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(21) International Application Number: PCT/US00/06518 (22) International Filing Date: 10 March 2000 (10.03.00) (30) Priority Data: 09/266,225 10 March 1999 (10.03.99) US (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 09/266,225 (CIP) Filed on 10 March 1999 (10.03.99) (71) Applicant (for all designated States except US): CURAGEN CORPORATION [US/US]; 555 Long Wharf Drive, 11th floor, New Haven, CT 06511 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): NANDABALAN, Krishnan [IN/US]; 228 Village Pond Road, Guilford, CT 06437 (US). YANG, Meijia [CN/US]; 6 Catbird Lane, East Lyme, CT 06533 (US). (74) Agent: ELRIFI, Ivor, R.; Mintz, Levin, Cohn, Ferris Glovsky and Popeo, P.C., One Financial Center, Boston, MA 02111 (US). (81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>																																												
(54) Title: HERMANSKY PUDLAK SYNDROME PROTEIN-INTERACTING PROTEINS AND METHODS OF USE THEREOF																																												
(57) Abstract Provided are complexes of polypeptides which include an HPS polypeptide and an HPS-interacting polypeptides (HPSIP). The HPSIP polypeptides include 14-3-3 protein, Hrs, atrophin-1, DGS-I, nuclear factor NF90, HPIP1 and human HN1 homolog protein. Also disclosed are nucleic acids encoding the HPIP1 and human HN1 homolog protein, or derivatives, fragments and analogs thereof. Methods of screening the complexes or proteins for efficacy in treating and/or preventing certain diseases and disorders, particularly atopic diseases, autoimmune diseases, neurodegenerative disease, cancer, pigmentation disorders, platelet dysfunction and viral diseases, are also disclosed.																																												
<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th></th> <th style="text-align: center;">HPS (forward)</th> <th style="text-align: center;">HPS (reverse)</th> </tr> </thead> <tbody> <tr><td>14-3-3 eta</td><td style="text-align: center;">-</td><td style="text-align: center;">+</td></tr> <tr><td>Hrs</td><td style="text-align: center;">-</td><td style="text-align: center;">+</td></tr> <tr><td>BMK1 alpha</td><td style="text-align: center;">-</td><td style="text-align: center;">+</td></tr> <tr><td>CDK2</td><td style="text-align: center;">-</td><td style="text-align: center;">+</td></tr> <tr><td>Nuclear factor NF90</td><td style="text-align: center;">-</td><td style="text-align: center;">+</td></tr> <tr><td>Atrophin-I</td><td style="text-align: center;">-</td><td style="text-align: center;">+</td></tr> <tr><td>DGS-I</td><td style="text-align: center;">-</td><td style="text-align: center;">+</td></tr> <tr><td>HPIP-1 (cg49368.b1, cg49367.h11, cg49424.c10)</td><td style="text-align: center;">+</td><td style="text-align: center;">-</td></tr> <tr><td>HN1 homolog (cgHs2950_0)</td><td style="text-align: center;">+</td><td style="text-align: center;">-</td></tr> <tr><td>Retinoblastoma</td><td style="text-align: center;">-</td><td style="text-align: center;">-</td></tr> <tr><td>p27(Kip1)</td><td style="text-align: center;">-</td><td style="text-align: center;">-</td></tr> <tr><td>RGL-2</td><td style="text-align: center;">-</td><td style="text-align: center;">-</td></tr> <tr><td>Vector control</td><td style="text-align: center;">-</td><td style="text-align: center;">-</td></tr> </tbody> </table>				HPS (forward)	HPS (reverse)	14-3-3 eta	-	+	Hrs	-	+	BMK1 alpha	-	+	CDK2	-	+	Nuclear factor NF90	-	+	Atrophin-I	-	+	DGS-I	-	+	HPIP-1 (cg49368.b1, cg49367.h11, cg49424.c10)	+	-	HN1 homolog (cgHs2950_0)	+	-	Retinoblastoma	-	-	p27(Kip1)	-	-	RGL-2	-	-	Vector control	-	-
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HERMANSKY PUDLAK SYNDROME PROTEIN-INTERACTING PROTEINS AND
METHODS OF USE THEREOF

GRANT SUPPORT

The invention disclosed herein was made with United States Government support under Grant Number 70NANB5H1066 awarded by the National Institute of Standards and Technology. Accordingly, the United States Government has certain rights in the invention.

FIELD OF THE INVENTION

The invention relates generally to polypeptides and nucleic acids and more particularly to polypeptides that interact with the Hermansky-Pudlak Syndrome-associated HPS polypeptide, and to complexes containing the HPS polypeptides and HPS-interacting polypeptides.

BACKGROUND OF THE INVENTION

Hermansky-Pudlak syndrome (HPS) is a genetic disorder characterized by defective lysosome-related organelles. In humans, HPS-afflicted individuals suffer from albinism, bleeding disorders, lung, and digestive disorders. The lung disorders can, for example, result in progressive pulmonary fibrosis leading to death in the fourth or fifth decade.

The primary defect in HPS is thought to arise from defects in one or more several subcellular organelles. These organelles can include, *e.g.*, lysosomes, melanosomes, and platelet-dense granules. Functional melanocytes in HPS patients are quantitatively reduced in number and/or are qualitatively abnormal. This is thought to result in albinism of tissues such as skin and hair.

Bleeding disorders associated with HPS can include prolonged bleeding time. The prolonged bleeding time associated with HPS is thought to be due to the lack of the storage organelles, platelet-dense granules, which are required for ADP-release and platelet aggregation.

The pulmonary and digestive disorders associated with HPS patients, such as fibrosis and granulomatous colitis, are thought to arise from the accumulation of the ceroid lipofuscin

in lysosomes of reticuloendothelial cells, bone marrow macrophages lung macrophages, gastrointestinal mucosal cell, and other cell types.

A gene responsible for HPS has been identified afflicted HPS families. The HPS gene has been reported to be altered in the DNA of Puerto Rican, Swiss, Irish, and Japanese HPS patients. The amino acid sequence of the HPS polypeptide suggests it is a transmembrane protein that is likely to be a component of multiple cytoplasmic organelles.

In mouse, a recessive mutation at the pale ear (ep) locus has been reported to be the murine homologue of human HPS. Mice carrying the ep mutation show defects similar to human HPS patients in melanosomes and plate-dense granules.

SUMMARY OF THE INVENTION

The invention is based in part on the discovery that certain proteins bind to, and form complexes with, the Hermansky-Pudlak Syndrome (HPS) protein. Accordingly, the invention discloses protein complexes which include the HPS protein bound to, *i.e.*, complexed with, a protein which recognizes and interacts with the HPS protein. A protein which forms a complex with HPS protein hereinafter will be designated an "HPS protein-IP" for HPS protein-Interacting Protein; and of the HPS protein and an HPS protein-IP hereinafter will be designated as "HPS protein•HPS protein-IP complexes." As used herein, an "HPSIP" or "HPSIP polypeptide" includes 14-3-3 *eta*, Hrs, BMK1 alpha kinase, CDK2, Nuclear factor NF90, Atrophin-1, DGS-1, HPIP1, or human HN1 homolog polypeptide.

In some embodiments, the invention is directed to complexes of HPS protein and complexes of the derivatives, fragments and/or analogs of HPS protein with HPIP1 and human HN1 homolog, as well as with the derivatives, fragments and/or analogs of these aforementioned HPS protein-IPs.

The invention further discloses methods of screening for proteins which interact with the HPS protein or derivatives, fragments and/or analogs, thereof. Preferably, the method of screening is a yeast two-hybrid assay system, or a variation thereof.

The invention further discloses the nucleotide and amino acid sequences of human HPIP1 (and homologs of other species) and of human HN1 homolog, as well as derivatives, fragments and analogs thereof. Nucleic acids which are complementary to (*i.e.*, possess the ability to hybridize to), specific nucleotide sequences (*e.g.*, the inverse complement of the foregoing sequences), are also provided. An inverse complement is a nucleic acid sequence

which possesses a complementary sequence, running in reverse orientation to the coding strand, such that the inverse complement would hybridize without mismatches to the nucleic acid strand. Thus, for example, where the coding nucleic acid strand is hybridizable to a nucleic acid sequence with no mismatches between the coding strand and the hybridizable strand, then the inverse complement of the hybridizable strand is identical to the coding strand.

The invention also discloses derivatives, fragments and/or analogs of HPIP1 and human HN1 homolog and which possess biological activity (*i.e.*, they are capable of displaying one or more known functional activities of the wild-type HPIP1 or human HN1 homolog protein. Such biological activities include, but are not limited to: (i) the ability to bind to, or compete for interaction with, the HPS protein; (ii) antigenicity (*i.e.*, the ability to bind to, or compete with, HPIP1 or human HN1 homolog for binding to an anti-HPIP1 or anti-human HN1 homolog antibody, respectively and (iii) immunogenicity (*i.e.*, the ability to generate an antibody which is specific for, and binds to, HPIP1 and human HN1 homolog protein.

Methods for the production of the HPS protein•HPS protein-IP complex and of the HPIP1 and human HN1 homolog protein, and derivatives and analogs of these individual proteins and/or protein complexes (*e.g.*, by recombinant means), are also disclosed. Pharmaceutical compositions comprising same are also provided herein.

The invention further discloses methods for the modulation (*i.e.*, the inhibition or enhancement) of the activity of HPS protein•HPS protein-IP complexes, and methods of modulating the HPIP1 and human HN1 homolog proteins. The individual protein components of these complexes have been implicated in various cellular functions, including, *e.g.*, to: physiological processes (*e.g.*, vesicular transport, protein trafficking, pigmentation regulation, and platelet function) and pathological processes (*e.g.*, oculocutaneous albinism, platelet dysfunction, neurodegenerative disease, and fibrotic lung disease).

The invention also discloses methods for screening specific proteins or protein complexes including, *e.g.*: (i) screening for the HPS protein•HPS protein-IP complex, the HPIP1 and human HN1 homolog proteins, as well derivatives and analogs of the HPS protein•HPS protein-IP complex; (ii) screening for HPIP1 and human HN1 homolog mRNA and (iii) screening the HPIP1 protein and human HN1 homolog for their ability to alter cell functions, particularly those cell functions in which the HPS protein and/or an HPS protein-IP have been implicated.

The invention further discloses diagnostic and prognostic screening methods, as well as therapeutic and prophylactic compositions which are based upon:

(i) HPS protein•HPS protein-IP complexes (including the nucleic acids encoding the individual proteins which participate in the formation of the complexes) and (ii) the HPIP1 protein and their encoding nucleic acid. Therapeutic compounds of the invention include, but are not limited to: (i) HPS protein•HPS protein-IP complexes where one or both members of said complex is a derivative, fragment or analog of the HPS protein and/or an HPS protein-IP; (ii) HPIP1 protein and human HN1 homolog and derivatives, fragments or analogs thereof; (iii) antibodies to the protein or the derivatives, fragments or analogs thereof and (iv) nucleic acids encoding the aforementioned proteins, or their derivatives, fragments or analogs.

Therapeutic compounds may also include the generation of antisense nucleic acids specific for the nucleotide sequences encoding both the HPS protein•HPS protein-IP components and the HPIP1 and human HN1 homolog protein. In addition, diagnostic, prognostic and screening kits are also disclosed herein.

In a further aspect, the invention includes a method of producing a complex of a HPS protein and an HPS-IP protein by growing a recombinant cell containing a nucleic acid expressing a chimeric HPS-HPSIP polypeptide such that the encoded HPS and HPS-IP proteins are expressed and bind to one another, and then recovering the expressed complex.

Also included in the invention is method of producing a HPIP1 or human HN1 homolog protein by growing a recombinant cell containing a nucleic acid encoding one or both of these proteins under conditions that allow for expression of the encoded protein, and recovering the expressed protein.

The invention further provides a method of diagnosing, or screening for the presence of, or a predisposition for, developing a disease or disorder characterized by an aberrant level of a complex of an HPS protein and a herein disclosed HPS-IP protein. The method includes measuring the level of one or more of the complex (*e.g.*, by measuring levels of one or more of the polypeptides in the complex), or RNA encoding polypeptide members of the complex, or measuring the functional activity of the complex, in a sample derived from the subject. An increase or decrease in the level of the complex, the RNA encoding HPS and HPS-IP, or functional activity of the complex in the sample, relative to the level of the complex, the RNA encoding HPS and HPS-IP, or functional activity of the complex, found in an analogous sample not having the disease or disorder or a predisposition for developing the disease or

disorder, indicates the presence of the disease or disorder, or a predisposition for developing the disease or disorder.

Also included in the invention is a kit that includes, in one or more containers, a complex of an HPS and an HPS-IP, an antibody against the complex, nucleic acid probes
5 capable of hybridizing to RNA of HPS and RNA of the HPS-IP, or pairs of nucleic acid primers capable of priming amplification of at least a portion of a gene for HPS and a gene for the HPS-IP.

In a further aspect, the invention includes a method of treating or preventing a disease or disorder involving aberrant levels of a complex of HPS and an HPS-IP in a subject. The
10 method includes administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a molecule or molecules that modulate the function of the complex.

The disease or disorder can include decreased levels of the complex, and the molecule or molecules promote the function of the complex of an HPS and an HPS-IP. The molecule or
15 molecules can be, *e.g.*, a complex of HPS and an HPS-IP; a derivative or analog of a complex of HPS and an HPS-IP, which complex is more stable or more active than the wild type complex; nucleic acids encoding the HPS and an HPS-IP protein, and nucleic acids encoding a derivative or analog of HPS and HPS-IP that form a complex that is more stable or more active than the wild type complex.

Alternatively, the disease or disorder can involve increased levels of the complex, and
20 the molecule or molecules inhibit the function of the complex. The molecule or molecules can be, *e.g.*, an antibody against the complex or a fragment or derivative thereof containing the binding region thereof; HPS and an HPS-IP antisense nucleic acids; and nucleic acids comprising at least a portion of an HPS and an HPS-IP gene into which a heterologous
25 nucleotide sequence has been inserted such that the heterologous sequence inactivates the biological activity of the HPS and HPS-IP genes, in which the HPS and the HPS-IP gene portions flank the heterologous sequences so as to promote homologous recombination with genomic HPS and HPS-IP genes.

In other embodiments, the disease or disorder involves a decreased level of the HPS-IP.
30 In this case, the molecule promotes the function of the HPS-IP and can be, *e.g.*, an HPS-IP protein, a derivative or analog of the HPS-IP that is active in binding HPS, a nucleic acid

encoding the HPS-IP protein, or a nucleic acid encoding a derivative or analog of the HPS-IP that is active in binding HPS.

In other embodiments, the disease or disorder involves an increased level of the HPS-IP. The administered molecule or molecule inhibits the HPS-IP function and can be, *e.g.*, an anti-HPS-IP antibody or a fragment or derivative thereof containing the binding region thereof.
5 an HPS-IP antisense nucleic acid, or a nucleic acid including at least a portion of the HPS-IP gene into which a heterologous nucleotide sequence has been inserted. The heterologous sequence inactivates the biological activity of the HPS-IP gene, in which the HPS-IP gene portion flanks the heterologous sequence so as to promote homologous recombination with a
10 genomic HPS-IP gene.

In a further aspect, the invention includes a method for screening a purified complex of HPS and an HPS-IP with a compound to identify a modulator of the activity of the complex for activity in treating or preventing a disease or disorder. The method includes contacting cultured cells that exhibit an indicator of a disease or disorder, *in vitro* with the complex,
15 derivative or modulator; and comparing the level of the indicator in the cells contacted with the complex, derivative, or modulator with the level of the indicator in cells not so contacted. A lower level in the contacted cells indicates that the complex, derivative or modulator has activity in treating or preventing the disease or disorder. Representative diseases and disorders include, for example, pigmentation disorders, platelet dysfunction, neurodegenerative disease
20 and fibrous lung disease.

In some embodiments, the method includes administering an HSP-HSPIP complex, derivative or modulator to a test animal exhibiting symptoms of a disease or disorder or which test animal is predisposed to develop symptoms of a disease or disorder. Symptoms of the disease or disorder after administration of the complex, derivative, or modulator are then
25 measured. A reduction in the severity of the symptoms of the disease or disorder or prevention of the symptoms of the disease or disorder indicates that the complex, derivative or modulator has activity in treating or preventing the disease or disorder. Representative diseases and disorders include, for example, pigmentation disorders, platelet dysfunction, neurodegenerative disease and fibrous lung disease.

Also included in the invention is a method of identifying a compound which is a
30 modulator of the activity of an HPS-HSPIP complex. The method includes contacting a purified complex of HPS and an HPS-IP with a test compound or agent and examining the

activity of the complex in the presence of, or subsequent to contacting, the compound or agent. An alteration in activity of the complex after contact with the compound or agent indicates the agent is a modulator of the activity of an HPS-HPSIP complex.

One embodiment of the method of screening for a molecule that modulates (directly or indirectly) the formation of an HPS-HPSIP involves measuring the levels of the complex formed from HPS and HPS-IP proteins in the presence of the molecule under conditions that allow for formation of the complex; and comparing the levels of the complex with the levels of the complex that are formed in the absence of the molecule. A lower or higher level of the complex in the presence of the molecule indicates that the molecule modulates formation of the complex.

