINE PHOSPHATASE STRIATUM ENRICHED EC 3.1.3.48 NEURAL SPECIFIC PD099306:M1-W172</p> <p>PHOSPHATASE PROTEIN PROTEIN TYROSINE PRECURSOR SIGNAL TYROSINE TRANSMEMBRANE GLYCOPROTEIN RECEPTOR PD000167:K301-G496</p> <p>HYDROLASE PHOSPHATASE PROTEIN TYROSINE PRECURSOR SIGNAL LCPTP HEMATOPOIETIC HEPTP STRIATUM ENRICHED: PD005701:K211-G297</p> <p>HYDROLASE PHOSPHATASE PROTEIN TYROSINE TYROSINE PRECURSOR SIGNAL TRANSMEMBRANE GLYCOPROTEIN RECEPTOR: PD000155:R425-Y531</p> <p>PROTEIN-TYROSINE-PHOSPHATASE</p> <p>DM00089 P35234 89-362:L261-L535</p> <p>DM00089 P54830 261-534:L261-L535</p> <p>DM00089 A55574 377-649:L261-L535</p> <p>DM00089 A55769 133-405:L261-L535</p>	<p>BLAST-PRODOM</p> <p>BLAST-PRODOM</p> <p>BLAST-PRODOM</p> <p>BLAST-PRODOM</p> <p>BLAST-DOMO</p>
8	3016191CD1	321	S299 S311 S70 T198 T285	N68 N69	<p>Nitrophenylphosphatase: DM02445 P19881 1-306: E19-L315</p> <p>Nitrophenylphosphatase: DM02445 P34492 1-292: L24-Q293</p> <p>Nitrophenylphosphatase: DM02445 Q00472 1-267; G60-L315</p> <p>Nitrophenylphosphatase: DM02445 P15302 1-249: L161-V289</p> <p>4-Nitrophenylphosphatase: PD005233: Q22-G142</p> <p>4-Nitrophenylphosphatase: PD149754:G192-L316</p>	<p>BLAST-DOMO</p> <p>BLAST-DOMO</p> <p>BLAST-DOMO</p> <p>BLAST-DOMO</p> <p>BLAST-PRODOM</p> <p>BLAST-PRODOM</p>

Table 3 (cont.)

SEQ ID NO:	Incyte Polypeptide ID	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Domains and Motifs	Analytical Methods and Databases
9	7476860CD1	320	S219 S239 S296 S55 S59 S82 T204 T23 T284	N57	PDZ domain (also known as DHR or GLGF): E90-P177 PDZ domain: PF00595: I137-N147 GLGF domain: DM00224 A54971 1496-1590: V83-C175	HMMER-PFAM BLIMPS-PFAM BLAST-DOMO

Table 4

Polynucleotide SEQ ID NO:	Incyte Polynucleotide ID	Sequence Length	Selected Fragment (s)	Sequence Fragments	5' Position	3' Position
10	8124196CB1	1803	1-62, 1399-1803	70528419V1	1004	1803
				8103438J1 (MIXDDIE02)	491	1059
				3203544T6 (PENCNOT02)	427	977
11	7473604CB1	1329	369-407, 1012-1329, 644-936	7667022H1 (TONSDIC01)	1	463
				FL7473604-	1	897
				g7329576_000027-g6692782		
12	1437588CB1	1236	1-393	4456407F6 (HEAADIR01)	835	1329
				1437588F6 (PANCNOT08)	74	604
				3973378H1 (ADRETUT06)	1	262
13	7476861CB1	1914	647-748, 1441-1808, 237-330, 1014-1144	441315H1 (MPHGNOT03)	1001	1236
				1532239F6 (SPLNNOT04)	355	879
				4067805H1 (SEMVNOT05)	858	1152
14	5320695CB1	1263		5508091F6 (BRADDIR01)	1385	1914
				70954791V1	815	1347
				71284965V1	473	1013
15	8116710CB1	2278	1-78, 408-555, 941-1552	70953936V1	1	629
				71285536V1	1158	1738
				5914035F7 (BRAIFEN03)	474	1263
15	8116710CB1	2278		71930652V1	1	644
				8037858H1 (SMCRUNE01)	1309	1946
				2899801F6 (DRGCNOT01)	903	1353
15	8116710CB1	2278		8116710H1 (TONSDIC01)	435	1031
				70818281V1	1733	2278
				71225945V1	1518	2180
15	8116710CB1	2278		6540118H1 (ADIPXT01)	1089	1375
				6491034H1 (MIXDUNB01)	1	428
				7408188H1 (UTREDME05)	383	1004

Table 4 (cont.)

Polynucleotide SEQ ID NO:	Incyte Polynucleotide ID	Sequence Length	Selected Fragment(s)	Sequence Fragments	5' Position	3' Position
16	5370008CB1	2904	1-129, 1686-2532, 769-853	6442313H1 (BRAENOT02)	2100	2746
				1287380F6 (BRAINOT11)	2175	2904
				7584530H1 (BRAIFEC01)	553	1230
				71878587V1	1723	2399
				7178936H1 (BRAXDIC01)	1	561
				71879372V1	1298	2029
				71880642V1	1133	1952
17	3016191CB1	1289	1-42, 1014-1289	7713736J2 (TESTTUE02)	421	1057
				FL3016191_g8575852_000	1	507
				002_g6572215_1_1		
				3016191F6 (MUSCNOT07)	549	1013
				6800702J1 (COLENOT03)	204	786
				3379750H1 (PENGNOT01)	957	1220
				946890H1 (RATRNOT02)	1021	1289
				70880855V1	1432	1950
				5205306F6 (BRAFNOT02)	997	1603
				70880928V1	791	1437
18	7476860CB1	1950	476-754, 398-420, 1913-1950, 1167-1226	6746531H1 (BRAFNOT02)	240	924
				6745671H1 (BRAFNOT02)	27	642
				5681178H1 (BRAENOT02)	1	242

Table 5

Polynucleotide SEQ ID NO:	Incyte Project ID	Representative Library
10	8124196CB1	HEAONOC01
11	7473604CB1	HEAADIR01
12	1437588CB1	LEUKNOT03
13	7476861CB1	BRADDIR01
14	5320695CB1	BRAIFEN03
15	8116710CB1	MIXDUNB01
16	5370008CB1	BSCNNOT03
17	3016191CB1	MUSCNOT07
18	7476860CB1	BRAFNOT02

Table 6

Library	Vector	Library Description
HEAONOC01	PSPORT1	Library was constructed using RNA isolated from the aorta of a 39-year-old Caucasian male, who died from a gunshot wound. Serology was positive for cytomegalovirus (CMV). Patient history included tobacco abuse (one pack of cigarettes per day for 25 years), and occasionally cocaine, marijuana, and alcohol use.
HEAADIR01	PINCY	The library was constructed using RNA isolated from diseased right atrium and heart muscle wall tissue removed from a 7-month-old Caucasian male who died from cardiopulmonary arrest due to Pompe's disease. Patient history included Pompe's disease, left ventricular hypertrophy, pyrexia, right complete cleft lip, cleft palate, chronic serous otitis media, hypertrophic cardiomyopathy, congestive heart failure, and developmental delays. Family history included acutemyocardial infarction, diabetes, cystic fibrosis, and Down's syndrome.
LEUKNOT03	PINCY	The library was constructed using RNA isolated from white blood cells of a 27-year-old female with blood type A+. The donor tested negative for cytomegalovirus (CMV).
BRADDIR01	PINCY	Library was constructed using RNA isolated from diseased choroid plexus tissue of the lateral ventricle, removed from the brain of a 57-year-old Caucasian male, who died from a cerebrovascular accident.
BRAIFEN03	PINCY	This normalized fetal brain tissue library was constructed from 3.26 million independent clones from a fetal brain tissue library. Starting RNA was made from brain tissue removed from a Caucasian male fetus, who was stillborn with a hypoplastic left heart at 23 weeks' gestation. The library was normalized in 2 rounds using conditions adapted from Soares et al., PNAS (1994) 91:9228 and Bonaldo et al., Genome Research 6 (1996):791, except that a significantly longer (48 hours/round) reannealing hybridization was used.

Table 6 (cont.)

Library	Vector	Library Description
MIXDUNB01	pINCY	Library was constructed using RNA isolated from myometrium removed from a 41-year-old Caucasian female (donor A) during vaginal hysterectomy with a dilation and curettage and untreated smooth muscle cells removed from the renal vein of a 57-year-old Caucasian male. Pathology for donor A indicated the myometrium and cervix were unremarkable. The endometrium was secretory and contained fragments of endometrial polyps. Benign endo- and ectocervical mucosa were identified in the endocervix. Pathology for the associated tumor tissue indicated uterine leiomyoma. Medical history included an unspecified menstrual disorder, ventral hernia, normal delivery, a benign ovarian neoplasm, and tobacco abuse in donor A. Previous surgeries included a bilateral destruction of fallopian tubes, removal of a solitary ovary, and an exploratory laparotomy in donor A.
BSCNNOT03	pINCY	Library was constructed using RNA isolated from caudate nucleus tissue removed from the brain of a 92-year-old male. Pathology indicated several small cerebral infarcts but no senile plaques or neurofibrillary degeneration. Patient history included throat cancer which was treated with radiation.
BRAFNOT02	pINCY	Library was constructed using RNA isolated from superior frontal cortex tissue removed from a 35-year-old Caucasian male who died from cardiac failure. Pathology indicated moderate leptomenigeal fibrosis and multiple microinfarctions of the cerebral neocortex. Microscopically, the cerebral hemisphere revealed moderate fibrosis of the leptomeninges with focal calcifications. There was evidence of shrunken and slightly eosinophilic pyramidal neurons throughout the cerebral hemispheres. In addition, scattered throughout the cerebral cortex, there were multiple small microscopic areas of cavitation with surrounding gliosis. Patient history included dilated cardiomyopathy, congestive heart failure, cardiomegaly, and an enlarged spleen and liver.
MUSCNOT07	pINCY	Library was constructed using RNA isolated from muscle tissue removed from the forearm of a 38-year-old Caucasian female during a soft tissue excision. Pathology for the associated tumor tissue indicated intramuscular hemangioma. Family history included breast cancer, benign hypertension, cerebrovascular disease, colon cancer, and type II diabetes.

Table 7

Program	Description	Reference	Parameter Threshold
ABI FACTURA	A program that removes vector sequences and masks ambiguous bases in nucleic acid sequences.	Applied Biosystems, Foster City, CA.	
ABI/PARACEL FDF	A Fast Data Finder useful in comparing and annotating amino acid or nucleic acid sequences.	Applied Biosystems, Foster City, CA; Paracel Inc., Pasadena, CA.	Mismatch <50%
ABI AutoAssembler	A program that assembles nucleic acid sequences.	Applied Biosystems, Foster City, CA.	
BLAST	A Basic Local Alignment Search Tool useful in sequence similarity search for amino acid and nucleic acid sequences. BLAST includes five functions: blastp, blastn, blastx, tblastn, and tblastx.	Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410; Altschul, S.F. et al. (1997) Nucleic Acids Res. 25:3389-3402.	ESTs: Probability value= 1.0E-8 or less Full Length sequences: Probability value= 1.0E-10 or less
FASTA	A Pearson and Lipman algorithm that searches for similarity between a query sequence and a group of sequences of the same type. FASTA comprises at least five functions: fasta, tfasta, fastx, tfastx, and ssearch.	Pearson, W.R. and D.J. Lipman (1988) Proc. Natl. Acad. Sci. USA 85:2444-2448; Pearson, W.R. (1990) Methods Enzymol. 183:63-98; and Smith, T.F. and M.S. Waterman (1981) Adv. Appl. Math. 2:482-489.	ESTs: fasta E value=1.06E-6 Assembled ESTs: fasta Identity= 95% or greater and Match length=200 bases or greater; fastx E value=1.0E-8 or less Full Length sequences: fastx score=100 or greater
BLIMPS	A BLocks IMProved Searcher that matches a sequence against those in BLOCKS, PRINTS, DOMO, PRODOM, and PFAM databases to search for gene families, sequence homology, and structural fingerprint regions.	Henikoff, S. and J.G. Henikoff (1991) Nucleic Acids Res. 19:6565-6572; Henikoff, J.G. and S. Henikoff (1996) Methods Enzymol. 266:88-105; and Attwood, T.K. et al. (1997) J. Chem. Inf. Comput. Sci. 37:417-424.	Probability value= 1.0E-3 or less
HMMER	An algorithm for searching a query sequence against hidden Markov model (HMM)-based databases of protein family consensus sequences, such as PFAM.	Krogh, A. et al. (1994) J. Mol. Biol. 235:1501-1531; Sonnhammer, E.L.L. et al. (1988) Nucleic Acids Res. 26:320-322; Durbin, R. et al. (1998) Our World View, in a Nutshell, Cambridge Univ. Press, pp. 1-350.	PFAM hits: Probability value= 1.0E-3 or less Signal peptide hits: Score= 0 or greater

Table 7 (cont.)

Program	Description	Reference	Parameter Threshold
ProfileScan	An algorithm that searches for structural and sequence motifs in protein sequences that match sequence patterns defined in Prosite.	Gribskov, M. et al. (1988) CABIOS 4:61-66; Gribskov, M. et al. (1989) Methods Enzymol. 183:146-159; Bairoch, A. et al. (1997) Nucleic Acids Res. 25:217-221.	Normalized quality score ≥ GCG-specified "HIGH" value for that particular Prosite motif. Generally, score = 1.4-2.1.
Phred	A base-calling algorithm that examines automated sequencer traces with high sensitivity and probability.	Ewing, B. et al. (1998) Genome Res. 8:175-185; Ewing, B. and P. Green (1998) Genome Res. 8:186-194.	
Phrap	A Phils Revised Assembly Program including SWAT and CrossMatch, programs based on efficient implementation of the Smith-Waterman algorithm, useful in searching sequence homology and assembling DNA sequences.	Smith, T.F. and M.S. Waterman (1981) Adv. Appl. Math. 2:482-489; Smith, T.F. and M.S. Waterman (1981) J. Mol. Biol. 147:195-197; and Green, P., University of Washington, Seattle, WA.	Score = 120 or greater; Match length = 56 or greater
Consed	A graphical tool for viewing and editing Phrap assemblies.	Gordon, D. et al. (1998) Genome Res. 8:195-202.	
SPScan	A weight matrix analysis program that scans protein sequences for the presence of secretory signal peptides.	Nielson, H. et al. (1997) Protein Engineering 10:1-6; Claverie, J.M. and S. Audic (1997) CABIOS 12:431-439.	Score = 3.5 or greater
TMAP	A program that uses weight matrices to delineate transmembrane segments on protein sequences and determine orientation.	Persson, B. and P. Argos (1994) J. Mol. Biol. 237:182-192; Persson, B. and P. Argos (1996) Protein Sci. 5:363-371.	
TMHMMER	A program that uses a hidden Markov model (HMM) to delineate transmembrane segments on protein sequences and determine orientation.	Sonnhammer, E.L. et al. (1998) Proc. Sixth Intl. Conf. on Intelligent Systems for Mol. Biol., Glasgow et al., eds., The Am. Assoc. for Artificial Intelligence Press, Menlo Park, CA, pp. 175-182.	
Motifs	A program that searches amino acid sequences for patterns that matched those defined in Prosite.	Bairoch, A. et al. (1997) Nucleic Acids Res. 25:217-221; Wisconsin Package Program Manual, version 9, page M51-59, Genetics Computer Group, Madison, WI.	