The invention further covers a recombinant, non-human animal in which both an endogenous HPS gene and an endogenous HPSIP gene have been deleted or inactivated by homologous recombination or insertional mutagenesis of the animal or its ancestor. In some cases, the recombinant, non-human animal contains an HPS-IP gene which is under the control of a promoter that is not the native HPS gene promoter or the native HPS-IP gene promoter. For example, the recombinant, non-human animal may contain a transgene such as the nucleic acid sequence encoding a chimeric HPS-HPSIP polypeptide.

Also included in the invention is a method of modulating the activity or levels of an HPSIP polypeptide in a cell by contacting the cell with HPS, a nucleic acid encoding HPS, an antibody that immunospecifically-binds HPS, or a fragment or derivative of the antibody containing the binding domain thereof, in an amount sufficient to modulate levels of the HPSIP polypeptide.

Also included in the invention is a method of modulating the activity or levels of a complex of HPS and an HPSIP polypeptide contacting a cell with a molecule that modulates the formation of the complex.

In a further embodiment, the invention includes a method for identifying a molecule that modulates activity of HPS or an HPSIP polypeptide, or a complex of HPS and HPSIP. The method includes contacting one or more candidate molecules with HPS in the presence of one or more of the polypeptides and measuring the amount of complex that forms between HPS and the protein. An increase or decrease in the amount of complex that forms relative to the amount that forms in the absence of the candidate molecules indicates that the molecules modulate the activity of HPS or the protein or the complex of HPS and the protein.

In some embodiments, the method is performed by administering the candidate molecules to a recombinant, non-human animal containing a HPS gene, HPSIP transgene, or both and measuring HPS or HPSIP activity, or both, in cells of the transgenic mammal.

Alternatively, the method can be performed *in vitro* on cells expressing HPS or HPSIP proteins.

In another aspect, the invention includes a method for screening a derivative or analog of HPS for biological activity. The method includes contacting the derivative or analog of HPS with an HPSIP polypeptide and detecting the formation of a complex between the derivative or analog of HPS and the protein. Formation of the complex indicates that the derivative or analog of HPS has biological activity.

Also provided by the invention is a method for screening a derivative or analog of an HPSIP polypeptide for its ability to form a complex with an HPS polypeptide. The method includes contacting the derivative or analog of the protein with HPS and detecting the formation of a complex between the derivative or analog of the protein and HPS. Formation of the complex indicates that the derivative or analog of the protein has biological activity.

In a further aspect, the invention provides a method of monitoring the efficacy of a treatment of a disease or disorder characterized by an aberrant level of a complex of HPS protein and a HPS-IP protein in a subject. The method includes measuring the level of the complex, RNA encoding the HPS and HPS-IP proteins, or functional activity of the complex, in a sample derived from the subject. A sample is taken from the subject after the administration of the treatment, and compared to: (i) the level in a sample taken from the subject prior to the administration of the treatment or (ii) a standard level associated with the pretreatment stage of the disease or disorder. The change, or lack of change in the level of the complex, the RNA encoding HPS and HPS-IP, or functional activity of the complex, in the sample taken after the administration of the treatment relative to the level of the complex, the RNA encoding HPS and HPS-IP or functional activity of the complex in the sample taken before the administration of the treatment or to the standard level, indicates whether the administration is effective for treating the disease or disorder.

Also provided by the invention is a method of treating or preventing a disorder associated with HPS syndrome in a subject, *e.g.*, a human. The method includes administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a molecule that modulates the function of a complex of HPS and a HPS-IP protein. The

HPS syndrome associated disorder can be, *e.g.*, a pigmentation disorder, platelet dysfunction, neurodegenerative disease, lung disease (including fibrous lung disease).

Animal models and methods related to the screening of modulators (*e.g.*, agonists, antagonists and inhibitors) of the biological activity of an HPS protein•HPS protein-IP complex, or of an HPIP1 protein, are also provided in the invention. Similarly, methods related to the identification of molecules which inhibit or, alternatively, increase formation of HPS protein•HPS protein-IP complex complexes are also disclosed.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a representation of the nucleic acid sequence [SEQ ID NO:1] and the encoded amino acid sequence [SEQ ID NO:2] of HPIP1.

Fig. 2 is a representation of the nucleic acid sequence [SEQ ID NO:3] and the encoded amino acid sequence [SEQ ID NO:4] of human HN1 homolog. The amino acid residue occupying position 126 could be Met (ATG), Leu (TTG), Val (GTG), or Leu (CTG).

Fig. 3 is a representation of the results obtained from the yeast two-hybrid system assays demonstrating the specificity of HPS protein interactions. The results of assays which utilized the HPS protein as the bait proteins are indicated above the columns. The HPS protein was used in a forward (HPS protein) screen. The prey proteins 14-3-3 *eta*, Hrs, BMK1 alpha kinase, CDK2, Nuclear factor NF90, Atrophin-1, DGS-1, HPIP1, human HN1 homolog, retinoblastoma, p27(Kip1), RGL-2 and vector control are indicated to the left of the columns. A positive interaction between the indicated bait and prey proteins is indicated as "+" sign in

the box forming the intersection between the particular bait and prey proteins, a lack of interaction between the particular bait and prey proteins is designated as a "-" sign.

DETAILED DESCRIPTION OF THE INVENTION

5 The invention is based upon the identification of various proteins which interact with the Hermansky-Pudlak Syndrome (HPS) protein (hereinafter designated as "HPS protein-interacting proteins" or "HPSIPS") using a yeast based system for identifying interacting polypeptides. The HPS protein-interacting proteins were demonstrated to form complexes under physiological conditions with HPS protein. Hereinafter the complexes of HPS protein with an HPS protein-IP will be designated as "HPS protein•HPS protein-IP complexes."
10 These HPS protein•HPS protein-IP complexes, by virtue of this interaction, are implicated in the modulation of functional activities of the HPS protein and its associated binding partners.

 HPS patients have been reported to suffer from several serious medical conditions, including oculocutaneous albinism, a bleeding diathesis, and ceroid deposition, often
15 accompanied by severe fibrotic lung disease and granulomatous colitis. However, despite the recognition of these HPS-associated syndromes, no curative therapeutic intervention currently exists for this disease. Currently, only symptomatic treatment can be offered. One potential area of difficulty involves a lack of understanding regarding the interaction of the HPS protein with other cellular proteins. The elucidation of these potential interactions may provide the
20 means for the subsequent development of an diagnostic assay and/or a therapeutic modality for HPS and its associated diseases.

 The HPSIP disclosed herein may be differentiated into proteins which are involved in signaling processes and protein trafficking (14-3-3 *etc.*, Hrs, BMK1 alpha, CDK2, NF90), those proteins involved in neurodegenerative and developmental disorders (Atrophin I, DGS-
25 1) and those previously-uncharacterized, novel proteins (HPIP1, HN1 homolog). Table I provides an overview of the HPS interacting proteins and their interacting domains disclosed herein.

(A) Proteins Involved Signaling Processes and Protein Trafficking(i) 14-3-3 *eta*

The caHPSoxy-terminal region (starting at nucleotide 764) of the 14-3-3 protein *eta* isoform (GenBank Accession Number X80536; Ichimura-Ohshima, *et al.*, 1992. *J. Neurosci. Res.* 31:600-605) was found to interact with HPS protein. The highly conserved 14-3-3 family of proteins is found in a broad range of organisms and tissues and have been associated with many diverse biological functions, including signal transduction, exocytosis and cell-cycle regulation. The 14-3-3 proteins have been demonstrated to associate with a wide-range of cellular and viral polypeptides involved in signal transduction, cell cycle regulation and/or oncogenesis, suggesting that they participate in cellular growth regulation. See *e.g.*, Aitken, 1995. *Trends Biochem. Sci.* 20:95-97. For example, the *eta* isoform interacts with several kinases, implicating 14-3-3 *eta* proteins in intracellular signal transduction cascades and cellular protein networks.

In addition, calcium-dependent exocytosis in permeabilized adrenal chromaffin cells has been demonstrated to be mediated by several proteins, including, *e.g.*, 14-3-3 proteins (see *e.g.*, Morgan & Burgoyne, 1992. *Nature* 355:833-836); alpha-SNAP proteins (see *e.g.*, Morgan & Burgoyne, 1995. *EMBO J.* 14:323-239); and protein kinase C (see *e.g.*, Morgan & Burgoyne, 1992. *Nature* 355:833-836). Furthermore, 14-3-3 proteins may enhance catecholamine release in permeabilized cells by reorganizing the cortical actin-barrier to allow the increased availability of secretory vesicles for exocytotic release. See *e.g.*, Roth & Burgoyne, 1995. *FEBS Letters* 374:77-81.

The 14-3-3 family of proteins (including the *eta* isoform), activate tryptophan and tryptophan hydroxylase in brain tissue, which is one of the rate-limiting steps in catecholamine and serotonin neurotransmitter biosynthesis (see *e.g.*, Banik, *et al.*, 1997. *J. Biol. Chem.* 272:26219-26225). 14-3-3 proteins have been demonstrated within the neurofibrillary tangles seen in Alzheimer's Disease. This association may be due to the 14-3-3 protein's affect on MAP kinase signaling, which causes hyper-phosphorylation of the *tau* protein. This *tau* protein hyper-phosphorylation is believed to lead to the formation of the paired helical filaments seen in the brains of Alzheimer's Disease victims. See *e.g.*, Layfield, *et al.*, 1996. *Neurosci. Lett.* 209:57-60. Moreover, 14-3-3 proteins have also been shown in the cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. See *e.g.*, Rosenmann, *et al.*, 1997. *Neurol.* 49:593-595.

These data suggest that the 14-3-3 family of proteins, including the *eta* isoform, in neurodegenerative disorders. In addition, 14-3-3 *eta* plays roles in signal transduction, cell cycle regulation and oncogenesis.

(ii) Hrs Protein

Another protein which was found to interact with the HPS protein was Hrs (hepatocyte growth factor-regulated tyrosine kinase substrate) protein [GenBank Accession No. D84064; Lu *et al.*, 1998. *Gene* 213:125-135]. Hrs is a 115 Kdal cytoplasmic protein with a structurally-conserved, putative zinc-finger binding domain and several proline-rich regions. See *e.g.*, Komada & Kitamura, 1995. *Mol. Cell. Biol.* 15:6213-6221. The phosphorylation of tyrosine residues within the Hrs protein may be induced by the treatment of cells with epidermal growth factor or platelet-derived growth factor. It is thus likely that Hrs plays a role in the intracellular signaling pathway of these aforementioned growth factors.

Hrs has been shown to exhibit an 80% homology with rat Hrs-2, an enzyme with ATPase catalytic activity. Hrs-2 was characterized as a brain protein which interacts with SNAP-25, a plasma membrane protein involved in vesicular transport (*i.e.*, vesicular docking and fusion). Synaptic vesicle docking and calcium-dependent exocytosis require the specific interaction of various synaptic vesicle membrane proteins (*e.g.*, VAMP and synaptogamin) with their plasma membrane-localized counterparts (*e.g.*, SNAP-25 and syntaxin). See Sollner, *et al.*, 1993. *Cell* 75:409-418. It was then demonstrated that Hrs-2 functioned to significantly inhibit secretion in a dose-dependent manner, and thus, may be a modulator of exocytosis. Specifically, the binding of Hrs-2 to SNAP-25 is inhibited by calcium at a concentration which is required to support synaptic transmission. Thus, Hrs-2 (and the homologous human protein Hrs) may act as regulators of secretory processes through calcium- and nucleotide-dependent modulation of vesicle-trafficking protein complexes (*e.g.*, SNAP-25). See *e.g.*, Bean, *et al.*, 1997 *Nature* 385:826-829.

(iii) BMK1 alpha kinase

The caHPSoxy-terminal region (starting at nucleotide 2431) of the BMK1 alpha kinase (GenBank Accession Number U29725) was found to interact with HPS protein. The mitogen-activated protein kinase BMK1 is part of a distinct signaling pathway that is required for proliferation and progression through the cell cycle (see, e.g., Lee, *et al.*, 1995, *Biochem. Biophys. Res. Comm.* 213(2):715-724.

(iv) CDK2

Another HPS protein-IP is the cell cycle regulator CDK2 (GenBank Accession Number X61622; Allege & Spottswood, 1991, *EMBO J.* 10:2653-2659). In the mammalian cell cycle, the transition from the G1 phase to DNA replication phase is regulated by the cyclin-dependent kinases (CDKs). Activities of CDKs are controlled by association with cyclins and reversible phosphorylation reactions. An additional level of regulation is provided by inhibitors of CDKs. CDK2 is expressed in, for example, the majority of squamous cell carcinomas, small cell carcinomas, and large cell carcinomas. Higher CDK2 kinase activity is critical for promoting cell cycle progression and unrestrained proliferation of tumor cells.

(v) Nuclear Factor NF-90

The caHPSoxy-terminal region (starting at nucleotide 1930) of nuclear factor NF90 [GenBank Accession Number U10324; See e.g., Kao, *et al.*, 1994, *J. Biol. Chem.* 269:20691-20699] was found to interact with the HPS protein. The nuclear factor of activated T cells (NFAT) has been shown to regulate gene expression of the lymphokine Interleukin-2 (IL-2) which is secreted following T-cell activation. See e.g., Marcoulato, *et al.*, 1998, *J. Interferon Cytokine Res.* 18:351-355. The 90 and 45 Kdal subunits (*i.e.*, NF90 and NF45) of NFAT specifically bind to the antigen receptor response element of the IL-2 promoter. NFAT is the nuclear-target of both T-cell stimulation signals and the immunosuppressant activity of the drugs cyclosporine and FK-506; whereas NF90 and NF45 are substrates for DNA-dependent protein kinase (DNA-PK) *in vitro*. In addition, recombinant NF90 has been found to promote the formation of a complex between the subunits of DNA-PK and DNA. See e.g., Lu, *et al.*, 1998, *J. Biol. Chem.* 273:2136-2145.

(B) Neurodegenerative and Developmental Disorder Proteins(i) Atrophin-I

The caHPSoxy-terminal region (starting at nucleotide 2649) of atrophin-I (GenBank
 5 Accession Number U23851; Margolis, *et al.*, 1996. *Brain Res. Mol. Brain Res.* 36:219-226)
 was found to interact with HPS protein. Although the exact function of atrophin-1 is
 unknown, atrophin-I is the protein encoded by the gene involved in dentatorubral
 pallidoluysian atrophy (DRPLA; Smith's Disease), a rare, progressive and fatal autosomal
 dominant neurological disorder. DRPLA is characterized by neuronal degeneration, especially
 10 in the cerebellar dentate nucleus. Clinical symptomology include variable combinations of
 myoclonus epilepsy, cerebellar ataxia, choreoathetosis and dementia. DRPLA has been shown
 to result from the expansion of a CAG trinucleotide repeat encoding the amino acid glutamine.
 The DRPLA gene product has been primarily localized within the neuronal cytoplasm by *in*
situ hybridization (see *e.g.*, Yazawa, *et al.*, 1995. *Nat. Genet.* 10:99-103) and it is wide-spread
 15 throughout the cerebral and cerebellar regions (see *e.g.*, Knight, *et al.*, 1997. *J. Neurol. Sci.*
146:19-26).

Atrophin-1 interacting proteins (AIPs) containing multiple WW domains were
 identified (see, *e.g.*, Wood *et al.*, 1998, *Mol. Cell. Neurosci.* 11:149-60). Two of these proteins
 are multidomain proteins containing a number of protein-protein interaction modules. The
 20 other three AIPs are highly homologous, each having four WW domains and a HECT domain
 characteristic of ubiquitin ligases.

(ii) DiGeorge Syndrome (DGS)-I Protein

A further HPS protein-IP disclosed herein is the DiGeorge Syndrome (DGS)-I protein
 25 (GenBank Accession Number L77566; see *e.g.*, Gong, *et al.*, 1996. *Hum. Mol. Genet.* 5:789-
 800). One in 4,000 children is born with chromosome 22 deletion syndrome (DiGeorge
 syndrome), making it one of the most common genetic abnormalities in children. Patients
 with DiGeorge Syndrome possess deletions of the chromosomal region 22q11.2. The DGS-I
 gene has been localized to a DGS-critical region of chromosome 22 which encodes for protein
 30 consisting of 476 amino acid residues. Clinical symptomology associated with DGS include
 cardiac defects, thymic hypoplasia cardiac defects, abnormal facial features, immune
 deficiencies, cleft palate and low blood calcium.

(C) HPS Protein-IPs Encoded by Novel Genes(i) HPIP1

One heretofore uncharacterized gene, named HPIP1, was identified as an HPS
5 interacting polypeptide.

(ii) Human HN1 Homolog

Another heretofore uncharacterized human gene (referred to as HN1 homolog) was
identified as the human homolog to mouse gene HN-1 (see Tang *et al.*, 1997. *Mamm. Genome*
10 8:695-6). Murine Hn1 is expressed in many *fetal* and adult tissues. The highest levels of
expression are found in hemapoietic cells, including day 10 yolk sac, blood islands-derived
circulating erythroblasts, day 13 *fetal* liver, adult bone marrow and spleen. The expression is
also very high in day 17 *fetal* brain, while the expression in adult brain is considerably lower.
The nucleotide and amino acid sequences for the human homolog of murine HN-1 are
15 disclosed by the invention.