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				20					25					30
Asp	Arg	Arg	Val	Thr	Pro	Thr	Cys	His	Ser	Ser	Thr	Ser	Glu	Pro
				35					40					45
Arg	Cys	Ser	Arg	Phe	Asp	Pro	Asp	Gly	Ser	Gly	Ser	Pro	Ala	Thr
				50					55					60
Trp	Asp	Asn	Phe	Gly	Ile	Trp	Asp	Asn	Arg	Ile	Asp	Glu	Pro	Ile
				65					70					75
Leu	Leu	Pro	Pro	Ser	Ile	Lys	Tyr	Gly	Lys	Pro	Ile	Pro	Lys	Ile
				80					85					90
Ser	Leu	Glu	Asn	Val	Gly	Cys	Ala	Ser	Gln	Ile	Gly	Lys	Arg	Lys
				95					100					105
Glu	Asn	Glu	Asp	Arg	Phe	Asp	Phe	Ala	Gln	Leu	Thr	Asp	Glu	Val
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Leu	Tyr	Phe	Ala	Val	Tyr	Asp	Gly	His	Gly	Gly	Pro	Ala	Ala	Ala
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Pro	Lys	Glu	Lys	Asn	Leu	Glu	Thr	Leu	Leu	Thr	Leu	Ala	Phe	Leu
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Glu	Ile	Asp	Lys	Ala	Phe	Ser	Ser	His	Ala	Arg	Leu	Ser	Ala	Asp
				170					175					180
Ala	Thr	Leu	Leu	Thr	Ser	Gly	Thr	Thr	Ala	Thr	Val	Ala	Leu	Leu
				185					190					195
Arg	Asp	Gly	Ile	Glu	Leu	Val	Val	Ala	Ser	Val	Gly	Asp	Ser	Arg
				200					205					210
Ala	Ile	Leu	Cys	Arg	Lys	Gly	Lys	Pro	Met	Lys	Leu	Thr	Ile	Asp
				215					220					225
His	Thr	Pro	Glu	Arg	Lys	Asp	Glu	Lys	Glu	Arg	Ile	Lys	Lys	Cys
				230					235					240
Gly	Gly	Phe	Val	Ala	Trp	Asn	Ser	Leu	Gly	Gln	Pro	His	Val	Asn
				245					250					255
Gly	Arg	Leu	Ala	Met	Thr	Arg	Ser	Ile	Gly	Asp	Leu	Asp	Leu	Lys
				260					265					270
Thr	Ser	Gly	Val	Ile	Ala	Glu	Pro	Glu	Thr	Lys	Arg	Ile	Lys	Leu
				275					280					285
His	His	Ala	Asp	Asp	Ser	Phe	Leu	Val	Leu	Thr	Thr	Asp	Gly	Ile
				290					295					300
Asn	Phe	Met	Val	Asn	Ser	Gln	Glu	Ile	Cys	Asp	Phe	Val	Asn	Gln
				305					310					315
Cys	His	Asp	Pro	Asn	Glu	Ala	Ala	His	Ala	Val	Thr	Glu	Gln	Ala
				320					325					330
Ile	Gln	Tyr	Gly	Thr	Glu	Asp	Asn	Ser	Thr	Ala	Val	Val	Val	Pro
				335					340					345
Phe	Gly	Ala	Trp	Gly	Lys	Tyr	Lys	Asn	Ser	Glu	Ile	Asn	Phe	Ser
				350					355					360
Phe	Ser	Arg	Ser	Phe	Ala	Ser	Ser	Gly	Arg	Trp	Ala			
				365					370					

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 <211> 405
 <212> PRT
 <213> Homo sapiens

<220>
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 <223> Incyte ID No: 7473604CD1

<400>	2													
Met	Leu	Glu	Ser	Ala	Glu	Gln	Leu	Leu	Val	Glu	Asp	Leu	Tyr	Asn
1				5					10					15
Arg	Val	Arg	Glu	Lys	Met	Asp	Asp	Thr	Ser	Leu	Tyr	Asn	Thr	Pro
				20					25					30
Cys	Val	Leu	Asp	Leu	Gln	Arg	Ala	Leu	Val	Gln	Asp	Arg	Gln	Glu
				35					40					45
Ala	Pro	Trp	Asn	Glu	Val	Asp	Glu	Val	Trp	Pro	Asn	Val	Phe	Ile
				50					55					60
Ala	Asp	Arg	Ser	Val	Ala	Val	Asn	Lys	Gly	Arg	Leu	Lys	Arg	Leu
				65					70					75
Gly	Ile	Thr	His	Ile	Leu	Asn	Ala	Ala	His	Gly	Thr	Gly	Val	Tyr
				80					85					90
Thr	Gly	Pro	Glu	Phe	Tyr	Thr	Gly	Leu	Glu	Ile	Gln	Tyr	Leu	Gly
				95					100					105
Val	Glu	Val	Asp	Asp	Phe	Pro	Glu	Val	Asp	Ile	Ser	Gln	His	Phe
				110					115					120
Arg	Lys	Ala	Tyr	Cys	His	Tyr	Ile	Ile	Phe	Ser	Cys	Val	Phe	Ile
				125					130					135
Ser	Gly	Lys	Val	Leu	Val	Ser	Ser	Glu	Met	Gly	Ile	Ser	Arg	Ser
				140					145					150
Ala	Val	Leu	Val	Val	Ala	Tyr	Leu	Met	Ile	Phe	His	Asn	Met	Ala
				155					160					165
Ile	Leu	Glu	Ala	Leu	Met	Thr	Val	Arg	Lys	Lys	Arg	Ala	Ile	Tyr
				170					175					180

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Pro	Asn	Glu	Gly	Phe	Leu	Lys	Gln	Leu	Arg	Glu	Leu	Asn	Glu	Lys
				185					190					195
Leu	Met	Glu	Glu	Arg	Glu	Glu	Asp	Tyr	Gly	Arg	Glu	Gly	Gly	Ser
				200					205					210
Ala	Glu	Ala	Glu	Glu	Gly	Glu	Gly	Thr	Gly	Ser	Met	Leu	Gly	Ala
				215					220					225
Arg	Val	His	Ala	Leu	Thr	Val	Glu	Glu	Glu	Asp	Asp	Ser	Ala	Ser
				230					235					240
His	Leu	Ser	Gly	Ser	Ser	Leu	Gly	Lys	Ala	Thr	Gln	Ala	Ser	Lys
				245					250					255
Pro	Leu	Thr	Leu	Ile	Asp	Glu	Glu	Glu	Glu	Glu	Lys	Leu	Tyr	Glu
				260					265					270
Gln	Trp	Lys	Lys	Gly	Gln	Gly	Leu	Leu	Ser	Asp	Lys	Val	Pro	Gln
				275					280					285
Asp	Gly	Gly	Gly	Trp	Arg	Ser	Ala	Ser	Ser	Gly	Gln	Gly	Gly	Glu
				290					295					300
Glu	Leu	Glu	Asp	Glu	Asp	Val	Glu	Arg	Ile	Ile	Gln	Glu	Trp	Gln
				305					310					315
Ser	Arg	Asn	Glu	Arg	Tyr	Gln	Ala	Glu	Gly	Tyr	Arg	Arg	Trp	Gly
				320					325					330
Arg	Glu	Glu	Glu	Lys	Glu	Glu	Glu	Ser	Asp	Ala	Gly	Ser	Ser	Val
				335					340					345
Gly	Arg	Arg	Arg	Arg	Thr	Leu	Ser	Glu	Ser	Ser	Ala	Trp	Glu	Ser
				350					355					360
Val	Ser	Ser	His	Asp	Ile	Trp	Val	Leu	Lys	Gln	Gln	Leu	Glu	Leu
				365					370					375
Asn	Arg	Pro	Asp	His	Gly	Arg	Arg	Arg	Arg	Ala	Asp	Ser	Met	Ser
				380					385					390
Ser	Glu	Ser	Thr	Trp	Gly	Arg	Met	Glu	Arg	Glu	Ala	Ala	Gly	Asp
				395					400					405

<210> 3
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 <212> PRT
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> Incyte ID No: 1437588CD1

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 Met Tyr Val Pro Val Cys Pro Pro His Ile Pro Glu Thr Cys Leu
 1 5 10
 Arg Ser Gly Gly Leu Ala Glu Glu Ser Ser Cys Leu Gly Gln Pro
 20 25 30
 Met Gly Ser Pro Pro Ala Ala Ala Pro Ala Leu Arg Gly Trp Ala
 35 40 45
 Gly Lys Ala Ser Pro Pro Leu Cys Ser Leu Gln Gly Gly Pro Val
 50 55 60
 Glu Ile Leu Pro Tyr Leu Phe Leu Gly Ser Cys Ser His Ser Ser
 65 70 75
 Asp Leu Gln Gly Leu Gln Ala Cys Gly Ile Thr Ala Val Leu Asn
 80 85 90
 Val Ser Ala Ser Cys Pro Asn His Phe Glu Gly Leu Phe Arg Tyr
 95 100 105
 Lys Ser Ile Pro Val Glu Asp Asn Gln Met Val Glu Ile Ser Ala
 110 115 120
 Trp Phe Gln Glu Ala Ile Gly Phe Ile Asp Trp Val Lys Asn Ser
 125 130 135
 Gly Gly Arg Val Leu Val His Cys Gln Ala Gly Ile Ser Arg Ser
 140 145 150
 Ala Thr Ile Cys Leu Ala Tyr Leu Met Gln Ser Arg Arg Val Arg
 155 160 165
 Leu Asp Glu Ala Phe Asp Phe Val Lys Gln Arg Arg Gly Val Ile
 170 175 180
 Ser Pro Asn Phe Ser Phe Met Gly Gln Leu Leu Gln Phe Glu Thr

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Gln Val Leu Cys His 185
 190
 195
 200

<210> 4
 <211> 420
 <212> PRT
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> Incyte ID No: 7476861CD1

<400> 4
 Met Ser Ser Pro Arg Asp Phe Arg Ala Glu Pro Val Asn Asp Tyr
 1 5 10 15
 Glu Gly Asn Asp Ser Glu Ala Glu Asp Leu Asn Phe Arg Glu Thr
 20 25 30
 Leu Pro Ser Ser Ser Gln Glu Asn Thr Pro Arg Ser Lys Val Phe
 35 40 45
 Glu Asn Lys Val Asn Ser Glu Lys Val Lys Leu Ser Leu Arg Asn
 50 55 60
 Phe Pro His Asn Asp Tyr Glu Asp Val Phe Glu Glu Pro Ser Glu
 65 70 75
 Ser Gly Ser Asp Pro Ser Met Trp Thr Ala Arg Gly Pro Phe Arg
 80 85 90
 Arg Asp Arg Trp Ser Ser Glu Asp Glu Glu Ala Ala Gly Pro Ser
 95 100 105
 Gln Ala Leu Ser Pro Leu Leu Ser Asp Thr Arg Lys Ile Val Ser
 110 115 120
 Glu Gly Glu Leu Asp Gln Leu Ala Gln Ile Arg Pro Leu Ile Phe
 125 130 135
 Asn Phe His Glu Gln Thr Ala Ile Lys Asp Cys Leu Lys Ile Leu
 140 145 150
 Glu Glu Lys Thr Ala Ala Tyr Asp Ile Met Gln Glu Phe Met Ala
 155 160 165
 Leu Glu Leu Lys Asn Leu Pro Gly Glu Phe Asn Ser Gly Asn Gln
 170 175 180
 Pro Ser Asn Arg Glu Lys Asn Arg Tyr Arg Asp Ile Leu Pro Tyr
 185 190 195
 Asp Ser Thr Arg Val Pro Leu Gly Lys Ser Lys Asp Tyr Ile Asn
 200 205 210
 Ala Ser Tyr Ile Arg Ile Val Asn Cys Gly Glu Glu Tyr Phe Tyr
 215 220 225
 Ile Ala Thr Gln Gly Pro Leu Leu Ser Thr Ile Asp Asp Phe Trp
 230 235 240
 Gln Met Val Leu Glu Asn Asn Ser Asn Val Ile Ala Met Ile Thr
 245 250 255
 Arg Glu Ile Glu Gly Gly Ile Ile Lys Cys Tyr His Tyr Trp Pro
 260 265 270
 Ile Ser Leu Lys Lys Pro Leu Glu Leu Lys His Phe Arg Val Phe
 275 280 285
 Leu Glu Asn Tyr Gln Ile Leu Gln Tyr Phe Ile Ile Arg Met Phe
 290 295 300
 Gln Val Val Glu Lys Ser Thr Gly Thr Ser His Ser Val Lys Gln
 305 310 315
 Leu Gln Phe Thr Lys Trp Pro Asp His Gly Thr Pro Ala Ser Ala
 320 325 330
 Asp Ser Phe Ile Lys Tyr Ile Arg Tyr Ala Arg Lys Ser His Leu
 335 340 345
 Thr Gly Pro Met Val Val His Cys Ser Ala Gly Ile Gly Arg Thr
 350 355 360
 Gly Val Phe Leu Cys Val Asp Val Val Phe Cys Ala Ile Val Lys
 365 370 375
 Asn Cys Ser Phe Asn Ile Met Asp Ile Val Ala Gln Met Arg Glu
 380 385 390
 Gln Arg Ser Gly Met Val Gln Thr Lys Glu Gln Tyr His Phe Cys

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Tyr	Asp	Ile	Val	Leu	Glu	Val	Leu	Arg	Lys	Leu	Leu	Thr	Leu	Asp
				395					400					405
				410					415					420

<210> 5
 <211> 313
 <212> PRT
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> Incyte ID No: 5320695CD1