The invention includes complexes of polypeptides that include the HPSIP binding
domains of an HPS polypeptide and an HPS binding domain of a herein disclosed HPSIP
polypeptide. These complexes can include, *e.g.*, derivatives, analogs and fragments of the HPS
20 protein with HPS protein-IPs, or derivatives, analogs and fragments thereof. In one
embodiment, such complexes bind an anti-HPS protein•HPS protein-IP antibody.
Specifically, complexes of human HPS protein with a human HPS protein-IP protein are
disclosed.

The invention also provides methods of producing and/or isolating HPS protein•HPS
25 protein-IP complexes. In a specific embodiment, the invention provides methods of using
recombinant DNA techniques to express both HPS protein and its binding partner (or
fragments, derivatives or homologs of one or both members of the complex) either where both
binding partners are under the control of one heterologous promoter (*i.e.*, a promoter not
naturally associated with the native gene encoding the particular complex component) or
30 where each is under the control of a separate heterologous promoter.

The invention also provides the nucleotide sequence of the partial HPIP1 gene and of
human HN1 homolog gene, respectively, and its respective, encoded amino acid sequences.
The invention further relates to the carboxyl-terminal region of the HPIP1 protein and to the

HN1 homolog protein, including derivatives, fragments, homologs or analogs thereof, as well as to the nucleic acid which encode the HPIP1 protein and HN1 homolog protein or its derivatives, fragments, homologs or analogs. The invention further provides for the HPIP1 protein and HN1 homolog protein and the nucleic acid sequences encoding these
5 aforementioned proteins, from many different species, preferably from vertebrates, and more preferably from mammals. For example, in one embodiment, the HPIP1 protein and HN1 homolog protein and genes are of human origin. Methods disclosing the production of the aforementioned protein, and their derivatives, fragments, homologs or analogs, are also provided in the invention.

10 The invention further relates to an HPIP1 and human HN1 homolog derivative or analog which is biologically active, that is, possessing one or more of the known functional activities associated with a full-length (wild-type) HPIP1 and human HN1 homolog proteins. Such biological activities include, but are not limited to: (i) the ability to bind to, or compete for interaction with, the HPS protein; (ii) antigenicity (*i.e.*, the ability to bind to, or compete
15 with, HPIP1 and human HN1 homolog for binding to an anti-HPIP1 and anti-human HN1 homolog antibody, respectively and (iii) immunogenicity (*i.e.*, the ability to generate an antibody which is specific for, and binds to, HPIP1 protein and human HN1 homolog, respectively.

Methods relating to diagnosis and prognosis, as well as those methods involved in the
20 screening for diseases and disorders associated with aberrant levels of an HPS protein•HPS protein-IP complex and/or HPIP1 and human HN1 homolog proteins, are provided. The invention also provides methods related to treating or preventing diseases or disorders associated with: (i) aberrant levels of an HPS protein•HPS protein-IP complex; (ii) aberrant levels of the HPIP1 and human HN1 homolog proteins or (iii) aberrant biological activity
25 levels of one or more of the components of the complex. These methods preferably include: (i) administering the HPS protein•HPS protein-IP complex; (ii) administering the HPIP1 and human HN1 homolog protein or (iii) administering modulators of HPS protein•HPS protein-IP complex formation or activity (*e.g.*, antibodies which bind the HPS protein•HPS protein-IP complex, non-complexed HPS protein, or their binding partner or a fragment thereof -
30 preferably the fragment containing the portion of HPS protein or the HPS protein-IP which is directly involved in complex formation). Methods disclosed herein also include, but are not limited to, the administration of: (i) mutants of the HPS protein or the HPS protein-IPs which

either increase or decrease binding affinity; (ii) small-molecule inhibitors or enhancers of protein complex formation or (iii) antibodies which either stabilize or neutralize the protein complex.

In order to quantitatively assess biological activity for use in therapeutics or diagnostics, methods related to the detection of HPS protein•HPS protein-IP complexes, HPIP1 and human HN1 homolog proteins, or modulators (*i.e.*, inhibitors, agonists and antagonists) thereof, are also provided.

(1) HPS Protein•HPS Protein-IP Complexes and HPIP1 and Human HN1 Homolog Proteins

The invention provides a complex of an HPSIP-binding domain of an HPS polypeptide and an HPS-binding domain of an HPSIP polypeptide.

By "HPSIP-binding domain" is meant a region of amino acids sufficient to allow the HPS polypeptide in which the region of amino acids is present to bind specifically to an HPSIP polypeptide. The encoded HPSIP-binding polypeptide can be derived from a full-length HPS polypeptide, or from a derivative, fragment, analog, homolog or paralog of an HPS polypeptide. Preferably, the derivative, fragment, analog, homolog or paralog has one or more of the following attributes: (i) is functionally active (*i.e.*, capable of exhibiting one or more functional activities associated with a full-length, wild-type HPS; (ii) possesses the ability to bind the HPSIP protein; (iii) is immunogenic or (iv) is antigenic.

In some embodiments, the fragment of an HPS polypeptide includes at least 10, 20, 30, 40, or 50 amino acid residues (preferably not larger than 35, 100 or 200 amino acid residues) of an HPS polypeptide. Derivatives or analogs of the encoded HPS polypeptide include, *e.g.*, molecules which include regions which are substantially homologous to the HPS in various embodiments, of at least 50%, 60%, 70%, 80%, 90% or 95% amino acid identity when: (i) compared to an amino acid sequence of identical size; (ii) compared to an aligned sequence in which the alignment is done by a computer homology program known within the art or (iii) the encoding nucleic acid is capable of hybridizing to a sequence encoding the HPS protein under stringent, moderately stringent, or non-stringent conditions, as is discussed below.

Thus, in some embodiments, the encoded HPSIP-binding domain is derived from an HPS polypeptide that includes a sequence that is at least 90% identical to a polypeptide which includes the amino acid sequence having protein accession number U65676. In some

embodiments, the domain is derived from an HPS polypeptide which is at least 95, 98 or even 99% identical to a polypeptide including the amino acid sequence of U65676.

By "HPS-binding domain" is meant a region of amino acids sufficient to allow an HPSIP polypeptide in which the region of amino acids is present to bind specifically to an HPS polypeptide. The encoded HPS-binding polypeptide can be derived from a full-length HPSIP polypeptide, or from a derivative, fragment, analog, homolog or paralog of an HPSIP polypeptide. Preferably, the derivative, fragment, analog, homolog or paralog has one or more of the following attributes: (i) is functionally active (*i.e.*, capable of exhibiting one or more functional activities associated with full-length, wild-type HPSIP; (ii) possesses the ability to bind the HPS protein; (iii) is immunogenic or (iv) is antigenic.

In some embodiments, the HPS binding domain is present in a polypeptide that is a fragment of an HPSIP polypeptide including at least 10, 20, 30, 40, or 50 amino acid residues (preferably not larger than 35, 100 or 200 amino acid residues) of an HPS polypeptide. Derivatives or analogs of the encoded HPSIP polypeptide include, *e.g.*, molecules which include regions which are substantially homologous to the HPSIP in various embodiments, of at least 50%, 60%, 70%, 80%, 90% or 95% amino acid identity when: (i) compared to an amino acid sequence of identical size; (ii) compared to an aligned sequence in which the alignment is done by a computer homology program known within the art or (iii) the encoding nucleic acid is capable of hybridizing to a sequence encoding the HPSIP protein under stringent, moderately stringent, or non-stringent conditions, as is discussed below.

Thus, in some embodiments, the encoded HPP-binding domain is derived from an HPSIP polypeptide that includes a sequence that is at least 90% identical to a polypeptide which includes the amino acid sequence of an HPSIP protein accession number recited in Table I. In some embodiments, the domain is derived from an HPS polypeptide which is at least 95, 98 or even 99% identical to a polypeptide including the referenced amino acid sequence.

The HPSIP polypeptide that provides the HPS-binding domain can be, *e.g.*, a 14-3-3 *eta*, Hrs, BMK1 alpha kinase, CDK2, Nuclear factor NF90, Atrophin-1, DGS-1, HPIP1 or human HN1 homolog protein. Examples of HPSIP polypeptides that include HPS binding region are those shown in Table I, column 4 (entitled "ORF of HPS-IP). In some embodiments, the HPS-binding domain is present in a polypeptide that includes the amino acid sequence of the polypeptide, *i.e.*, the HPSIP polypeptide in the complex can be the full-

length HPSIP polypeptide. *e.g.*, a human or rodent (rat or mouse) HPSIP polypeptide. Alternatively, the HPSIP polypeptide, can be a fragment, homolog, or analog of an HPSIP polypeptide as described herein.

In some embodiments, the HPSIP-binding domain is present in a polypeptide that includes the complete amino acid sequence of an HPS polypeptide, *i.e.*, the HPS polypeptide can be a full-length polypeptide, *e.g.*, a human or rodent (rat or mouse) HPS polypeptide. In some embodiments the HPSIP-binding domain is present in an HPS polypeptide that includes the amino acid sequence of the polypeptide having accession number U65676, *e.g.*, the polypeptide encoded by nucleotides 1272-2306; 1271-2357; 210-1292; 1272-2306, as shown in Table I, column 2. In some embodiments, the HPS polypeptide containing the HPSIP-binding domain is a fragment, homolog, or analog of an HPS polypeptide.

If desired, the polypeptide providing the HPSIP binding domain, or the polypeptide providing the HPS binding domain, or both, can be labeled, *i.e.*, attached to one or more detectable substances. Labeling can be performed using any art recognized method for labeling polypeptides. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

Thus, the invention also relates to complexes which include (i) derivatives, fragments and analogs of the HPS protein interacting with an HPS protein-IP; (ii) the HPS protein interacting with derivatives, fragments and analogs of a HPS protein-IP and (iii) derivatives, fragments and analogs of the HPS protein interacting with derivatives, fragments and analogs of a HPS protein-IP. Accordingly, the invention provides methods for the screening of HPS protein•HPS protein-IP complexes, the HPIP1 and human HN1 homolog proteins, and their various derivatives, fragments and analogs for the ability to alter cell functions, particularly those cell functions in which the HPS protein and/or a HPS protein-IP has been implicated.

Such functions include, but are not limited to, physiological processes (*e.g.*, vesicular transport, protein trafficking, pigmentation regulation, and platelet function) and pathological processes (*e.g.*, oculocutaneous albinism, platelet dysfunction, neurodegenerative disease and fibrotic lung disease).

5 In addition to the ability to alter cellular functions, other functions of these protein complexes include, but are not limited to: binding to an anti-HPS protein•HPS protein-IP complex antibody, as well as other activities as described in the art. For example, derivatives or analogs of the HPS protein•HPS protein-IP complex which have the desired immunogenicity or antigenicity can be used in immunoassays, for immunization, for inhibition
10 of HPS protein•HPS protein-IP complex activity, and the like. Derivatives or analogs of the HPS protein•HPS protein-IP complex which *retain* or enhance, or alternatively lack or inhibit, a property of interest (*e.g.*, participation in an HPS protein•HPS protein-IP complex), can be used as inducers, or inhibitors, respectively, of such a property and its physiological correlates. A specific embodiment relates to an HPS protein•HPS protein-IP complex of a fragment of the
15 HPS protein and/or a fragment of a HPS protein-IP protein which can be bound by an anti-HPS protein, an anti-HPS protein-IP antibody, or by an antibody specific for a HPS protein•HPS protein-IP complex, when such fragment is included within an HPS protein•HPS protein-IP complex.

Also included in the invention is a chimeric polypeptide that includes a region of an
20 HPS polypeptide covalently linked, *e.g.*, via a peptide bond, to a region of an HPSIP polypeptide. Preferably, at least six amino acids each from the HPS and HPSIP polypeptide are included in the chimeric polypeptide. The HPSIP polypeptide can be, *e.g.*, one or more of the 14-3-3 *eta*, Hrs, BMK1 alpha kinase, CDK2, Nuclear factor NF90, Atrophin-1, DGS-1, HPIP1 or human HN1 homolog protein. In some embodiments, the amino acids derived from
25 the HPS polypeptide include an HPSIP-binding domain. In other embodiments, the amino acids derived from the HPSIP polypeptide include an HPS binding domain or region.

The invention further relates to the HPIP1 and human HN1 homolog protein, as well as their derivatives, homologs and analogs. The native protein, fragment, derivative or analog of the HPIP1 and human HN1 homolog protein may be derived from a variety of sources
30 including, *e.g.*, to: human, mouse, rat, pig, cow, dog, monkey, fly, frog, or plant.

The nucleotide sequences which encode the human HPS protein, 14-3-3 *eta*, Hrs, BMK1 alpha kinase, CDK2, Nuclear factor NF90, Atrophin-1, and DGS-1 are known. The

nucleic acids which encode the HPS protein and the HPS protein-IP s may be obtained by any method known in the art (*e.g.*, by PCR amplification using synthetic primers hybridizable to the 3'- and 5'-termini of the sequence or by cloning from a cDNA or genomic library using an oligonucleotide specific for the gene sequence). Homologs (*i.e.*, nucleic acids encoding HPS protein or HPS protein-IPs derived from species other than human) or other related sequences (*e.g.*, paralogs) may be obtained by use of all or a portion of the particular human nucleotide sequence as a probe under low, moderate or high stringency hybridization conditions, followed by cloning.

The HPS protein and HPS protein-IPs, either alone or in a complex, may be obtained by methods well known in the art for protein purification and *in vitro* transcription/translation. The expression of one or more of the aforementioned proteins may be facilitated by the insertion of the nucleic acid containing all or a portion of the nucleotide sequence encoding the protein of interest into an appropriate expression vector (*i.e.*, a vector which possesses the necessary elements for the transcription and translation of the inserted protein coding sequence). Additionally, the necessary transcriptional and translational signals may also be supplied by the native promoter for the HPS protein, any HPS protein-IP gene, or their flanking regions. A variety of host-vector systems may be utilized to express the protein coding sequence. These include, but are not limited to: (i) mammalian cell systems infected with virus (*e.g.*, vaccinia virus, adenovirus, etc.); (ii) insect cell systems infected with virus (*e.g.*, baculovirus); (iii) yeast containing yeast vectors or (iv) bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA. The associated expression elements of these vectors vary in their strengths and specificities.

In one embodiment, an HPS protein•HPS protein-IP complex is obtained by expressing the entire HPS protein sequence and an HPS protein-IP coding sequence within the same cell, either under the control of the same promoter or under two separate promoters. In another embodiment, a derivative, fragment or homolog of the HPS protein and/or a derivative, fragment or homolog of an HPS protein-IP are recombinantly expressed. Preferably the derivative, fragment or homolog of the HPS protein or of the HPS protein-IP protein forms a complex with a binding partner identified by a binding assay (*e.g.*, a modified yeast two-hybrid system) and, more preferably, forms a complex which also binds to an anti-HPS protein•HPS protein-IP complex antibody.

Any of the methods known in the art for the insertion of DNA fragments into a vector may be utilized to construct expression vectors containing a chimeric gene possessing not only a protein coding sequence but the appropriate transcriptional/translational control signals. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of nucleotide sequences encoding the HPS protein and an HPS protein-IP may be regulated by a second nucleotide sequence so that the gene or gene fragment thereof is expressed in a host transformed with the recombinant DNA molecule(s) of interest.

Expression of the proteins may be controlled by any promoter/enhancer known in the art. Promoters which may be utilized include, but are not limited to: (i) the SV40 early promoter (see *e.g.*, Bernoist & Chambon, 1981. *Nature* 290:304-310); (ii) the promoter contained in the 3'-terminus long terminal repeat of Rous Sarcoma Virus (see *e.g.*, Yamamoto, *et al.*, 1980. *Cell* 22:787-797); (iii) the Herpes Simplex Virus thymidine kinase promoter (see *e.g.*, Wagner, *et al.*, 1981. *Proc. Natl. Acad. Sci. USA* 78:1441-1445); (iv) the regulatory sequences of the metallothionein gene (Brinster, *et al.*, 1982. *Nature* 296:39-42); (v) prokaryotic expression vectors such as the B-lactamase promoter (see *e.g.*, Villa-Kamaroff, *et al.*, 1978. *Proc. Natl. Acad. Sci. USA* 75:3727-3731) or the *tac* promoter (see *e.g.*, DeBoer, *et al.*, 1983. *Proc. Natl. Acad. Sci. USA* 80:21-25); (vi) plant expression vectors comprising the nopaline synthetase promoter (see *e.g.*, Herrar-Estrella, *et al.*, 1984. *Nature* 303:209-213) or the Cauliflower Mosaic Virus 35S RNA promoter (see *e.g.*, Garder, *et al.*, 1981. *Nuc. Acids Res.* 9:2871-2879); (vii) the promoter of the photosynthetic enzyme ribulose biphosphate carboxylase (see *e.g.*, Herrera-Estrella, *et al.*, 1984. *Nature* 310:115-120); (viii) promoter elements from yeast and other fungi such as the Gal4 promoter, the alcohol dehydrogenase promoter, the phosphoglycerol kinase promoter, the alkaline phosphatase promoter and (ix) animal transcriptional control regions which exhibit tissue specificity and have been utilized in transgenic animals such as the elastase I gene control region which is active in pancreatic acinar cells (see *e.g.*, Swift, *et al.*, 1984. *Cell* 38:639-646).