<400> 5
 Met Glu Pro Met Asn Val Asp Asn Gly Gly Cys Gly Gly Leu Asp
 1 5 10 15
 Ala Gln Ile Glu Gln Leu Met Gln Cys Arg Pro Leu Ala Glu Gln
 20 25 30
 Glu Val Lys Ala Leu Cys Glu Lys Ala Lys Glu Ile Leu Met Glu
 35 40 45
 Glu Ser Asn Val Gln Pro Val Lys Ser Pro Val Thr Ile Cys Gly
 50 55 60
 Asp Ile His Gly Gln Phe His Asp Leu Val Glu Leu Phe Arg Ile
 65 70 75
 Gly Gly Lys Cys Pro Asp Thr Asn Tyr Leu Phe Met Gly Asp Tyr
 80 85 90
 Val Asp Arg Gly Tyr Tyr Ser Val Glu Thr Val Thr Met Leu Val
 95 100 105
 Ala Leu Lys Val Arg Tyr Pro His Arg Ile Thr Ile Leu Arg Gly
 110 115 120
 Asn His Glu Ser Arg Gln Ile Thr Gln Val Tyr Gly Phe Tyr Asp
 125 130 135
 Glu Cys Leu Arg Lys Tyr Gly Asn Ala Asn Val Trp Lys Thr Phe
 140 145 150
 Thr Asp Leu Phe Asp Tyr Phe Pro Leu Thr Ala Leu Val Glu Ser
 155 160 165
 Glu Ile Phe Cys Leu His Gly Gly Leu Ser Pro Ser Ile Glu Asn
 170 175 180
 Leu Asp Ser Val Arg Ser Leu Asp Arg Val Gln Glu Val Pro His
 185 190 195
 Glu Gly Pro Met Cys Asp Leu Leu Trp Ser Asp Pro Asp Asp Arg
 200 205 210
 Cys Gly Trp Gly Ile Ser Pro Arg Gly Ala Gly Tyr Thr Phe Gly
 215 220 225
 Gln Asp Ile Ser Glu Gln Phe Asn His Thr Asn Asn Leu Lys Leu
 230 235 240
 Val Ala Arg Ala His Gln Leu Val Met Glu Gly Tyr Asn Trp Ala
 245 250 255
 His Glu Gln Lys Val Val Thr Ile Phe Ser Ala Pro Asn Tyr Cys
 260 265 270
 Tyr Arg Cys Gly Asn Met Ala Ser Ile Leu Glu Val Asp Asp Cys
 275 280 285
 Asn Ser His Thr Phe Ile Gln Phe Glu Pro Ala Pro Arg Arg Gly
 290 295 300
 Glu Pro Asp Val Thr Arg Arg Thr Pro Asp Tyr Phe Leu
 305 310

<210> 6
 <211> 622
 <212> PRT
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> Incyte ID No: 8116710CD1

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<400> 6
 Met Asn Lys Leu Asn Phe His Asn Asn Arg Val Met Gln Asp Arg
 1 5 10 15
 Arg Ser Val Cys Ile Phe Leu Pro Asn Asp Glu Ser Leu Asn Ile
 20 25 30
 Ile Ile Asn Val Lys Ile Leu Cys His Gln Leu Leu Val Gln Val
 35 40 45
 Cys Asp Leu Leu Arg Leu Lys Asp Cys His Leu Phe Gly Leu Ser
 50 55 60
 Val Ile Gln Asn Asn Glu His Val Tyr Met Glu Leu Ser Gln Lys
 65 70 75
 Leu Tyr Lys Tyr Cys Pro Lys Glu Trp Lys Lys Glu Ala Ser Lys
 80 85 90
 Val Arg Gln Tyr Glu Val Thr Trp Gly Ile Asp Gln Phe Gly Pro
 95 100 105
 Pro Met Ile Ile His Phe Arg Val Gln Tyr Tyr Val Glu Asn Gly
 110 115 120
 Arg Leu Ile Ser Asp Arg Ala Ala Arg Tyr Tyr Tyr Tyr Trp His
 125 130 135
 Leu Arg Lys Gln Val Leu His Ser Gln Cys Val Leu Arg Glu Glu
 140 145 150
 Ala Tyr Phe Leu Leu Ala Ala Phe Ala Leu Gln Ala Asp Leu Gly
 155 160 165
 Asn Phe Lys Arg Asn Lys His Tyr Gly Lys Tyr Phe Glu Pro Glu
 170 175 180
 Ala Tyr Phe Pro Ser Trp Val Val Ser Lys Arg Gly Lys Asp Tyr
 185 190 195
 Ile Leu Lys His Ile Pro Asn Met His Lys Asp Gln Phe Ala Leu
 200 205 210
 Thr Ala Ser Glu Ala His Leu Lys Tyr Ile Lys Glu Ala Val Arg
 215 220 225
 Leu Asp Asp Val Ala Val His Tyr Tyr Arg Leu Tyr Lys Asp Lys
 230 235 240
 Arg Glu Ile Glu Ala Ser Leu Thr Leu Gly Leu Thr Met Arg Gly
 245 250 255
 Ile Gln Ile Phe Gln Asn Leu Asp Glu Glu Lys Gln Leu Leu Tyr
 260 265 270
 Asp Phe Pro Trp Thr Asn Val Gly Lys Leu Val Phe Val Gly Lys
 275 280 285
 Lys Phe Glu Ile Leu Pro Asp Gly Leu Pro Ser Ala Arg Lys Leu
 290 295 300
 Ile Tyr Tyr Thr Gly Cys Pro Met Arg Ser Arg His Leu Leu Gln
 305 310 315
 Leu Leu Ser Asn Ser His Arg Leu Tyr Met Asn Leu Gln Pro Val
 320 325 330
 Leu Arg His Ile Arg Lys Leu Glu Glu Asn Glu Glu Lys Lys Gln
 335 340 345
 Tyr Arg Glu Ser Tyr Ile Ser Asp Asn Leu Asp Leu Asp Met Asp
 350 355 360
 Gln Leu Glu Lys Arg Ser Arg Ala Ser Gly Ser Ser Ala Gly Ser
 365 370 375
 Met Lys His Lys Arg Leu Ser Arg His Ser Thr Ala Ser His Ser
 380 385 390
 Ser Ser His Thr Ser Gly Ile Glu Ala Asp Thr Lys Pro Arg Asp
 395 400 405
 Thr Gly Pro Glu Asp Ser Tyr Ser Ser Ser Ala Ile His Arg Lys
 410 415 420
 Leu Lys Thr Cys Ser Ser Met Thr Ser His Gly Ser Ser His Thr
 425 430 435
 Ser Gly Val Glu Ser Gly Gly Lys Asp Arg Leu Glu Glu Asp Leu
 440 445 450
 Gln Asp Asp Glu Ile Glu Met Leu Val Asp Asp Pro Arg Asp Leu
 455 460 465
 Glu Gln Met Asn Glu Glu Ser Leu Glu Val Ser Pro Asp Met Cys
 470 475 480
 Ile Tyr Ile Thr Glu Asp Met Leu Met Ser Arg Lys Leu Asn Gly
 485 490 495

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His Ser Gly Leu Ile Val Lys Glu Ile Gly Ser Ser Thr Ser Ser
 500 505 510
 Ser Ser Glu Thr Val Val Lys Leu Arg Gly Gln Ser Thr Asp Ser
 515 520 525
 Leu Pro Gln Thr Ile Cys Arg Lys Pro Lys Thr Ser Thr Asp Arg
 530 535 540
 His Ser Leu Ser Leu Asp Asp Ile Arg Leu Tyr Gln Lys Asp Phe
 545 550 555
 Leu Arg Ile Ala Gly Leu Cys Gln Asp Thr Ala Gln Ser Tyr Thr
 560 565 570
 Phe Gly Cys Gly His Glu Leu Asp Glu Glu Gly Leu Tyr Cys Asn
 575 580 585
 Ser Cys Leu Ala Gln Gln Cys Ile Asn Ile Gln Asp Ala Phe Pro
 590 595 600
 Val Lys Arg Thr Ser Lys Tyr Phe Ser Leu Asp Leu Thr His Asp
 605 610 615
 Glu Val Pro Glu Phe Val Val
 620