In one embodiment of the invention, a vector is utilized which comprises a promoter operably- linked to nucleotide sequences encoding the HPS protein and/or an HPS protein-IP or a fragment, derivative or homolog thereof, one or more origins of replication and, optionally, one or more selectable markers (*e.g.*, an antibiotic resistance gene). In another embodiment, an expression vector containing the coding sequence, or a portion thereof, of the

HPS protein and an HPS protein-IP, either together or separately, is made by subcloning the aforementioned gene sequences into the EcoRI restriction site of one of the commercially-available pGEX vectors (glutathione S-transferase expression vectors; Promega Corp.; Madison, WI) See *e.g.*, Smith & Johnson, 1988. *Gene* 7:31-40. This allows for the expression
5 of gene products in the correct reading frame.

Expression vectors which contain the nucleic acid sequences of interest may be identified by three general methods: (i) nucleic acid hybridization; (ii) presence or absence of marker gene function and (iii) expression of the inserted sequences. In the first method, the HPS protein or HPS protein-IP sequences are detected by nucleic acid hybridization with
10 probes possessing sequences homologous and complementary to the inserted sequences. In the second method, the recombinant vector/host system may be identified and selected based upon the presence or absence of certain marker functions (*e.g.*, binding to an anti-HPS protein, anti-HPSIP, or anti-HPS protein•HPS protein-IP complex antibody; resistance to antibiotics, occlusion body formation in baculovirus, or the like) caused by insertion of the sequences of
15 interest into the vector. In the third method, recombinant expression vectors may be identified by assaying for the HPS protein or HPS protein-IP products expressed by the recombinant vector. Such assays may be based on, for example, the physical or functional properties of the interacting species in *in vitro* assay systems (*e.g.*, the formation of an HPS protein•HPS
protein-IP complex or immunoreactivity to antibodies specific for the protein).

20 Once the recombinant protein molecules are identified and the complexes or individual proteins are isolated, numerous methods known in the art may be used to propagate/amplify them. In addition, a host cell strain may be chosen which serves to modulate the expression of the inserted sequence, or which modifies or processes the expressed protein in the specific manner desired. Expression from certain promoters may be elevated in the presence of certain
25 inducers, thus controlling the expression of the genetically-engineered HPS protein and/or HPS protein-IP. Furthermore, different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (*e.g.*, glycosylation, phosphorylation, etc.) of proteins.

In other embodiments, the HPS protein and/or HPS protein-IP, or fragment, homolog
30 or derivative thereof, may be expressed as a fusion or chimeric protein product which includes the protein joined via a peptide bond to a heterologous protein sequence of a different protein. Such chimeric products may be generated by ligation of the appropriate nucleic acid sequences

encoding the desired amino acids to each one another in the proper reading frame and expressing the chimeric products in a suitable host by methods well known in the art. Alternatively, such chimeric products may be generated by protein synthetic techniques (e.g., by use of a peptide synthesizer) wherein chimeric genes comprising portions of the HPS protein and/or an HPS protein-IP fused to any heterologous protein-encoding sequences may be constructed. A specific embodiment relates to a chimeric protein comprising a fragment of the HPS protein and/or an HPS protein-IP of at least six amino acids.

In one embodiment disclosed herein, fusion proteins are generated which contain the interacting domains of the HPS protein and an HPS protein-IP and/or, optionally, a hetero-functional reagent, such as a peptide linker between the two domains, wherein the use of the hetero-functional reagent promotes the interaction of the HPS protein and HPS protein-IP binding domains. These fusion proteins may be particularly useful where thermodynamic stability of the interaction is desirable (e.g., in production of antibodies specific to the HPS protein•HPS protein-IP complex). Additionally, HPS protein and/or HPS protein-IPs derivatives may be generated by altering their respective sequence by the use of conservative substitutions, additions or deletions which provide for functionally equivalent molecules. Due to the degeneracy of nucleotide coding sequences, other DNA sequences which encode substantially the same amino acid sequence as the HPS protein or HPS protein-IP genes may be used in the practice of the invention. In a specific embodiment of the invention, proteins consisting of at least 6 (contiguous) amino acids of the HPS protein or an HPS protein-IP are provided. In other embodiments, the fragment consists of at least about 10, 20, 30, 40, or 50 contiguous amino acids of the HPS protein or an HPS protein-IP.

The HPS protein or HPS protein-IP derivatives and analogs of the invention may be produced by various methods known in the art. The manipulations which result in their production may occur at either the gene or protein level. For example, the cloned HPS protein or HPS protein-IP gene sequence can be modified by any of numerous strategies known in the art (see e.g., Sambrook, *et al.*, 1989, *Molecular Cloning, A Laboratory Manual, 2d Ed.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). The sequences can then be cleaved at appropriate sites with restriction endonuclease(s), isolated, and ligated *in vitro*. Additionally, the HPS protein and/or HPS protein-IP-encoding nucleotide sequence may be mutated *in vitro* or *in vivo*, so as to: (i) create and/or destroy translation, initiation, and/or termination sequences or (ii) create variations in coding regions and/or form new restriction

endonuclease sites or destroy pre-existing ones, to facilitate further *in vitro* modification. Any technique for mutagenesis known in the art may be used, including, *e.g.*, chemical mutagenesis and *in vitro* site-directed mutagenesis (see *e.g.*, Hutchinson, *et al.*, 1978. *J. Biol. Chem.* 253:6551-6558); use of TAB™ Linkers (Pharmacia; Upsala, Sweden), and the like.

5 Once the recombinant cell expressing the HPS protein and/or an HPS protein-IP protein, or fragment or derivative thereof, is identified, the individual gene product or complex may be isolated and analyzed. This is facilitated through the use of assays based upon the physical and/or functional properties of the protein or complex, including, *e.g.*, radioactive labeling of the product followed by gel electrophoresis analysis, immunoassay, cross-linking
10 to marker-labeled product, and the like.

 The HPS protein•HPS protein-IP complex or HPIP1 and human HN1 homolog protein, may be isolated and purified by standard methods known in the art (either from natural sources or recombinant host cells expressing the complexes or proteins), including, *e.g.*: (i) column chromatography (*e.g.*, ion exchange, affinity, gel exclusion, reversed-phase high pressure, fast
15 protein liquid, etc); (ii) differential centrifugation; (iii) differential solubility or by any other standard technique utilized for the purification of proteins. Additionally, biological functionality may be evaluated using any suitable assay known in the art. Alternatively, once an HPS protein-IP or its derivative is identified, the amino acid sequence of the protein can be deduced from the nucleotide sequence of the chimeric gene from which it was encoded. As a
20 result, the protein (or its derivative) may be synthesized by standard chemical methods known in the art. See *e.g.*, Hunkapiller, *et al.*, 1984. *Nature* 310: 105-111.

 Manipulations of the HPS protein and/or HPS protein-IP sequences may be made at the protein level. Included within the scope of the invention are derivatives of complexes of the HPS protein, an HPS protein-IP, or fragments, derivatives or analogs thereof, that are
25 differentially modified during or after translation (*e.g.*, by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc). Any of numerous chemical modifications may be carried out by known techniques including, *e.g.*, specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH₄,
30 acetylation, formylation, oxidation, reduction, metabolic synthesis in the presence of tunicamycin, and the like.

In a specific embodiment, the HPS protein and/or HPS protein-IP sequences are modified to include a fluorescent label. In another specific embodiment, the HPS protein and/or the HPS protein-IP are modified to include a heterofunctional reagent, which can be used to cross-link the protein to other members of the complex or to other HPS protein-IPs. In addition, analogs and derivatives of the HPS protein, an HPS protein-IP, or their analogs and derivatives can be chemically synthesized. For example, a peptide corresponding to a portion of the HPS protein and/or an HPS protein-IP which either comprises the desired domain or mediates the desired activity *in vitro* (e.g., HPS protein•HPS protein-IP complex formation) may be synthesized by use of a peptide synthesizer. Furthermore, if so desired, non-classical amino acids or chemical amino acid analogs may be introduced as a substitution or addition into the HPS protein and/or an HPS protein-IP.

In addition to standard sequencing techniques, the HPS protein•HPS protein-IP complex or HPIP1 and human HN1 homolog proteins may be analyzed by hydrophilicity analysis. See e.g., Hopp & Woods, 1981. *Proc. Natl. Acad. Sci. USA* 78:3824-3828. Secondary structural analysis can also be done to identify regions of the HPS protein and/or a HPS protein-IP that assume specific structures. See e.g., Chou & Fasman, 1974. *Biochemistry* 13:222-223. The methods of manipulation, translation, secondary structure prediction, hydrophilicity and hydrophobicity profiles, open reading frame prediction and plotting, and determination of sequence homologies, can all be accomplished using computer software programs currently available in the art. Other methods of structural analysis include, but are not limited to: X-ray crystallography (see e.g., Engstrom, 1974. *Biochem. Exp. Biol.* 11:7-13); mass spectroscopy and gas chromatography (see e.g., *Methods in Protein Science*, J. Wiley and Sons, New York, 1997) and computer modeling (see e.g., Fletterick & Zoller, eds., 1986. *Computer Graphics and Molecular Modeling*, In: *Current Communications in Molecular Biology*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY) may also be employed.

(2) Identification and Isolation of the Gene Encoding the HPIP1 and Human HN1 Homolog Protein

The invention discloses the nucleotide sequences which encode the HPIP1, and human HN1 homolog proteins. In specific embodiments, the HPIP1 and human HN1 homolog nucleic acid sequence comprises the sequence of SEQ ID NOS: 1 and 3, respectively, or a

portion thereof, or a nucleotide sequence encoding, in whole or in part, an HPIP1 and human HN1 homolog protein (*e.g.*, a protein comprising the amino acid sequence of SEQ ID NOS: 2 and 4, respectively, or a portion thereof). The invention also provides purified nucleic acids consisting of at least 8 nucleotides (*i.e.*, a hybridizable portion) of an HPIP1 and human HN1 homolog sequence. In other embodiments, the nucleic acids consist of at least about 25 (contiguous) nucleotides, 50 nucleotides, 100 nucleotides, 150 nucleotides, or 200 nucleotides of a HPIP1 and human HN1 homolog gene sequence, or a full-length HPIP1 and human HN1 homolog gene sequence. In yet another embodiment, the nucleic acids are smaller than 35, 200 or 500 nucleotides in length. The nucleic acids may be single or double stranded.

The invention also relates to nucleic acids which are hybridizable or complementary to the aforementioned nucleic acid sequences, in particular, the invention provides the inverse complement to nucleic acids which are hybridizable to the aforementioned sequences. More specifically, the inverse complement of a nucleic acid strand has the complementary sequence running in reverse orientation to the strand so that the inverse complement would hybridize without mismatches to the nucleic acid strand. In one embodiment, nucleic acids are generated which comprise a sequence complementary to (specifically are the inverse complement of) at least about 10, 25, 50, 100, or 200 nucleotides or the entire coding region of an HPIP1 and human HN1 homolog gene.

In one embodiment, a nucleic acid which is hybridizable to an HPIP1 and human HN1 homolog nucleic acid sequence (*e.g.*, possessing the sequence set forth in SEQ ID NOS: 1 and 3, respectively), or to a nucleic acid sequence encoding an HPIP1 and human HN1 homolog protein derivative (or a complement thereof), under conditions of low stringency, is disclosed. By way of example and not of limitation, the procedure using conditions of low, moderate or high stringency can be as described, see *e.g.*, Shilo & Weinberg, 1981, *Proc. Natl. Acad. Sci. USA* 78:6789-6792. Other conditions of low, moderate or high stringency hybridization (*e.g.*, as employed for cross-species hybridizations) are well known within the art and may be utilized.

Nucleic acid molecules which encode derivatives and analogs of HPIP1 and human HN1 homolog protein, or HPIP1 and human HN1 homolog antisense nucleic acids, are additionally disclosed. By "nucleic acid encoding a fragment or portion of an HPIP1 and human HN1 homolog protein" is meant referring to a nucleic acid encoding only the recited

fragment or portion of the HPIP1 and human HN1 homolog protein, and not the other contiguous portions of the HPIP1 and human HN1 homolog as a continuous sequence.

Within a given nucleotide sequence, potential open reading frames (ORFs) can be identified using the NCBI BLAST ORF Finder computer program which is currently commercially available. Due to the fact that all known protein translation products are at least 60 amino acids or longer (see *e.g.*, Creighton, 1992. *Proteins*, 2nd Ed., W.H. Freeman and Co., New York, NY), only those ORFs potentially encoding a protein of 60 amino acids or more were considered. The nucleic acid sequence encoding HPIP1 possesses a stop codon. The ORF encoding HPIP1 (nucleotide 1 to 519, 173 amino acids) putatively predicts the carboxyl-terminal of a longer protein. The ORF encoding human HN1 homolog (nucleotide 106 to 564 - 153 amino acid reading frame, starting with a methionine start codon and ending with a stop codon) predicts a protein of 153 amino acids, with a calculated molecular weight of 15946.4.

Any method available within the art may be utilized to obtain a full-length (*i.e.*, encompassing the entire coding region) cDNA clone encoding an HPIP1 and human HN1 homolog protein. In particular, the polymerase chain reaction (PCR) can be utilized to amplify sequences *in silico* from a cDNA library. Oligonucleotide primers which hybridize to sequences at the 3'- and 5'-termini of the identified sequences may be used as primers to facilitate amplification by PCR those sequences of interest from a nucleic acid sample (cDNA or DNA), preferably a cDNA library, from an appropriate source (*e.g.*, the sample from which the initial cDNA library for the modified yeast two-hybrid assay fusion population was derived). For example, PCR may be carried out through use of a Perkin-Elmer Cetus Thermal Cycler and *Taq* polymerase. The DNA being amplified may include genomic DNA or cDNA sequences obtained from any eukaryotic species. One can choose to synthesize several different degenerate primers, for use in the PCR reactions and it is also possible to vary the stringency of hybridization conditions used in priming the PCR reactions, to amplify nucleic acid homologs (*e.g.*, to obtain HPIP1 and human HN1 homolog protein sequences from species other than humans, or to obtain human sequences with homology to HPIP1 and human HN1 homolog protein) by allowing for greater or lesser degrees of nucleotide sequence homology between the known nucleotide sequence and the nucleic acid homolog being isolated. For cross species hybridization, low stringency conditions are generally preferred. In contrast, for same species hybridization, moderately stringent conditions are generally preferred.

Following successful amplification of the nucleic acid which contained all or a portion of the HPIP1 and human HN1 homolog protein sequences, those amplified sequence may be subsequently cloned and sequenced, and, if desired, utilized as a probe to isolate a complete cDNA or genomic clone. This, in turn, permits the determination of the gene's complete
5 nucleotide sequence, the analysis of its expression, and the production of its protein product for functional analysis. In this manner, the nucleotide sequences of HPIP1 and human HN1 homolog protein were identified.

Any eukaryotic cell potentially can serve as the nucleic acid source for the cloning of the HPIP1 and human HN1 homolog protein genes. The DNA may be obtained by standard
10 procedures known in the art from cloned DNA (e.g., a DNA "library"), by chemical synthesis, by cDNA cloning or by the cloning of genomic DNA, or fragments thereof, purified from the desired cell (see e.g., Sambrook, *et al.*, 1989. *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY); 1985. *DNA Cloning: A Practical Approach*, MRL Press, Ltd., Oxford, U.K., Vols. I & II). Clones derived from
15 genomic DNA may contain regulatory and intronic DNA regions in addition to the coding exonic regions; whereas clones derived from cDNA will contain only exonic sequences.

In the cloning of a sequence of interest (i.e., a gene) from genomic DNA, DNA fragments may be generated by numerous methods which are well-known within the art. These methods include, but are not limited to: (i) cleavage of the DNA at specific sites using
20 one or more restriction endonucleases, (ii) fragmentation of the DNA by use of DNase in the presence of manganese or (iii) physically shearing of the DNA by, for example, sonication. The linear, double-stranded DNA fragments may then be separated as a function of size by standard techniques, including, e.g., agarose and/or polyacrylamide gel electrophoresis, gradient ultracentrifugation or column chromatography.