<210> 7
 <211> 541
 <212> PRT
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> Incyte ID No: 5370008CD1

<400> 7
 Met Cys Cys Ser Glu Arg Leu Pro Gly Leu Pro Gln Pro Ile Val
 1 5 10 15
 Met Glu Ala Leu Asp Glu Ala Glu Gly Leu Gln Asp Ser Gln Arg
 20 25 30
 Glu Met Pro Pro Pro Pro Pro Pro Ser Pro Pro Ser Asp Pro Ala
 35 40 45
 Gln Lys Pro Pro Pro Arg Gly Ala Gly Ser His Ser Leu Thr Val
 50 55 60
 Arg Ser Ser Leu Cys Leu Phe Ala Ala Ser Gln Phe Leu Leu Ala
 65 70 75
 Cys Gly Val Leu Trp Phe Ser Gly Tyr Gly His Ile Trp Ser Gln
 80 85 90
 Asn Ala Thr Asn Leu Val Ser Ser Leu Leu Thr Leu Leu Lys Gln
 95 100 105
 Leu Glu Pro Thr Ala Trp Leu Asp Ser Gly Thr Trp Gly Val Pro
 110 115 120
 Ser Leu Leu Leu Val Phe Leu Ser Val Gly Leu Val Leu Val Thr
 125 130 135
 Thr Leu Val Trp His Leu Leu Arg Thr Pro Pro Glu Pro Pro Thr
 140 145 150
 Pro Leu Pro Pro Glu Asp Arg Arg Gln Ser Val Ser Arg Gln Pro
 155 160 165
 Ser Phe Thr Tyr Ser Glu Trp Met Glu Glu Lys Ile Glu Asp Asp
 170 175 180
 Phe Leu Asp Leu Asp Pro Val Pro Glu Thr Pro Val Phe Asp Cys
 185 190 195
 Val Met Asp Ile Lys Pro Glu Ala Asp Pro Thr Ser Leu Thr Val
 200 205 210
 Lys Ser Met Gly Leu Gln Glu Arg Arg Gly Ser Asn Val Ser Leu
 215 220 225
 Thr Leu Asp Met Cys Thr Pro Gly Cys Asn Glu Glu Gly Phe Gly
 230 235 240
 Tyr Leu Met Ser Pro Arg Glu Glu Ser Ala Arg Glu Tyr Leu Leu
 245 250 255
 Ser Ala Ser Arg Val Leu Gln Ala Glu Glu Leu His Glu Lys Ala
 260 265 270
 Leu Asp Pro Phe Leu Leu Gln Ala Glu Phe Phe Glu Ile Pro Met
 275 280 285

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Asn	Phe	Val	Asp	Pro	Lys	Glu	Tyr	Asp	Ile	Pro	Gly	Leu	Val	Arg
				290					295					300
Lys	Asn	Arg	Tyr	Lys	Thr	Ile	Leu	Pro	Asn	Pro	His	Ser	Arg	Val
				305					310					315
Cys	Leu	Thr	Ser	Pro	Asp	Pro	Asp	Asp	Pro	Leu	Ser	Ser	Tyr	Ile
				320					325					330
Asn	Ala	Asn	Tyr	Ile	Arg	Gly	Tyr	Gly	Gly	Glu	Glu	Lys	Val	Tyr
				335					340					345
Ile	Ala	Thr	Gln	Gly	Pro	Ile	Val	Ser	Thr	Val	Ala	Asp	Phe	Trp
				350					355					360
Arg	Met	Val	Trp	Gln	Glu	His	Thr	Pro	Ile	Ile	Val	Met	Ile	Thr
				365					370					375
Asn	Ile	Glu	Glu	Met	Asn	Glu	Lys	Cys	Thr	Glu	Tyr	Trp	Pro	Glu
				380					385					390
Glu	Gln	Val	Ala	Tyr	Asp	Gly	Val	Glu	Ile	Thr	Val	Gln	Lys	Val
				395					400					405
Ile	His	Thr	Glu	Asp	Tyr	Arg	Leu	Arg	Leu	Ile	Ser	Leu	Lys	Ser
				410					415					420
Gly	Thr	Glu	Glu	Arg	Gly	Leu	Lys	His	Tyr	Trp	Phe	Thr	Ser	Trp
				425					430					435
Pro	Asp	Gln	Lys	Thr	Pro	Asp	Arg	Ala	Pro	Pro	Leu	Leu	His	Leu
				440					445					450
Val	Arg	Glu	Val	Glu	Glu	Ala	Ala	Gln	Gln	Glu	Gly	Pro	His	Cys
				455					460					465
Ala	Pro	Ile	Ile	Val	His	Cys	Ser	Ala	Gly	Ile	Gly	Arg	Thr	Gly
				470					475					480
Cys	Phe	Ile	Ala	Thr	Ser	Ile	Cys	Cys	Gln	Gln	Leu	Arg	Gln	Glu
				485					490					495
Gly	Val	Val	Asp	Ile	Leu	Lys	Thr	Thr	Cys	Gln	Leu	Arg	Gln	Asp
				500					505					510
Arg	Gly	Gly	Met	Ile	Gln	Thr	Cys	Glu	Gln	Tyr	Gln	Phe	Val	His
				515					520					525
His	Val	Met	Ser	Leu	Tyr	Glu	Lys	Gln	Leu	Ser	His	Gln	Ser	Pro
				530					535					540

Glu

<210> 8
 <211> 321
 <212> PRT
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> Incyte ID No: 3016191CD1

<400> 8

Met	Ala	Ala	Ala	Glu	Ala	Gly	Gly	Asp	Asp	Ala	Arg	Cys	Val	Arg
1				5					10					15
Leu	Ser	Ala	Glu	Arg	Ala	Gln	Ala	Leu	Leu	Ala	Asp	Val	Asp	Thr
				20					25					30
Leu	Leu	Phe	Asp	Cys	Asp	Gly	Val	Leu	Trp	Arg	Gly	Glu	Thr	Ala
				35					40					45
Val	Pro	Gly	Ala	Pro	Glu	Ala	Leu	Arg	Ala	Leu	Arg	Ala	Arg	Gly
				50					55					60
Lys	Arg	Leu	Gly	Phe	Ile	Thr	Asn	Asn	Ser	Ser	Lys	Thr	Arg	Ala
				65					70					75
Ala	Tyr	Ala	Glu	Lys	Leu	Arg	Arg	Leu	Gly	Phe	Gly	Gly	Pro	Ala
				80					85					90
Gly	Pro	Gly	Ala	Ser	Leu	Glu	Val	Phe	Gly	Thr	Ala	Tyr	Cys	Thr
				95					100					105
Ala	Leu	Tyr	Leu	Arg	Gln	Arg	Leu	Ala	Gly	Ala	Pro	Ala	Pro	Lys
				110					115					120
Ala	Tyr	Val	Leu	Gly	Ser	Pro	Ala	Leu	Ala	Ala	Glu	Leu	Glu	Ala
				125					130					135
Val	Gly	Val	Ala	Ser	Val	Gly	Val	Gly	Pro	Glu	Pro	Leu	Gln	Gly
				140					145					150

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Glu Gly Pro Gly Asp Trp Leu His Ala Pro Leu Glu Pro Asp Val
 155 160 165
 Arg Ala Val Val Val Gly Phe Asp Pro His Phe Ser Tyr Met Lys
 170 175 180
 Leu Thr Lys Ala Leu Arg Tyr Leu Gln Gln Pro Gly Cys Leu Leu
 185 190 195
 Val Gly Thr Asn Met Asp Asn Arg Leu Pro Leu Glu Asn Gly Arg
 200 205 210
 Phe Ile Ala Gly Thr Gly Cys Leu Val Arg Ala Val Glu Met Ala
 215 220 225
 Ala Gln Arg Gln Ala Asp Ile Ile Gly Lys Pro Ser Arg Phe Ile
 230 235 240
 Phe Asp Cys Val Ser Gln Glu Tyr Gly Ile Asn Pro Glu Arg Thr
 245 250 255
 Val Met Val Gly Asp Arg Leu Asp Thr Asp Ile Leu Leu Gly Ala
 260 265 270
 Thr Cys Gly Leu Lys Thr Ile Leu Thr Leu Thr Gly Val Ser Thr
 275 280 285
 Leu Gly Asp Val Lys Asn Asn Gln Glu Ser Asp Cys Val Ser Lys
 290 295 300
 Lys Lys Met Val Pro Asp Phe Tyr Val Asp Ser Ile Ala Asp Leu
 305 310 315
 Leu Pro Ala Leu Gln Gly
 320

<210> 9
 <211> 320
 <212> PRT
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> Incyte ID No: 7476860CD1

<400> 9
 Met Thr Ser Ile Pro Phe Pro Gly Asp Arg Leu Leu Gln Val Asp
 1 5 10 15
 Gly Val Ile Leu Cys Gly Leu Thr His Lys Gln Ala Val Gln Cys
 20 25 30
 Leu Lys Gly Pro Gly Gln Val Ala Arg Leu Val Leu Glu Arg Arg
 35 40 45
 Val Pro Arg Ser Thr Gln Gln Cys Pro Ser Ala Asn Asp Ser Met
 50 55 60
 Gly Asp Glu Arg Thr Ala Val Ser Leu Val Thr Ala Leu Pro Gly
 65 70 75
 Arg Pro Ser Ser Cys Val Ser Val Thr Asp Gly Pro Lys Phe Glu
 80 85 90
 Val Lys Leu Lys Lys Asn Ala Asn Gly Leu Gly Phe Ser Phe Val
 95 100 105
 Gln Met Glu Lys Glu Ser Cys Ser His Leu Lys Ser Asp Leu Val
 110 115 120
 Arg Ile Lys Arg Leu Phe Pro Gly Gln Pro Ala Glu Glu Asn Gly
 125 130 135
 Ala Ile Ala Ala Gly Asp Ile Ile Leu Ala Val Asn Gly Arg Ser
 140 145 150
 Thr Glu Gly Leu Ile Phe Gln Glu Val Leu His Leu Leu Arg Gly
 155 160 165
 Ala Pro Gln Glu Val Thr Leu Leu Leu Cys Arg Pro Pro Pro Gly
 170 175 180
 Ala Leu Pro Glu Leu Glu Gln Glu Trp Gln Thr Pro Glu Leu Ser
 185 190 195
 Ala Asp Lys Glu Phe Thr Arg Ala Thr Cys Thr Asp Ser Cys Thr
 200 205 210
 Ser Pro Ile Leu Asp Gln Glu Asp Ser Trp Arg Asp Ser Ala Ser
 215 220 225
 Pro Asp Ala Gly Glu Gly Leu Gly Leu Arg Pro Glu Ser Ser Gln
 230 235 240

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Lys Ala Ile Arg Glu Ala Gln Trp Gly Gln Asn Arg Glu Arg Pro
 245 250 255
 Trp Ala Ser Ser Leu Thr His Ser Pro Glu Ser His Pro His Leu
 260 265 270
 Cys Lys Leu His Gln Glu Arg Asp Glu Ser Thr Leu Ala Thr Ser
 275 280 285
 Leu Glu Lys Asp Val Arg Gln Asn Cys Tyr Ser Val Cys Asp Ile
 290 295 300
 Met Arg Leu Gly Arg Tyr Ser Phe Ser Ser Pro Leu Thr Arg Leu
 305 310 315
 Ser Thr Asp Ile Phe
 320

<210> 10
 <211> 1803
 <212> DNA
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> Incyte ID No: 8124196CB1

<400> 10
 cgagtgcgga ctggccggat ctgctgtcag tcagcgggaa cagacttctc cctctccatc 60
 tgggtcaactg cgggagaaaa attttcgaga atttccagca ggcaaggcag tggccgcttt 120
 gactgcttgc ttcggagatc cgagacgacg gagaaggcac tcttatttac cgaccaagaa 180
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<220>

<221> misc_feature

<223> Incyte ID No: 8116710CB1

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<211> 2904

<212> DNA

<213> Homo sapiens

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<223> Incyte ID No: 5370008CB1

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What is claimed is:

1. An isolated polypeptide selected from the group consisting of:
 - a) a polypeptide comprising an amino acid sequence selected from the group consisting of
5 SEQ ID NO:1-9,
 - b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-7,
 - c) a polypeptide comprising a naturally occurring amino acid sequence at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:8-9,
10
 - d) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, and
 - e) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-9.
- 15 2. An isolated polypeptide of claim 1 selected from the group consisting of SEQ ID NO:1-9.
3. An isolated polynucleotide encoding a polypeptide of claim 1.
4. An isolated polynucleotide encoding a polypeptide of claim 2.
20
5. An isolated polynucleotide of claim 4 selected from the group consisting of SEQ ID NO:10-18.
6. A recombinant polynucleotide comprising a promoter sequence operably linked to a
25 polynucleotide of claim 3.
7. A cell transformed with a recombinant polynucleotide of claim 6.
8. A transgenic organism comprising a recombinant polynucleotide of claim 6.
30
9. A method for producing a polypeptide of claim 1, the method comprising:
 - a) culturing a cell under conditions suitable for expression of the polypeptide, wherein said cell is transformed with a recombinant polynucleotide, and said recombinant polynucleotide comprises a promoter sequence operably linked to a polynucleotide encoding the polypeptide of claim

1, and

b) recovering the polypeptide so expressed.

10. An isolated antibody which specifically binds to a polypeptide of claim 1.

5

11. An isolated polynucleotide selected from the group consisting of:

a) a polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:10-18,

b) a polynucleotide comprising a naturally occurring polynucleotide sequence at least 90% identical to a polynucleotide sequence selected from the group consisting of SEQ ID NO:10-16,

c) a polynucleotide comprising a naturally occurring polynucleotide sequence at least 80% identical to a polynucleotide sequence selected from the group consisting of SEQ ID NO:17-18,

d) a polynucleotide complementary to a polynucleotide of a),

e) a polynucleotide complementary to a polynucleotide of b),

f) a polynucleotide complementary to a polynucleotide of c), and

g) an RNA equivalent of a)-f).