Once the DNA fragments have been generated, identification of the specific DNA
25 fragment containing the desired gene may be accomplished in a variety of ways. For example, a portion of the HPIP1 gene and human HN1 homolog protein (generated by, for example, PCR amplification or an oligonucleotide having a sequence of a portion of the known nucleotide sequence) or its specific mRNA, or a fragment or derivative thereof, may be
30 purified and labeled, and the generated DNA fragments may then be screened by nucleic acid hybridization to the labeled probe molecule (see e.g., Benton & Davis, 1977. *Science* 196:180-182; Grunstein & Hogness, 1975. *Proc. Natl. Acad. Sci. U.S.A.* 72:3961-3964). It is also

possible to identify the appropriate fragment by restriction enzyme digestion(s) and comparison of fragment sizes with those expected according to a known restriction map if such is available, or by DNA sequence analysis and comparison to the known nucleotide sequence of HPIP1 and human HN1 homolog protein. Further selection may be performed on the basis of the specific properties of the gene of interest or, alternately, its expressed gene product through assays based upon the physical, chemical, or immunological properties of the product. For example, cDNA clones, or DNA clones which hybrid-select the proper mRNAs, may be selected on the basis of specific protein production (*i.e.*, selection of those clones which produce a protein producing similar or identical electrophoretic migration, isoelectric focusing behavior, proteolytic digestion maps, antigenic properties or ability to bind the HPS protein, as has been demonstrated for HPIP1 and human HN1 homolog protein. In addition, clones putatively producing the HPIP1 protein may be identified by the binding of a labeled antibody (specific for HPIP1 and human HN1 homolog protein) to the putatively clone(s), in an ELISA (enzyme-linked immunosorbent assay)-type procedure. An alternative method to the isolation of an HPIP1 and human HN1 homolog protein cDNA, includes, but is not limited to, chemically synthesizing the gene sequence itself from a known sequence. Other methods are possible and within the scope of the invention.

Subsequently, the identified and isolated nucleic acids may then be ligated into an appropriate cloning vector. A large number of vector-host systems are known within the art. Examples of vectors which may be utilized in the invention include, but are not limited to: bacteriophage vectors (*e.g.*, lambda derivatives) or bacterial or yeast plasmids (*e.g.*, pBR322). The insertion of the DNA fragment of interest into a cloning vector may be accomplished by the use of a variety of methods including, for example: (i) the use of complementary cohesive termini; (ii) the use of an enzyme (Klenow fragment of DNA polymerase I to make the insert termini "blunt-ended" (iii) the use of "linker" nucleotide sequences (*e.g.*, specific, chemically-synthesized oligonucleotides possessing, for example, RE sequences) ligated to the termini of the insert DNA fragment or (iv) the use of complementary, homopolymeric tailing of both the vector and DNA insert, etc.

To facilitate the production of numerous copies of the gene sequence of interest, the recombinant molecules are then be introduced into host cells by, for example, transformation, transfection, infection, electroporation, and the like. In an alternative method, the desired gene may be identified and isolated in a "shotgun" cloning approach whereby the gene of interest is

enriched by, for example, size fractionation, prior to its insertion into a suitable cloning vector. Large quantities of the nucleic acid of interest can be generated by transformation of host cells with: (i) recombinant DNA molecules which possess sequences (*i.e.*, the gene) encoding HPIP1 and human HN1 homolog protein; (ii) an HPIP1 and human HN1 homolog protein cDNA or (iii) a chemically-synthesized DNA sequence.

The HPIP1 and human HN1 homolog protein nucleic acid sequences provided by the invention includes those nucleotide sequences which encode: (i) substantially the same amino acid sequence as found within the native HPIP1 and human HN1 homolog protein; (ii) amino acid sequences possessing functionally-equivalent amino acid substitutions and (iii) other HPIP1 and human HN1 homolog protein derivatives, fragments or analogs.

(3) Antibodies Specific for the HPS Protein•HPS Protein-IP Complex and HPIP1 and Human HN1 Homolog Protein Proteins

As disclosed by the invention herein, the HPS protein•HPS protein-IP complex or fragments, derivatives, analogs or homologs thereof, or the HPIP1 and human HN1 homolog protein, or fragments, derivatives, analogs or homologs thereof, may be utilized as immunogens to generate antibodies which immunospecifically-bind these aforementioned immunogenic molecules. Such antibodies include, but are not limited to: polyclonal, monoclonal, chimeric, and single-chain antibodies, F_{ab} fragments, and F_{ab} expression libraries. In one embodiment, antibodies specific for complexes of the human HPS protein and a human HPS protein-IP are generated. Various methods known within the art may be utilized for the production of polyclonal antibodies to a HPS protein•HPS protein-IP complex, or to a derivative, homolog or analog thereof, or to HPIP1 and human HN1 homolog protein, or a derivative, fragment, homolog or analog thereof. For production of the antibody, various host animals can be immunized by injection with the native HPS protein•HPS protein-IP complex, the HPIP1 and human HN1 homolog protein, or a synthetic version or derivative thereof (*e.g.*, a cross-linked HPS protein/HPS protein-IP).

In an embodiment of the invention, epitopes encompassed by the antigenic peptide are regions of HPSIP polypeptides that are located on the surface of the protein, *e.g.*, hydrophilic regions. As a means for targeting antibody production, hydropathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, including, for example, the Kyte Doolittle or the Hopp Woods methods, either with or without

Fourier transformation. See, e.g., Hopp and Woods, 1981, Proc. Nat. Acad. Sci. USA 78: 3824-3828; Kyte and Doolittle 1982, J. Mol. Biol. 157: 105-142, each incorporated herein by reference in their entirety.

For preparation of monoclonal antibodies directed towards an HPS protein•HPS protein-IP complex, to HPIP1 and human HN1 homolog protein, or derivatives, fragments, homologs or analogs thereof, any method which provides for the production of antibody molecules by continuous *in vitro* cell line culture may be utilized. Such methods include, but are not limited to: (i) the hybridoma technique (see Kohler & Milstein, 1975, *Nature* 256:495-497); (ii) the trioma technique (see Rosen, *et al.*, 1977, *Cell* 11:139-147); (iii) the human B-cell hybridoma technique (see Kozbor, *et al.*, 1983, *Immunol. Today* 4:7284) and (iv) the EBV hybridoma technique utilized to produce human monoclonal antibodies (see Cole, *et al.*, 1985, In: *Monoclonal Antibodies and Cancer Therapy* (Alan R. Liss, Inc., pp. 77-96)).

In one embodiment of the invention, human monoclonal antibodies obtained by using human hybridomas (see e.g., Cole, *et al.*, 1983, *Proc. Natl. Acad. Sci. USA* 80:2026-2030) or by the transformation of human B-cells with Epstein-Barr Virus (EBV) *in vitro* (see e.g., Cole, *et al.*, 1985, In: *Monoclonal Antibodies and Cancer Therapy* (Alan R. Liss, Inc., pp. 77-96)). In another embodiment, techniques which were developed for the production of chimeric antibodies (see e.g., Morrison, *et al.*, 1984, *Proc. Natl. Acad. Sci. USA* 81:6851-6855; Takeda, *et al.*, 1985, *Nature* 314:452-454) by splicing the genes from a murine antibody specific for the HPS protein•HPS protein-IP complex or HPIP1 and human HN1 homolog protein, together with genes encoding a human antibody molecule of the appropriate specificity and biological activity, may be utilized. In another embodiment, methods for the production of single-chain antibodies (see e.g., U.S. Patent No. 4,946,778) may be utilized to produce HPS protein•HPS protein-IP complex-specific and an HPIP1 and human HN1 homolog protein-specific, single-chain antibody. Alternatively, methods for the construction of F_{ab} expression libraries (see e.g., Huse, *et al.*, 1989, *Science* 246:1275-1281) are disclosed so as to allow the rapid and efficacious identification of monoclonal F_{ab} fragments with the desired specificity for the HPS protein•HPS protein-IP complex, or an individual HPIP1 and human HN1 homolog protein, derivative, homolog or analog. Non-human antibodies may be "humanized" by several methods known within the art (see e.g., U.S. Patent No. 5,225,539).

Similarly, antibody fragments which contain the idiotypes of an HPS protein•HPS protein-IP complex or of an HPIP1 and human HN1 homolog protein may be generated by

techniques known in the art including, *e.g.*: (i) production of an $F(ab)_2$ fragment by pepsin digestion of an intact antibody molecule; (ii) F_{ab} fragment production by reduction of the disulfide bridges of an $F(ab)_2$ fragment; (iii) F_{ab} fragment generation by treatment of an antibody molecular with papain and a reducing agent; and (iv) F_v fragments.

5 The invention includes antibodies specific to a domain of the HPS protein•HPS protein-IP complex are disclosed, as are antibodies to specific domains of the HPIP1 and human HN1 homolog protein. In some embodiments, antibodies raised against the complex bind with higher affinity than to one or more members of the complex, when the member is not present in the complex. In the production of antibodies, screening for the desired antibody
10 specificity may be accomplished by use of any of the known techniques within the art (*e.g.*, enzyme-linked immunosorbent assay; ELISA). In order to select antibodies which are specific for a particular domain of the HPS protein•HPS protein-IP complex or the HPIP1 and human HN1 homolog protein, one may screen hybridomas for the production of an antibody which binds to the fragment of the HPS protein•HPS protein-IP complex, or the HPIP1 and human
15 HN1 homolog protein, containing the domain. The aforementioned antibodies may be utilized for the localization and/or quantitation of a HPS protein•HPS protein-IP complex or of an HPIP1 and human HN1 homolog protein of the invention (*e.g.*, measuring levels in appropriate physiological samples, in diagnostic methods, etc). In another embodiment of the invention, anti-HPS protein•HPS protein-IP complex antibodies, and fragments or derivatives
20 thereof, as well as antibodies specific for HPIP1 protein, and fragments or derivatives thereof which contain a binding domain, are disclosed.

(4) Diagnostic and Prognostic Uses of Proteins and Nucleic Acids Associated with the HPS Protein•HPS Protein-IP Complex and HPIP1 and Human HN1 Homolog Protein Proteins

25 HPS protein•HPS protein-IP complexes may be used as “markers” of normal physiological processes, and thus have diagnostic utility. These processes include, but are not limited to: (i) physiological processes such as vesicular transport, protein trafficking, pigmentation regulation, and platelet function and (ii) pathological processes such as oculocutaneous albinism, platelet dysfunction, neurodegenerative disease and fibrotic lung
30 disease. Furthermore, characterization of a particular patients subpopulation with elevated or deficient levels of an HPS protein•HPS protein-IP complex or HPIP1 and human HN1 homolog protein may lead to new disease classifications, thus furthering diagnostic ability. In

addition, detection of levels of the aforementioned proteins, their associated mRNAs or antibodies directed against them, may also be utilized in assays (e.g., immunoassays) to detect, prognose, diagnose, or monitor various conditions, diseases, and disorders, and treatments thereof, which are characterized by aberrant levels of HPS protein•HPS protein-IP complexes or by aberrant levels of HPIP1 and human HN1 homolog protein.

5 In one embodiment, an antibodies specific for an HPS protein•HPS protein-IP complex or the HPIP1 and human HN1 homolog proteins are utilized to assay patient tissue or serum samples for the presence of the HPS protein•HPS protein-IP complex or the HPIP1 protein; wherein an aberrant level of the proteins or complex is an indication of a disease condition.

10 "Aberrant levels" is defined as increased or decreased levels relative to those actually present, or in relation to a standard level representing those levels which are present in an analogous sample from a portion of the body or from another individual not having the disorder. The immunoassays which can be utilized include, but are not limited to competitive and non-

15 linked immunosorbent assay (ELISA); "sandwich" immunoassays; immunoprecipitation assays; precipitin reactions; gel diffusion precipitin reactions; immunodiffusion assays; agglutination assays; complement-fixation assays; immunoradiometric assays; fluorescent immunoassays; protein-A immunoassays and the like.

Nucleic acids encoding the various components of the HPS protein•HPS protein-IP

20 complexes, the nucleic acids encoding an HPIP1 and human HN1 homolog protein and related nucleotide sequences, subsequences and complementary sequences thereof, comprising a minimum length of at least 8 nucleotides, may also be utilized in hybridization assays as probes. Such hybridization assays may be used to detect, prognose, diagnose, or monitor the various conditions, disorders, or disease states associated with aberrant levels of the mRNAs

25 encoding the components of an HPS protein•HPS protein-IP complex or HPIP1 and human HN1 homolog protein, as described *supra*. In one embodiment, the hybridization assay is carried out using nucleic acid probes which are capable of hybridizing to the HPS protein and to a binding partner of the HPS protein in order to concurrently measure the expression of both members of an HPS protein•HPS protein-IP complex. Similarly, in another embodiment, the

30 expression of mRNAs encoding HPIP1 and human HN1 homolog protein are measured.

Accordingly, diseases and disorders involving or characterized by aberrant levels of HPS protein•HPS protein-IP complexes may be diagnosed, their suspected presence can be

ascertained by screened procedures or a predisposition to the development of such disorders may be detected, by quantitating aberrant levels of an HPS protein•HPS protein-IP complex, non-complexed HPS protein and/or a HPS protein-IP. In addition, functional activities, including, *e.g.*: binding to an HPS protein-IP, detection of mutations in the HPS protein and/or in an HPS protein-IP RNA, DNA or protein (*e.g.*, translocations, truncations, changes in nucleotide or amino acid sequence relative to the wild-type HPS protein and/or HPS protein-IP) which cause an increase or decrease in the expression or activity of an HPS protein•HPS protein-IP complex, the HPS protein and/or an HPS protein-IP, may also be utilized.

Additionally, various immunoassays known within the art may be utilized to determine whether the ratio of the HPS protein•HPS protein-IP complex to the non-complexed components of the HPS protein•HPS protein-IP complex (*i.e.*, the HPS protein and/or the specific HPS protein-IP in the complex of interest) is increased or decreased within samples from patients suffering from a particular disease or disorder, or having a predisposition to develop such a disease or disorder, as compared to the identical ratio within samples from subjects not having such a disease or disorder.

The use of detection techniques, especially those involving antibodies against HPS protein•HPS protein-IP complexes, or against HPIP1 and human HN1 homolog protein, provides a method of detecting specific cells that express the complex or protein. Using such assays, specific cell types can be defined in which one or more particular HPS protein•HPS protein-IP complex, or HPIP1 and human HN1 homolog protein, is expressed, and the presence of the complex or protein can be correlated with cell viability.

Also embodied herein are methods for the detection of HPS protein•HPS protein-IP complexes or HPIP1 and human HN1 homolog protein, within cell culture models which express particular HPS protein•HPS protein-IP complex, HPIP1 and human HN1 homolog protein, or derivatives thereof, for the purpose of characterizing or preparing these aforementioned proteins for harvest. This embodiment includes methods involving: (i) cell-sorting of prokaryotes (see *e.g.*, Davey & Kell, 1996. *Microbiol. Rev.* 60: 641-696); (ii) primary cultures and tissue specimens from eukaryotes (see *e.g.*, Steele, *et al.*, 1996. *Clin. Obstet. Gynecol.* 39:801-813) and continuous cell cultures (see *e.g.*, Orfao & Ruiz-Arguelles, 1996. *Clin. Biochem.* 29:5-9).

The use of kits for diagnostic purposes is disclosed herein. In one embodiment, the kit includes, in one or more containers, of an anti-HPS protein•HPS protein-IP complex antibody

or an antibody specific for one of HPIP1 and human HN1 homolog protein and, optionally, a labeled binding partner to the antibody. The antibody may be detectably-labeled by any means known in the art including, *e.g.*: chemiluminescent, enzymatic, fluorescent, colorimetric or radioactive labels. A second kit embodiment, comprising, in one or more containers, a nucleic acid probe capable of hybridizing to the HPS protein and/or an HPS protein-IP mRNA. Specifically, the kit may include a pair of primers (each in the size range of approximately 6-30 nucleotides) which are capable of priming a PCR amplification (see *e.g.*, Innis, *et al.*, 1990. *PCR Protocols*, Academic Press, Inc., San Diego, CA), a ligase chain reaction (see PCT Publication EP 320,308) or other methods known within the art (*e.g.*, Q β replicase, cyclic probe reaction, etc). A kit may, optionally, further contain a predetermined amount of a purified HPS protein•HPS protein-IP complex, the HPS protein, or an HPS protein-IP for use as a standard or control.

(5) Therapeutic Uses of the HPS Protein•HPS Protein-IP Complex and HPIP1 and Human HN1 Homolog Protein

The invention provides for treatment or prevention of various diseases and disorders by the administration of therapeutic compounds (referred to hereinafter as "Therapeutics"). Such Therapeutics include, but are not limited to: HPS protein•HPS protein-IP complexes; the HPS protein and the individual HPS protein-IP proteins, and derivatives, fragments and analogs thereof; antibodies thereto; nucleic acids encoding the HPS protein and/or an HPS protein-IP; the HPS protein and/or HPS protein-IP antisense nucleic acids and the HPS protein•HPS protein-IP complex and HPIP1 and human HN1 homolog protein modulators (*i.e.*, inhibitors, agonists and antagonists).

As reviewed in Section 2, *supra*, the HPS protein is centrally implicated in physiological processes such as vesicular transport, protein trafficking, pigmentation regulation, and platelet function. Similarly, the HPS protein has also been strongly implicated in protection from pathological conditions including, but not restricted to: oculocutaneous albinism, platelet dysfunction, neurodegenerative disease, and fibrotic lung disease.

The majority of characterized HPS protein-IPs (particularly 14-3-3 *eta*, Hrs, BMK1 alpha, CDK2, and NF90), as disclosed in the invention, are involved in signal transduction and secretion processes. A linkage has been demonstrated between signal transduction disorders and induction of cellular apoptosis, which may relate to HPS protein and to the HPS protein-

IPs. The invention also discloses an HPS protein-IP (*e.g.*, 14-3-3 *eta*) that plays a role in autoimmune-diseases and inflammation. The 14-3-3 family of proteins are important in autoimmune-diseases, for example, by interacting with insulin receptor substrate 1.

Furthermore, the HPS protein, and binding partners as identified herein (*e.g.*, 14-3-3 *eta*, Hrs, atrophin-1, and DGS-I) are significantly implicated in disorders of neurodegeneration. For example, 14-3-3 *eta* has been shown to be present in Alzheimer's Disease neurofibrillary tangles and within the cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. The ATPase Hrs has been implicated in calcium-regulated secretion. Atrophin-I is the protein product of the gene which has been implicated in dentatorubral pallidolusian atrophy (DRPLA; Smith's disease) and is ubiquitously expressed in neuronal tissues. DGS-I is the protein associated with the developmental defect DiGeorge Syndrome.

The HPS protein-IPs encoded by the genes for HPIP1 and human HN1 homolog protein, may also be related to the implicated functions of the HPS protein and the HPS protein-IPs described above.

(a) Treatment of Diseases and Disorders with Increased Levels of the HPS Protein and the HPS Protein•HPS Protein-IP Complex

A wide range of cellular diseases which are affected by intracellular signal transduction, vesicle transport and protein trafficking may be treated or prevented by the administration of a Therapeutic which modulates (*i.e.*, inhibits, antagonizes, enhances or promotes) the biological of the HPS protein•HPS protein-IP complex and/or HPIP1 and human HN1 homolog protein. Similarly, diseases or disorders which are associated with aberrant HPS protein•HPS protein-IP complex levels or activity, or aberrant levels of HPIP1 and human HN1 homolog protein, may also be treated by administration of a modulating Therapeutic. In a specific embodiment, the activity or level of HPS protein is modulated by administration of a HPS protein-IP. In another specific embodiment, the activity or level of a HPS protein-IP is modulated by administration of the HPS protein.

(b) Antagonizing HPS Protein•HPS Protein-IP Complex Formation or Activity

Diseases and disorders which are characterized by increased (relative to an individual who is not suffering from the disease or disorder) HPS protein•HPS protein-IP complex levels or activity, or increased HPIP1 and human HN1 homolog protein levels or activity, may be

treated with Therapeutics which antagonize (*i.e.*, reduce or inhibit) the levels or activity of these aforementioned proteins or protein complexes. Therapeutics that can may be utilized include, but are not limited to: (i) the HPS protein or an HPS protein-IP (or analogs, derivatives or fragments thereof); (ii) anti-HPS protein•HPS protein-IP complex antibodies; (iii) nucleic acids encoding the HPS protein or an HPS protein-IP; (iv) the concurrent administration of the HPS protein and HPS protein-IP antisense nucleic acids, or HPIP1 and human HN1 homolog protein antisense nucleic acids; (v) the HPS protein and/or HPS protein-IP, or HPIP1 and human HN1 homolog protein nucleic acids, which are dysfunctional due to a heterologous [non-HPS-related] insertion within the coding sequences of the HPS protein or HPS protein-IP coding sequences, which are used to "knockout" endogenous HPS protein and/or HPS protein-IP function by homologous recombination (see *e.g.*, Capecchi, 1989. *Science* 244:1288-1292.

In a specific embodiment of the invention, a nucleic acid containing a portion of a HPS protein and/or a HPS protein-IP gene in which the these aforementioned sequences flank a different gene sequence, is utilized as an HPS protein and/or a HPS protein-IP antagonist or to promote HPS protein and/or HPS protein-IP inactivation by homologous recombination. See *e.g.*, Koller & Smithies, 1989. *Proc. Natl. Acad. Sci. USA* 86:8932-8935; Zijlstra, *et al.*, 1989. *Nature* 342:435-438. Additionally, mutants or derivatives of a first HPS protein-IP which possess greater affinity for the HPS protein than a second HPS protein-IP may be administered to compete with the second HPS protein-IP protein for HPS protein binding, thereby reducing the levels of HPS protein complexes containing the second HPS protein-IP. Other Therapeutics which inhibit HPS protein•HPS protein-IP complex or HPIP1 and human HN1 homolog protein function may be identified by use of known *in vitro* assays, which are based, for example, on their ability to inhibit HPS protein:HPS protein-IP binding.

In further specific embodiments, Therapeutics which antagonize HPS protein•HPS protein-IP complex formation or activity, or HPIP1 and human HN1 homolog protein activity, are administered therapeutically (including prophylactically) within: (i) diseases or disorders involving an increased level of an HPS protein•HPS protein-IP complex or an HPIP1 and human HN1 homolog protein or (ii) diseases or disorders wherein *in vitro* or *in vivo* assays indicate the utility of an HPS protein•HPS protein-IP complex or HPIP1 and human HN1 homolog protein antagonist administration. Increased levels of HPS protein•HPS protein-IP complexes or HPIP1 and human HN1 homolog proteins, may be readily detected by methods

standard in the art, which include, but are not limited to: immunoassays to detect and/or visualize HPS protein•HPS protein-IP complexes, or HPIP1 and human HN1 homolog proteins (e.g., Western blot, immunoprecipitation followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, and the like) and/or hybridization assays to detect concurrent expression of the HPS protein and an HPS protein-IP, or individual HPIP1 and human HN1 homolog protein mRNAs (e.g., Northern blot assays, dot blots, *in situ* hybridization, and the like).

(c) Reducing the Expression of the HPS Protein•HPS Protein-IP Complex

In one embodiment, reduction of HPS protein•HPS protein-IP complex expression (i.e., the expression of the two components of the HPS protein•HPS protein-IP complex and/or formation of the complex) or reducing HPIP1 and human HN1 homolog protein expression, is achieved by targeting mRNAs encoding those protein moieties. RNA therapeutics currently fall within three classes, antisense species, ribozymes, or RNA aptamers. See e.g., Good, *et al.*, 1997. *Gene Therapy* 4:45-54.

Antisense oligonucleotides have been the most-widely utilized method and will be discussed *infra*. Ribozyme therapy involves the administration, induced expression, etc., of small RNA molecules with enzymatic ability to cleave, bind, or otherwise inactivate specific RNAs to reduce or eliminate expression of particular proteins. See e.g., Grassi & Marini, 1996. *Annals of Med.* 28:499-510; Gibson, 1996. *Cancer and Metastasis Rev.* 15:287-299. Currently, the generation of "hairpin" and "hammerhead" RNA ribozymes is necessary to specifically-target a particular mRNA, such as the mRNA encoding the HPS protein. RNA aptamers are specific RNA ligands for proteins, such as for *Tat* and *Rev* RNA (see e.g., Good, *et al.*, 1997. *Gene Therapy* 4:45-54), which can specifically inhibit their translation.

In another embodiment of the invention, the activity or level of the HPS protein is reduced by administration of an HPS protein-IP, a nucleic acid which encodes an HPS protein-IP or an antibody which immunospecifically-binds to an HPS protein-IP, or a fragment or derivative of the antibody containing the binding domain thereof. In still another aspect of the invention, diseases or disorders associated with increased levels of the HPS protein or a particular HPS protein-IP (e.g., HPIP1 and human HN1 homolog protein) may be treated or prevented by administration of a Therapeutic which increases HPS protein•HPS protein-IP

complex formation, if the complex formation acts to reduce or inactivate the HPS protein or the particular HPS protein-IP through HPS protein•HPS protein-IP complex formation.

(d) Treatment of Diseases and Disorders with Decreased Levels of the HPS Protein and the HPS Protein•HPS Protein-IP Complex

5 Diseases and disorders associated with under-expression of an HPS protein•HPS protein-IP complex, the HPS protein or a particular HPS protein-IP, are treated or prevented by administration of a Therapeutic which promotes (*i.e.*, increases or supplies) HPS protein•HPS protein-IP complexes or function. Examples of such a Therapeutic include, but are not limited to: HPS protein•HPS protein-IP complexes and derivatives, analogs and
10 fragments thereof which are functionally active (*e.g.*, are active to form HPS protein•HPS protein-IP complexes), non-complexed HPS protein and HPS protein-IP proteins, and derivatives, analogs, and fragments thereof, and nucleic acids encoding the members of an HPS protein•HPS protein-IP complex, or functionally-active derivatives or fragments thereof (*e.g.*, for use in gene therapy).

15

(e) Determination of the Physiological Effects of a Therapeutic

Preferably, suitable *in vitro* or *in vivo* assays are utilized to determine the effect of a specific Therapeutic and whether its administration is indicated for treatment of the affected tissue. In various specific embodiments, *in vitro* assays can be carried out with representative
20 cells or cell types involved in a patient's disorder to determine if a Therapeutic has a desired effect upon such cell types.

Compounds for use as Therapeutics can be tested in suitable animal model systems prior to testing in humans. The systems can include, *e.g.*, rats, mice, chicken, cows, monkeys, rabbits, etc. For *in vivo* testing, prior to administration to humans, any animal model system
25 known in the art may be used.

(6) Diseases and Disorders Associated with the HPS Protein or HPS Protein Complex

(a) Pigmentation Disorders

30 The HPS protein is strongly implicated in pigmentation disorders, such as oculocutaneous albinism and hypopigmentation. Therapeutics of the invention, particularly those which modulate (or supply) HPS protein•HPS protein-IP complex activity, may be effective in treating or preventing pigmentation diseases or disorders. Therapeutics which

modulate the levels or activity of HPS protein•HPS protein-IP complexes may be assayed by any method known in the art including, *e.g.*, *in vitro* assays using cell culture models and *in vivo* assays using animal models of pigmentation diseases or disorders. See *e.g.*, McGeoch, *et al.*, 1986. *J. Gen. Virol.* 67:813-825.

5 Accordingly, once a pigmentation disease or disorder has been shown to be amenable to treatment by modulation of HPS protein•HPS protein-IP complex activity, the pigmentation disease or disorder can be treated or prevented by administration of a Therapeutic which modulates HPS protein•HPS protein-IP complex formation, including supplying a HPS protein•HPS protein-IP complex.

10 (b) Platelet Dysfunction

Platelet dysfunction which may be treated by modulation of HPS protein•HPS protein-IP complex activity is differentiated into two general classes: (i) diseases associated with platelet storage pool deficiency (*e.g.*, Hermansky-Pudlak Syndrome, Chediak-Higashi Syndrome, gray platelet syndrome) or thrombocytopenia and (ii) diseases associated with thrombocytosis or increased clotting tendency (*e.g.*, myocardial infarction, deep venous thrombosis, cardiovascular accident, transient ischemic attack).

Therapeutics of the invention, particularly those which modulate (or supply) HPS protein•HPS protein-IP complex activity may be effective in treating or preventing platelet dysfunction diseases or disorders. Therapeutics can, for example, reduce platelet dysfunction diseases or disorders in animal models in comparison to controls. Accordingly, once a platelet dysfunction disease or disorder has been shown to be amenable to treatment by modulation of HPS protein•HPS protein-IP complex activity, that platelet dysfunction disease or disorder may be treated or prevented by administration of a Therapeutic which modulates HPS protein•HPS protein-IP complex formation, including supplying an HPS protein•HPS protein-IP complex.

25 (c) Neurodegenerative Disorders

The HPS protein has been implicated to play a role in neurodegenerative disease. Accordingly, Therapeutics of the invention, *e.g.*, those that modulate (or supply) HPS proteins and/or protein complexes may be effective in treating or preventing neurodegenerative disease. Therapeutics of the invention which modulate HPS protein•HPS protein-IP complexes

involved in neurodegenerative disorder may be assayed by any method known in the art for efficacy in treating or preventing such neurodegenerative diseases and disorders. Such assays include, but are not limited to: *in vitro* assays for regulated cell secretion, protein trafficking, and/or folding or inhibition of apoptosis or *in vivo* assays using animal models of neurodegenerative diseases or disorders, or the like. Therapeutics can, for example, but not by way of limitation, promote regulated cell maturation and prevent cell apoptosis in culture, or reduce neurodegeneration in animal models in comparison to controls.

(d) Fibrous Lung Disease

The HPS protein is also strongly implicated in the etiology of severe fibrous lung disease. Therapeutics of the invention, particularly those which modulate the levels or activity of HPS protein•HPS protein-IP complexes may be effective in treating or preventing fibrous lung disease. Therapeutics may be assayed by any method known within the art for efficacy in treating or preventing fibrous lung disease including, *e.g.*, *in vitro* assays using cell culture models, and *in vivo* assays using animal models of fibrous lung disease.

Once a fibrous lung disease has been shown to be amenable to treatment by modulation of HPS protein•HPS protein-IP complexes, that fibrous lung disease can be treated or prevented by administration of a Therapeutic which modulates HPS protein•HPS protein-IP complex formation, including supplying an HPS protein•HPS protein-IP complexes.

(7) Methods of Treating HPS-Related Diseases

(a) Gene Therapy

Gene therapy refers to therapy performed by the administration of a nucleic acid molecule, of a known, specific nucleotide sequence, to a subject. Any of the methods currently known within the art may be use for gene therapy in the practice of the invention. For general reviews of the methods of gene therapy, see *e.g.*, Goldspiel, *et al.*, 1993. *Clinical Pharmacy* 12:488-505; Wu & Wu, 1991. *Biotherapy* 3:87-95; Tolstoshev, 1993. *Ann. Rev. Pharmacol. Toxicol.* 32:573-596; Mulligan, 1993. *Science* 260:926-932; Morgan & Anderson, 1993. *Ann. Rev. Biochem.* 62:191-217.

In one embodiment of the invention, a nucleic acid comprising a sequence which encodes the HPS protein, an HPS protein-IP, or a functional derivative thereof, are administered to modulate HPS protein•HPS protein-IP complexes, or to modulate HPIP1 and

human HN1 homolog protein function, by way of gene therapy. In other embodiments, a nucleic acid or nucleic acids encoding both the HPS protein and an HPS protein-IP, functional derivatives or chimeric protein thereof, are administered by use of gene therapy. In these embodiments, the nucleic acid molecule produces its encoded protein(s) which subsequently mediates a therapeutic effect by modulating the HPS protein•HPS protein-IP complex, or by modulating HPIP1 and human HN1 homolog protein function. In particular, this aforementioned nucleic acid possesses a promoter(s) operably linked to the HPS protein and/or the HPS protein-IP coding region(s), the promoter(s) being inducible or constitutive, and optionally, tissue-specific. In another particular embodiment, a nucleic acid molecule is used in which the HPS protein and/or HPS protein-IP coding sequence, or the HPIP1 and human HN1 homolog protein coding sequences, and any other desired sequences, are flanked by regions which promote homologous recombination at a desired site in the genome, thus providing for intra-chromosomal expression of the HPS protein and the HPS protein-IP nucleic acids. See *e.g.*, Koller & Smithies, 1989. *Proc. Natl. Acad. Sci. USA* 86:8932-8935; Zijlstra, *et al.*, 1989. *Nature* 342:435-438. In yet another specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

Delivery of the nucleic acid into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vector, or indirect, in which case, cells are first transformed with the nucleic acid *in vitro*, then transplanted into the patient. These two approaches are known, respectively, as *in vivo* and *ex vivo* gene therapy. In a specific embodiment, the nucleic acid is directly administered *in vivo*, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art including, *e.g.*: (i) by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular; (ii) by infection using a defective or attenuated retroviral or other viral vector (see *e.g.*, U.S. Patent No. 4,980,286); (iii) by direct injection of naked DNA, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, DuPont); (iv) by coating with lipids or cell-surface receptors or transfecting agents; (v) by encapsulation in liposomes, microparticles, or microcapsules, or by administering it in linkage to a peptide which is known to enter the nucleus; (vi) by administering it in linkage to

a ligand subject to receptor-mediated endocytosis (see *e.g.*, Wu & Wu, 1987. *J. Biol. Chem.* 262:4429-4432), which can be used to target cell types specifically expressing the receptors, etc. In another embodiment, a nucleic acid-ligand complex can be formed in which the ligand comprises a fusogenic viral peptide that disrupts endosomes, preventing lysosomal
5 degradation of the nucleic acid. In yet another embodiment, the nucleic acid may be targeted *in vivo* for cell specific uptake and expression by targeting a specific receptor (see *e.g.*, U.S. Patent No. 5,844,107). Alternatively, the nucleic acid may be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination. See *e.g.*, Koller & Smithies, 1989. *Proc. Natl. Acad. Sci. USA* 86:8932-8935; Zijlstra, *et al.*, 1989.
10 *Nature* 342:435-438.

In a specific embodiment, a viral vector which contains the HPS protein and/or the HPS protein-IP encoding nucleic acid sequence is utilized. For example, a retroviral vector can be used which has been modified to delete retroviral sequences that are not necessary for packaging of the viral genome and integration into host cell DNA. See *e.g.*, Miller, *et al.*,
15 1993. *Meth. Enzymol.* 217:581-599. The HPS protein and/or HPS protein-IP (preferably both the HPS protein and HPS protein-IP) encoding nucleic acids, to be used in gene therapy is/are cloned into the vector, which facilitates delivery of the gene into a patient. See *e.g.*, Clowes, *et al.*, 1994. *J. Clin. Invest.* 93:644-651; Kiem, *et al.*, 1994. *Blood* 83:1467-1473.