12. An isolated polynucleotide comprising at least 60 contiguous nucleotides of a polynucleotide of claim 11.

20

13. A method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide of claim 11, the method comprising:

a) hybridizing the sample with a probe comprising at least 20 contiguous nucleotides comprising a sequence complementary to said target polynucleotide in the sample, and which probe specifically hybridizes to said target polynucleotide, under conditions whereby a hybridization complex is formed between said probe and said target polynucleotide or fragments thereof, and

b) detecting the presence or absence of said hybridization complex, and, optionally, if present, the amount thereof.

14. A method of claim 13, wherein the probe comprises at least 60 contiguous nucleotides.

30

15. A method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide of claim 11, the method comprising:

a) amplifying said target polynucleotide or fragment thereof using polymerase chain reaction

amplification, and

b) detecting the presence or absence of said amplified target polynucleotide or fragment thereof, and, optionally, if present, the amount thereof.

5 16. A composition comprising a polypeptide of claim 1 and a pharmaceutically acceptable excipient.

17. A composition of claim 16, wherein the polypeptide has an amino acid sequence selected from the group consisting of SEQ ID NO:1-9.

10

18. A method for treating a disease or condition associated with decreased expression of functional PP, comprising administering to a patient in need of such treatment the composition of claim 16.

15 19. A method for screening a compound for effectiveness as an agonist of a polypeptide of claim 1, the method comprising:

- a) exposing a sample comprising a polypeptide of claim 1 to a compound, and
- b) detecting agonist activity in the sample.

20 20. A composition comprising an agonist compound identified by a method of claim 19 and a pharmaceutically acceptable excipient.

21. A method for treating a disease or condition associated with decreased expression of functional PP, comprising administering to a patient in need of such treatment a composition of claim 20.

25

22. A method for screening a compound for effectiveness as an antagonist of a polypeptide of claim 1, the method comprising:

- a) exposing a sample comprising a polypeptide of claim 1 to a compound, and
- b) detecting antagonist activity in the sample.

30

23. A composition comprising an antagonist compound identified by a method of claim 22 and a pharmaceutically acceptable excipient.

35 24. A method for treating a disease or condition associated with overexpression of functional

PP, comprising administering to a patient in need of such treatment a composition of claim 23.

25. A method of screening for a compound that specifically binds to the polypeptide of claim 1, said method comprising the steps of:

5 a) combining the polypeptide of claim 1 with at least one test compound under suitable conditions, and

b) detecting binding of the polypeptide of claim 1 to the test compound, thereby identifying a compound that specifically binds to the polypeptide of claim 1.

10 26. A method of screening for a compound that modulates the activity of the polypeptide of claim 1, said method comprising:

a) combining the polypeptide of claim 1 with at least one test compound under conditions permissive for the activity of the polypeptide of claim 1,

b) assessing the activity of the polypeptide of claim 1 in the presence of the test compound, and

15 c) comparing the activity of the polypeptide of claim 1 in the presence of the test compound with the activity of the polypeptide of claim 1 in the absence of the test compound, wherein a change in the activity of the polypeptide of claim 1 in the presence of the test compound is indicative of a compound that modulates the activity of the polypeptide of claim 1.

20 27. A method for screening a compound for effectiveness in altering expression of a target polynucleotide, wherein said target polynucleotide comprises a sequence of claim 5, the method comprising:

a) exposing a sample comprising the target polynucleotide to a compound, under conditions suitable for the expression of the target polynucleotide,

25 b) detecting altered expression of the target polynucleotide, and

c) comparing the expression of the target polynucleotide in the presence of varying amounts of the compound and in the absence of the compound.

28. A method for assessing toxicity of a test compound, said method comprising:

30 a) treating a biological sample containing nucleic acids with the test compound;

b) hybridizing the nucleic acids of the treated biological sample with a probe comprising at least 20 contiguous nucleotides of a polynucleotide of claim 11 under conditions whereby a specific hybridization complex is formed between said probe and a target polynucleotide in the biological sample, said target polynucleotide comprising a polynucleotide sequence of a polynucleotide of claim

11 or fragment thereof;

c) quantifying the amount of hybridization complex; and

d) comparing the amount of hybridization complex in the treated biological sample with the amount of hybridization complex in an untreated biological sample, wherein a difference in the amount of hybridization complex in the treated biological sample is indicative of toxicity of the test compound.

29. A diagnostic test for a condition or disease associated with the expression of PP in a biological sample comprising the steps of:

10 a) combining the biological sample with an antibody of claim 10, under conditions suitable for the antibody to bind the polypeptide and form an antibody:polypeptide complex; and

b) detecting the complex, wherein the presence of the complex correlates with the presence of the polypeptide in the biological sample.

15 30. The antibody of claim 10, wherein the antibody is:

a) a chimeric antibody,

b) a single chain antibody,

c) a Fab fragment,

d) a F(ab')₂ fragment, or

20 e) a humanized antibody.

31. A composition comprising an antibody of claim 10 and an acceptable excipient.

32. A method of diagnosing a condition or disease associated with the expression of PP in a subject, comprising administering to said subject an effective amount of the composition of claim 31.

33. A composition of claim 31, wherein the antibody is labeled.

34. A method of diagnosing a condition or disease associated with the expression of PP in a subject, comprising administering to said subject an effective amount of the composition of claim 33.

35. A method of preparing a polyclonal antibody with the specificity of the antibody of claim 10 comprising:

a) immunizing an animal with a polypeptide having an amino acid sequence selected from

the group consisting of SEQ ID NO:1-9, or an immunogenic fragment thereof, under conditions to elicit an antibody response;

b) isolating antibodies from said animal; and

c) screening the isolated antibodies with the polypeptide, thereby identifying a polyclonal antibody which binds specifically to a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-9.

36. An antibody produced by a method of claim 35.

37. A composition comprising the antibody of claim 36 and a suitable carrier.

38. A method of making a monoclonal antibody with the specificity of the antibody of claim 10 comprising:

a) immunizing an animal with a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-9, or an immunogenic fragment thereof, under conditions to elicit an antibody response;

b) isolating antibody producing cells from the animal;

c) fusing the antibody producing cells with immortalized cells to form monoclonal antibody-producing hybridoma cells;

d) culturing the hybridoma cells; and

e) isolating from the culture monoclonal antibody which binds specifically to a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-9.

39. A monoclonal antibody produced by a method of claim 38.

40. A composition comprising the antibody of claim 39 and a suitable carrier.

41. The antibody of claim 10, wherein the antibody is produced by screening a Fab expression library.

42. The antibody of claim 10, wherein the antibody is produced by screening a recombinant immunoglobulin library.

43. A method for detecting a polypeptide having an amino acid sequence selected from the

group consisting of SEQ ID NO:1-9 in a sample, comprising the steps of:

a) incubating the antibody of claim 10 with a sample under conditions to allow specific binding of the antibody and the polypeptide; and

b) detecting specific binding, wherein specific binding indicates the presence of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-9 in the sample.

44. A method of purifying a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-9 from a sample, the method comprising:

a) incubating the antibody of claim 10 with a sample under conditions to allow specific binding of the antibody and the polypeptide; and

b) separating the antibody from the sample and obtaining the purified polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-9.

45. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:1.

46. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:2.

47. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:3.

48. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:4.

49. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:5.

50. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:6.

51. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:7.

52. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:8.

53. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:9.

54. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID NO:10.

55. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID NO:11.

56. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID NO:12.

57. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID NO:13.

58. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID NO:14.

59. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID NO:15.

60. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID NO:16.

61. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID NO:17.

62. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID NO:18.