In another embodiment of the invention, adenovirus may be utilized as a viral vector in
20 gene therapy and are especially attractive "vehicles" for the delivery of genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease, although other targets for adenovirus-based delivery systems include, but are not limited to, the liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the additional advantage of being capable of infecting non-dividing cells. See *e.g.*, Kozarsky &
25 Wilson, 1993. *Curr. Opin. Gen. Develop.* 3:499-503. Adeno-associated virus (AAV) has also been proposed for use in gene therapy. See *e.g.*, Walsh, *et al.*, 1993. *Proc. Soc. Exp. Biol. Med.* 204:289-300.

Another approach to gene therapy in the practice of the invention involves transferring
30 a gene into cells in *in vitro* tissue culture. Usually, the method of transfer includes the transfer of a selectable marker to the cells to facilitate the isolation of those cells which have taken-up and are expressing the transferred gene. In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell by any method

known in the art, including, *e.g.*, transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. See *e.g.*, Loeffler & Behr, 1993. *Meth. Enzymol.* 217:599-618; Cohen, *et al.*, 1993. *Meth. Enzymol.* 217:618-644; Cline, 1985. *Pharmacol. Ther.* 29:69-92. The transfer technique should be selected to provide for the stable transfer of the nucleic acid to the cell so that the nucleic acid is expressible by the cell, and is heritable and expressible by its cell progeny, as well as ensuring that the that the necessary developmental and physiological functions of the recipient cells are not disrupted. The resulting selected, recombinant cells may be delivered to a patient by various methods known in the art. In one embodiment, epithelial cells are injected subcutaneously or applied as a skin graft onto the patient. Recombinant blood cells (*e.g.*, hematopoietic stem or progenitor cells) are preferably administered intravenously. The total concentration of cells utilized, as well as the delivery route, depend upon the desired effect, patient state, and the like, and can be ascertained by those individuals skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type and include but are not limited to, epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes, T-lymphocytes, B-lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; and various hematopoietic stem or progenitor cells (as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, and the like. Preferably, the cell used for gene therapy is autologous to the patient.

In one embodiment of the invention in which recombinant cells are used in gene therapy, the HPS protein- and/or HPS protein-IP- (preferably both HPS protein and HPS protein-IP) encoding nucleic acid molecule is/are introduced into the cells such that the gene or genes are expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In one embodiment, stem or progenitor cells including, *e.g.*, hematopoietic stem cells (HSC), stem cells of epithelial tissues such as the skin and the lining of the gut, embryonic heart muscle cells, liver stem cells (see *e.g.*, PCT Patent Publication WO 94/08598), and neural stem cells (see *e.g.*, Stemple & Anderson, 1992. *Cell* 71:973-985) may be utilized. Epithelial stem cells (ESCs) or keratinocytes can be obtained from tissues such as the skin and the lining of the gut by known procedures (see *e.g.*, Rheinwald, 1980. *Meth. Cell Bio.* 21:229-247) and can be grown in tissue culture (see *e.g.*,

Pittelkow & Scott, 1986. *Mayo Clinic Proc.* 61:771). If the ESCs are provided by a donor, a method for suppression of host versus graft reactivity (e.g., irradiation, drug or antibody administration to promote moderate immunosuppression) can also be utilized.

With respect to hematopoietic stem cells (HSC), any technique which provides for the isolation, propagation, and maintenance *in vitro* of HSCs can be used in this embodiment of the invention. Techniques by which this may be accomplished include, but are not limited to: (i) the isolation and establishment of HSC cultures from bone marrow cells isolated from the future host, or a donor, or (ii) the use of previously established long-term HSC cultures, which may be allogeneic or xenogeneic. Non-autologous HSC are used preferably in conjunction with a method of suppressing transplantation immune reactions of the future host/patient. In one specific embodiment of the invention, human bone marrow cells can be obtained from the posterior iliac crest by needle aspiration (see e.g., Kodo, *et al.*, 1984. *J. Clin. Invest.* 73:1377-1384) and can be made highly enriched or in substantially pure form by any technique known in the art. Long-term cultures of bone marrow cells may be established and maintained by using, for example, modified Dexter cell culture techniques (see Dexter, *et al.*, 1977. *J. Cell Physiol.* 91:335) or Witlock-Witte culture techniques (see Witlock & Witte, 1982. *Proc. Natl. Acad. Sci. USA* 79:3608-3612).

(b) Use of Antisense Oligonucleotides

embodiment of the invention, HPS protein•HPS protein-IP complex formation and function may be inhibited by the use of anti-sense nucleic acids for the HPS protein and/or HPS protein-IP, and is preferably includes both the HPS protein and HPS protein-IP. In addition, the invention discloses the therapeutic or prophylactic use of nucleic acids (of at least six nucleotides in length) which are anti-sense to a genomic sequence (gene) or cDNA encoding the HPS protein and/or HPS protein-IP, or portions thereof. Such anti-sense nucleic acids have utility as Therapeutics which inhibit HPS protein•HPS protein-IP complex formation or activity, and may be utilized in a therapeutic or prophylactic manner.

Another specific embodiment of the invention discloses methods for the inhibition of the expression of the HPS protein and HPS protein-IPs nucleic acid sequences, within a prokaryotic or eukaryotic cell, which includes providing the cell with an therapeutically-effective amount of an anti-sense nucleic acid of the HPS protein and HPS protein-IP, or derivatives thereof.

The anti-sense nucleic acids of the invention may be oligonucleotides which may either be directly administered to a cell or which may be produced *in vivo* by transcription of the exogenous, introduced sequences. In addition, the anti-sense nucleic acid may be complementary to either a coding (*i.e.*, exonic) and/or non-coding (*i.e.*, intronic) region of the HPS protein or HPS protein-IPs mRNAs. The HPS protein and HPS protein-IPs anti-sense nucleic acids are, at least, six nucleotides in length and are, preferably, oligonucleotides ranging from 6-200 nucleotides in length. In specific embodiments, the anti-sense oligonucleotide is at least 10 nucleotides, at least 15 nucleotides, at least 100 nucleotides, or at least 200 nucleotides. The anti-sense oligonucleotides may be DNA or RNA (or chimeric mixtures, derivatives or modified versions thereof), may be either single-stranded or double-stranded and may be modified at a base, sugar or phosphate backbone moiety.

In addition, the anti-sense oligonucleotide of the invention may include other associated functional groups, such as peptides, moieties which facilitate the transport of the oligonucleotide across the cell membrane, a hybridization-triggered cross-linking agent, a hybridization-triggered cleavage-agent, and the like. See *e.g.*, Letsinger, *et al.*, 1989. *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556; PCT Publication No. WO 88/09810. In a specific embodiment, the HPS protein and HPS protein-IPs antisense oligonucleotides comprise catalytic RNAs or ribozymes. See, *e.g.*, Sarver, *et al.*, 1990. *Science* 247:1222-1225.

The anti-sense oligonucleotides of the invention may be synthesized by standard methods known within the art including, *e.g.*: (i) automated phosphorothioate-mediated oligonucleotide synthesis (see *e.g.*, Stein, *et al.*, 1988. *Nuc. Acids Res.* 16:3209) or (ii) methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (see *e.g.*, Sarin, *et al.*, 1988. *Proc. Natl. Acad. Sci. U.S.A.* 85:7448-7451).

In an alternative embodiment, the HPS protein and HPS protein-IPs antisense nucleic acids are produced intracellularly by transcription of an exogenous sequence. For example, a vector may be produced which (upon being exocytosed by the cell) is transcribed *in vivo*, thus producing an antisense nucleic acid (RNA) species. The aforementioned vector may either remain episomal or become chromosomally-integrated, so long as it can be transcribed to produce the desired antisense RNA. The vectors utilized in the practice of the invention may be derived from bacterial, viral, yeast or other sources known within the art, which are utilized for replication and expression in mammalian cells. Expression of the sequences encoding the HPS protein and HPS protein-IPs antisense RNAs may be facilitated by any promoter known

within the art to function in mammalian, preferably, human cells. Such promoters may be inducible or constitutive and include, but are not limited to: (i) the SV40 early promoter region; (ii) the promoter contained in the 3'-terminus long terminal repeat of Rous sarcoma virus (RSV); (iii) the Herpesvirus thymidine kinase promoter and (iv) the regulatory sequences
5 of the metallothionein gene.

The HPS protein and HPS protein-IPs antisense nucleic acids may be utilized prophylactically or therapeutically in the treatment or prevention of disorders of a cell type which expresses (or preferably over-expresses) the HPS protein•HPS protein-IP complex. Cell
10 types which express or over-express the HPS protein and HPS protein-IPs RNA may be identified by various methods known within the art including, but are not limited to, hybridization with HPS protein- and HPS protein-IP-specific nucleic acids (*e.g.*, by Northern hybridization, dot blot hybridization, *in situ* hybridization) or by observing the ability of RNA from the specific cell type to be translated *in vitro* into the HPS protein and the HPS protein-IPs by immunohistochemistry. For example, primary tissue from a patient may be assayed for
15 the HPS protein and/or HPS protein-IPs expression prior to actual treatment by, for example, immunocytochemistry or *in situ* hybridization.

Pharmaceutical compositions of the invention, comprising an effective amount of a HPS protein and HPS protein-IPs antisense nucleic acid contained within a pharmaceutically-acceptable carrier may be administered to a patient having a disease or disorder which is of a
20 type that expresses or over-expresses HPS protein•HPS protein-IP complex RNA or protein. The amount of HPS protein and/or HPS protein-IPs antisense nucleic acid which will be effective in the treatment of a particular disorder or condition will be dependant upon the nature of the disorder or condition, and may be determined by standard clinical techniques. Where possible, it is desirable to determine the antisense cytotoxicity *in vitro*, and then in
25 useful animal model systems prior to testing and use in humans. In a specific embodiment, pharmaceutical compositions comprising HPS protein and HPS protein-IPs antisense nucleic acids may be administered via liposomes, microparticles, or microcapsules. See *e.g.*, Leonetti, *et al.*, 1990, *Proc. Natl. Acad. Sci. U.S.A.* 87:2448-2451.

(8) HPS Protein and HPS Protein•HPS Protein-IP Complex Assays

The functional activity of an HPS protein•HPS protein-IP, HPIP1 and human HN1 homolog protein, or derivatives, fragments and analogs thereof, may be assayed by various methods known within the art. Potential functional modulators (*e.g.*, inhibitors, agonists and antagonists) of HPS protein•HPS protein-IP complex or HPIP1 and human HN1 homolog protein activity (*e.g.*, antibodies or antisense nucleic acids specific for the HPS protein, HPS protein-IPs, or HPS protein-IP complex) may be assayed for by their ability to modulate HPS protein•HPS protein-IP complex formation and/or activity, and for the ability to modulate HPIP1 and human HN1 homolog protein activity.

(a) Immunoassays

In one embodiment of the invention, where one is assaying for the ability to bind or compete with wild-type HPS protein or HPS protein-IP protein, for binding to antibodies specific for the aforementioned proteins or protein complexes, various immunoassay methods known in the art may be used, including, *e.g.*, competitive and non-competitive assay systems using techniques such as radioimmunoassays, enzyme linked immunosorbent assay (ELISA), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions, immunodiffusion assays, *in situ* immunoassays, Western blots, precipitation reactions, agglutination assays, complement fixation assays, immunofluorescence assays, protein-A assays, immunoelectrophoresis assays, and the like.

(b) Gene Expression Assays

The expression of the HPS protein or HPS protein-IPs genes (both endogenous genes and those expressed from recombinant DNA) may be detected using techniques known within the art including, *e.g.*, Southern hybridization, Northern hybridization, restriction endonuclease mapping, DNA sequence analysis and polymerase chain reaction amplification (PCR) followed by Southern hybridization or RNase protection (see *e.g.*, *Current Protocols in Molecular Biology*, 1997, (John Wiley and Sons, New York, NY)) with probes specific for the HPS protein and HPS protein-IPs genes in various cell types.

In one specific embodiment of the invention, Southern hybridization may be used to detect genetic linkage of the HPS protein and/or HPS protein-IPs gene mutations to physiological or pathological states. Numerous cell types, at various stages of development,

may be characterized for their expression of the HPS protein and HPS protein-IPs (particularly the concomitant expression of the HPS protein and HPS protein-IPs within the same cells). The stringency of the hybridization conditions for Northern or Southern blot analysis may be manipulated to ensure detection of nucleic acids with the desired degree of relatedness to the specific probes used. Modification of these aforementioned methods, as well as other methods well-known within the art, may be utilized in the practice of the invention.

(c) Binding Assays

Derivatives (*e.g.*, fragments), analogs and homologs of HPS protein-IPs may be assayed for their ability to bind to the HPS protein by any method known within the art, including, *e.g.*, the modified yeast two hybrid assay system, immunoprecipitation with an antibody which binds to the HPS protein in a complex followed by size fractionation analysis of the immunoprecipitated proteins by denaturing or non-denaturing polyacrylamide gel electrophoresis, Western analysis, non-denaturing gel electrophoresis, and similar methods.

(d) Biological Activity Assays

One embodiment of the invention provides a method for the screening of a derivative, analog or homolog of the HPS protein for biological activity comprising contacting the derivative, analog or homolog of the HPS protein with a protein selected from the group consisting of HPIP1 and human HN1 homolog protein, and detecting the subsequent formation of a complex between the derivative, analog or homolog of the HPS protein and the protein; wherein detecting formation of the complex indicates that the derivative, analog or homolog of the HPS protein possesses biological (*e.g.*, binding) activity.

An additional embodiment discloses a method for the screening of a derivative, analog or homolog of a protein selected from the group consisting of 14-3-3 protein, Hrs, BMK1 alpha, CDK2, NF90, atrophin-1, DGS-I, or HPIP1 and human HN1 homolog protein for biological activity comprising contacting the derivative, analog or homolog of the protein with the HPS protein; and detecting the subsequent formation of a complex between the derivative, analog or homolog of the protein and the HPS protein; wherein detecting the formation of the complex indicates that the derivative, analog or homolog of the protein possesses biological (*e.g.*, binding) activity.

(e) Modulation of Protein Biological Activity

The invention also discloses methods for the modulation of the biological activity of a protein of interest which can participate in an HPS protein•HPS protein-IP by the administration of a binding partner of that protein of interest, or a derivative or analog thereof. For example, the ability of the HPS protein (and derivatives or analogs thereof) to modulate the activity or level of an HPS protein-IP may be ascertained by such methods as contacting a cell, administering to an animal, expressing an HPS protein-IP gene with a HPS protein, or a nucleic acid encoding a HPS protein, or an antibody which immunospecifically binds the HPS protein, and measuring a change in HPS protein-IP levels or activity. A change in HPS protein-IP levels or activity is indicative of the HPS protein possessing the ability to modulate HPS protein-IP levels or activity.

In an alternative embodiment of the invention, an HPS protein-IP may be assayed for the ability to modulate the activity or levels of the HPS protein by contacting a cell, administering to an animal, expressing the HPS protein gene with: (i) an HPS protein-IP; (ii) a nucleic acid encoding an HPS protein-IP or (iii) an antibody which immunospecifically binds to a HPS protein-IP, or a fragment or derivative of the antibody containing the binding domain thereof, wherein a change in the HPS protein levels or activity indicates that the HPS protein-IP can modulate HPS protein levels or activity.

The HPS protein•HPS protein-IP complex, or HPIP1 and human HN1 homolog protein, or derivative, analog, or fragment thereof, may also be screened for activity in modulating the activity of the HPS protein and the HPS protein binding partners (*i.e.*, the HPS protein-IPs), particularly, HPIP1 and human HN1 homolog protein. The proteins and protein complexes of the invention may be screened for the ability to modulate (*i.e.*, increase or decrease) HPS protein•HPS protein-IP complexes, as discussed *infra*.

(f) Assays for the Treatment of Pigmentation Disorders

The HPS protein has been implicated in etiology of pigmentation disorders, including oculocutaneous albinism and hypopigmentation. Accordingly, HPS protein•HPS protein-IP complexes, and derivatives, analogs, and fragments thereof, nucleic acids encoding the HPS protein genes, anti-HPS protein•HPS protein-IP complexes, and other modulators, may be tested for activity in treating or preventing pigmentation disorders in both *in vitro* and *in vivo* assays.

In one embodiment, a Therapeutic can be assayed for activity in treating or preventing pigmentation disorders by contacting cultured cells which exhibit an indicator of an pigmentation reaction, *in vitro*, with the Therapeutic, and comparing the level of indicator in the cells contacted with the Therapeutic with the level of the indicator in cells not so contacted, wherein a lower level in the contacted cells indicates that the Therapeutic has activity in treating or preventing pigmentation disorders. Cell models that can be used for such assays include, but are not limited to, *in vitro* studies using cultured vitiligo melanocytes and keratinocytes (see *e.g.*, Bessou, *et al.*, 1997, *Br. J. Dermatol.* 137:890-897); lines of immortal, severely hypopigmented melanocytes and melanoblasts from mice of the null genotype p(cp)/p(25H) (see *e.g.*, Sviderskaya, *et al.*, 1997, *J. Invest. Dermatol.* 108:30-34) and organotypic culture of human skin to study melanocyte migration (see *e.g.*, Le Poole, *et al.*, 1994, *Pigment. Cell Res.* 7:33-43).

(g) Assays for the Treatment of Platelet Dysfunction

The HPS protein has been implicated in platelet dysfunction. Accordingly, the HPS-associated proteins and protein complexes may be tested for activity in treating or preventing such platelet dysfunction in *in vitro* and *in vivo* assays.

In one embodiment, a Therapeutic of the invention can be assayed for activity in treating or preventing platelet dysfunction by contacting cultured cells *in vitro* that exhibit an indicator of an platelet reaction with the Therapeutic, and comparing the level of indicator in the cells contacted with the Therapeutic with the level of the indicator in cells not so contacted. In another embodiment, a Therapeutic of the invention can be assayed for activity in treating or preventing platelet dysfunction by administering the Therapeutic to a test animal exhibiting an platelet reaction or which test animal does not exhibit an platelet reaction and is

subsequently challenged with an agent that elicits an platelet reaction; and measuring the change in the platelet reaction after the administration of the Therapeutic.

A number of animal models of platelet dysfunction, which accurately mimic natural human platelet dysfunction, are known within the art. Examples of specific models include, but are not limited to, a skin bleeding-time test in a rat model (see MacDonald, *et al.*, 1994. *Thromb. Res.* 76:535-540); correlation between bleeding-time and antithrombotic effect of platelet-suppressive agents in rat experimental model (see Suehiro, *et al.*, 1994. *Res. Commun. Chem. Pathol. Pharmacol.* 83:157-163) and others similar assays.

(h) Assays for the Treatment of Neurodegenerative Diseases

HPS is associated with a variety of neurodegenerative disorders. In one embodiment of the invention, a Therapeutic of the invention may be assayed for by its activity in treating or preventing neurodegenerative disease by contacting cultured cells which exhibit an indicator of a neurodegenerative disease, such as over-expression of the α -A4 peptide, *in vitro*, with the Therapeutic, and comparing the level of the indicator in the cells contacted with the Therapeutic with the level of the indicator in cells not so contacted, wherein a lower level in the contacted cells indicates that the Therapeutic has activity in treating or preventing neurodegenerative disease. Specific examples of cell culture models for neurodegenerative disease include, but are not limited to, cultured rat endothelial cells from affected and non-affected individuals (see *e.g.*, Maneiro, *et al.*, 1997. *Meth. Find. Exp. Clin. Pharmacol.* 19:5-12); P19 murine embryonal carcinoma cells (see *e.g.*, Hung, *et al.*, 1992. *Proc. Natl. Acad. Sci. USA* 89:9439-9443) and dissociated cell cultures of cholinergic neurons from the nucleus basalis of Meynert (see *e.g.*, Nakajima, *et al.*, 1985. *Proc. Natl. Acad. Sci. USA*, 82:6325-6329).

In another embodiment, a Therapeutic of the invention can be assayed for activity in treating or preventing neurodegenerative disease by administering the Therapeutic to a test animal that exhibits symptoms of a neurodegenerative disease, such as premature development of cognitive deficiencies in transgenic animals expressing β -APP, or that is predisposed to develop symptoms of a neurodegenerative disease; and measuring the change in the symptoms of the neurodegenerative disease after administration of the Therapeutic. Examples of specific neurodegenerative disease animal models include, but are not limited to, the partial trisomy 16 mouse (see Holtzman, *et al.*, 1996. *Proc. Natl. Acad. Sci. USA* 93:13333-13338); bilateral nucleus basalis magnocellularis-lesioned rats (see Popovic, *et al.*, 1996. *Int. J. Neurosci.*

86:281-299); the aged rat (Muir, 1997, *Pharmacol. Biochem. Behav.* 56:687-696), the PDAPP transgenic mouse model of Alzheimer's disease (Johnson-Wood, *et al.*, 1997, *Proc. Natl. Acad. Sci. USA* 94:1550-1555), and experimental autoimmune dementia (Oron, *et al.*, 1997, *J. Neural Transm. Suppl.* 49:77-84).

(i) Screening of HPS Protein and Protein Complex Antagonists and

Agonists

The invention provides assays for the detection of molecules which specifically bind to HPS protein•HPS protein-IP complexes, the HPS protein, or HPS protein-IP nucleic acids, proteins or derivatives. For example, recombinant cells expressing both HPS protein and/or HPS protein-IP nucleic acids, or expressing HPIP1 and human HN1 homolog protein nucleic acids, can be used to recombinantly produce the complexes or proteins used in these assays, to screen for molecules which bind or interfere with HPS protein•HPS protein-IP complexes, or which interfere with HPIP1 and human HN1 homolog protein function.

Alternatively, modulators are identified by administering a candidate molecule to a transgenic, non-human animal expressing both the HPS protein and a HPS protein-IP from promoters that are not the native the HPS protein or the native HPS protein-IP promoters, more preferably where the candidate molecule is also recombinantly expressed in the transgenic non-human animal. Alternatively, the method for identifying such modulators can be carried out *in vitro*, preferably with purified HPS protein purified HPS protein-IP, and a purified candidate molecule. Agents to be screened may also include all forms of antisera, antisense nucleic acids, etc., which can modulate HPS protein•HPS protein-IP complex activity, or modulate an HPIP1 and human HN1 homolog protein activity.

By way of example, and not of limitation, diversity libraries, such as random or combinatorial peptide or non-peptide libraries may be screened for molecules that specifically bind to an HPS protein•HPS protein-IP complex, or to an HPIP1 and human HN1 homolog protein. Many libraries are known within the art including, *e.g.*, chemically synthesized libraries, recombinant libraries (*e.g.*, phage display libraries) and *in vitro* translation-based libraries. Screening the libraries can be accomplished by any of a variety of commonly employed methods known methods. See *e.g.*, Bock, *et al.*, 1992, *Nature* 355:564-566; Tuerk, *et al.*, 1992, *Proc. Natl. Acad. Sci. USA* 89:6988-6992; U.S. Patent No. 5,096,815; U.S. Patent No. 5,223,409; U.S. Patent No. 5,198,346; PCT Publication No. WO 94/18318.

In one embodiment, agents which modulate (*i.e.*, inhibit, antagonize or agonize) HPS protein•HPS protein-IP complex activity may be screened using a binding-inhibition assay, wherein agents are screened for their ability to inhibit formation of a HPS protein•HPS protein-IP complex under aqueous, physiological binding conditions in which HPS protein•HPS protein-IP complex formation occurs in the absence of the agent to be tested. Agents which interfere with the formation of HPS protein•HPS protein-IP complexes are identified as antagonists of complex formation. Agents which eliminate the formation of HPS protein•HPS protein-IP complexes are identified as inhibitors of complex formation. Agents which enhance the formation of HPS protein•HPS protein-IP complexes are identified as agonists of complex formation.

Methods utilized in the practice of the invention s for screening may involve labeling the complex proteins with: (i) radioligands (*e.g.*, ^{125}I or ^3H); (ii) magnetic ligands (*e.g.*, paramagnetic beads covalently attached to photobiotin acetate); (iii) fluorescent ligands (*e.g.*, fluorescein or rhodamine) or (iv) enzyme ligands (*e.g.*, luciferase or beta-galactosidase). The reactants which bind in solution may then be isolated by one of many techniques known in the art, including but not restricted to, co-immunoprecipitation of the labeled moiety using antisera against the unlabeled binding partner (or a binding partner labeled with a distinguishable marker from that used on the labeled moiety); immunoaffinity chromatography; size exclusion chromatography and gradient density centrifugation. Upon binding, the labeled species is rendered unable to pass through the filter, providing for a simple assay of complex formation.

Typical binding assays are performed, for example, but not by way of limitation, in an aqueous salt solution of 10-250 mM NaCl, 5-50 mM Tris-HCl, pH 5-8, and 0.5% Triton X-100 or other detergent which improves the specificity of interaction. Metal chelators and/or divalent cations may be added to improve binding and/or reduce proteolysis. Reaction temperatures may include 4, 10, 15, 22, 25, 35, or 42°C, and time of incubation is typically at least 15 seconds, but longer times are preferred so as to allow binding equilibrium to occur. Particular HPS protein•HPS protein-IP complexes may be assayed using routine protein binding assays to determine optimal binding conditions for reproducible binding. The physical parameters of complex formation may then be analyzed by quantitation of complex formation using assay methods specific for the particular label being utilized (*i.e.*, liquid scintillation spectroscopy for radioactivity detection). The reaction results are then quantitatively analyzed using Scatchard analysis, Hill analysis, and other methods commonly

known in the art. See *e.g.*, *Proteins. Structures. and Molecular Principles*. 2nd Ed.(1993) Creighton. Ed., (W.H. Freeman and Company. New York, NY).

In a second common approach to binding assays, one of the binding species is immobilized on a solid-state platform (*e.g.*, a filter, a microtiter plate well, a test tube, a chromatography matrix, and the like) either by covalent or non-covalent means. In one embodiment, immobilized HPS protein is utilized to assay for binding with a radioactively-labeled HPS protein-IP in the presence and absence of a compound to be tested for its ability to modulate HPS protein•HPS protein-IP complex formation. The binding partners are allowed to bind under aqueous, physiological conditions (*i.e.*, the conditions under which the original interaction was detected). Conversely, in yet another embodiment, the HPS protein-IP is immobilized and contacted with the labeled HPS protein, or derivative thereof, under binding conditions.

(j) Assays for Protein-Protein Interactions

The invention discloses methods for assaying and screening derivatives, fragments, analogs and homologs of HPS protein-IPs for binding to HPS protein. The derivatives, fragments, analogs and homologs of the HPS protein-IPs which interact with HPS protein may be identified by means of a yeast two hybrid assay system (see *e.g.*, Fields & Song, 1989. *Nature* 340:245-246) or; preferably, a modification and improvement thereof, as described in U.S. Patent Application Serial Nos. 08/663,824 (filed June 14, 1996) and 08/874,825 (filed June 13, 1997), both of which are entitled "Identification and Comparison of Protein-Protein Interactions that Occur in Populations and Identification of Inhibitors of These Interactions," to Nandabalan, *et al.*, and which are incorporated by reference herein in their entireties.

The identification of interacting proteins by the improved yeast two hybrid system is based upon the detection of the expression of a reporter gene (hereinafter "Reporter Gene"), the transcription of which is dependent upon the reconstitution of a transcriptional regulator by the interaction of two proteins, each fused to one half of the transcriptional regulator. The bait HPS protein (or derivative, fragment, analog or homolog) and prey protein (proteins to be tested for ability to interact with the bait protein) are expressed as fusion proteins to a DNA-binding domain, and to a transcriptional regulatory domain, respectively, or *vice versa*. In a specific embodiment of the invention, the prey population may be one or more nucleic acids encoding mutants of HPS protein-IPs (*e.g.*, as generated by site-directed mutagenesis or

another method of producing mutations in a nucleotide sequence). Preferably, the prey populations are proteins encoded by DNA (e.g., cDNA, genomic DNA or synthetically generated DNA). For example, the populations may be expressed from chimeric genes comprising cDNA sequences derived from a non-characterized sample of a population of cDNA from mammalian RNA. In another specific embodiment, recombinant biological libraries expressing random peptides may be used as the source of prey nucleic acids.

The invention discloses methods for the screening for inhibitors of HPS protein-IP. In brief, the protein-protein interaction assay may be performed as previously described herein, with the exception that it is performed in the presence of one or more candidate molecules. A resulting increase or decrease in Reporter Gene activity, in relation to that which was present when the one or more candidate molecules are absent, indicates that the candidate molecule exerts an effect on the interacting pair. In one embodiment, inhibition of the protein interaction is necessary for the yeast cells to survive, for example, where a non-attenuated protein interaction causes the activation of the *URA3* gene, causing yeast to die in medium containing the chemical 5-fluoroorotic acid. See e.g., Rothstein, 1983. *Meth. Enzymol.* 101:167-180.

In general, the proteins comprising the bait and prey populations are provided as fusion (chimeric) proteins, preferably by recombinant expression of a chimeric coding sequence containing each protein contiguous to a pre-selected sequence. For one population, the pre-selected sequence is a DNA-binding domain that may be any DNA-binding domain, so long as it specifically recognizes a DNA sequence within a promoter (e.g., a transcriptional activator or inhibitor). For the other population, the pre-selected sequence is an activator or inhibitor domain of a transcriptional activator or inhibitor, respectively. The regulatory domain alone (not as a fusion to a protein sequence) and the DNA-binding domain alone (not as a fusion to a protein sequence) preferably, do not detectably interact, so as to avoid false-positives in the assay. The assay system further includes a reporter gene operably linked to a promoter that contains a binding site for the DNA-binding domain of the transcriptional activator (or inhibitor). Accordingly, in the practice of the invention, the binding of the HPS protein fusion protein to a prey fusion protein leads to reconstitution of a transcriptional activator (or inhibitor), which concomitantly activates (or inhibits) expression of the Reporter Gene.

In a specific embodiment, the invention discloses a method for detecting one or more protein-protein interactions comprising the following steps: (i) recombinantly-expressing the

HPS protein (or a derivative, fragment, analog or homolog thereof) in a first population of yeast cells of a first mating type and possessing a first fusion protein containing the HPS protein sequence and a DNA-binding domain; wherein the first population of yeast cells contains a first nucleotide sequence operably-linked to a promoter which is "driven" by one or more DNA-binding sites recognized by the DNA-binding domain such that an interaction of the first fusion protein with a second fusion protein (comprising a transcriptional activation domain) results in increased transcription of the first nucleotide sequence; (ii) negatively selecting to eliminate those yeast cells in the first population in which the increased transcription of the first nucleotide sequence occurs in the absence of the second fusion protein; (iii) recombinantly expressing in a second population of yeast cells of a second mating type different from the first mating type, a plurality of the second fusion proteins; wherein the second fusion protein includes a sequence of a derivative, fragment, analog or homolog of aHPS protein-IPs and an activation domain of a transcriptional activator, in which the activation domain is the same in each the second fusion protein; (iv) mating the first population of yeast cells with the second population of yeast cells to form a third population of diploid yeast cells, wherein the third population of diploid yeast cells contains a second nucleotide sequence operably linked to a promoter "driven" by a DNA-binding site recognized by the DNA-binding domain such that an interaction of a first fusion protein with a second fusion protein results in increased transcription of the second nucleotide sequence, in which the first and second nucleotide sequences can be the same or different and (v) detecting the increased transcription of the first and/or second nucleotide sequence, thereby detecting an interaction between a first fusion protein and a second fusion protein.

In one embodiment, the bait (a HPS protein sequence) and the prey (a library of chimeric genes) are combined by mating the two yeast strains on solid media for a period of approximately 6-8 hours. Alternatively, the mating is performed in liquid media. The resulting diploids contain both types of chimeric genes (*i.e.*, the DNA-binding domain fusion and the activation domain fusion). After an interactive population is obtained, the DNA sequences encoding the pairs of interactive proteins are isolated by a method wherein either the DNA-binding domain hybrids or the activation domain hybrids are amplified, in separate reactions. Preferably, the amplification is carried out by polymerase chain reaction (PCR; see *e.g.*, Innis, *et al.*, 1990. *PCR Protocols* (Academic Press, Inc., San Diego, CA)) using pairs of oligonucleotide primers specific for either the DNA-binding domain hybrids or the activation

domain hybrids. The PCR amplification reaction may also be performed on pooled cells expressing interacting protein pairs, preferably pooled arrays of interactants. Other amplification methods known within the art may also be used including, *e.g.*, ligase chain reaction; Q β -replicase or the like. See *e.g.*, Kricka, *et al.*, 1995. *Molecular Probing, Blotting, and Sequencing* (Academic Press, New York, NY).

In an additional embodiment of the invention, the plasmids encoding the DNA-binding domain hybrid and the activation domain hybrid proteins may also be isolated and cloned by any of the methods well-known within the art. For example, but not by way of limitation, if a shuttle (yeast to *E. coli*) vector is used to express the fusion proteins, the genes may be subsequently recovered by transforming the yeast DNA into *E. coli* and recovering the plasmids from the bacteria. See *e.g.*, Hoffman, *et al.*, 1987. *Gene* 57:267-272.

(9) Pharmaceutical Compositions and Administration of Therapeutics

The invention discloses methods of treatment and prophylaxis by the administration to a subject of an pharmaceutically-effective amount of a Therapeutic of the invention. In one embodiment, the Therapeutic is substantially purified and the subject is a mammal, and most preferably, human.

Formulations and methods of administration that can be employed when the Therapeutic comprises a nucleic acid are described, *supra*. Various delivery systems are known and can be used to administer a Therapeutic of the invention including, *e.g.*: (i) encapsulation in liposomes, microparticles, microcapsules; (ii) recombinant cells capable of expressing the Therapeutic; (iii) receptor-mediated endocytosis (see, *e.g.*, Wu & Wu, 1987. *J. Biol. Chem.* 262:4429-4432); (iv) construction of a Therapeutic nucleic acid as part of a retroviral or other vector, and the like.

Methods of administration include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The Therapeutics of the invention may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically-active agents. Administration can be systemic or local. In addition, it may be advantageous to administer the Therapeutic into the central nervous system by any suitable route, including intraventricular and intrathecal injection. Intraventricular injection may be

facilitated by an intraventricular catheter attached to a reservoir (*e.g.*, an Ommaya reservoir). Pulmonary administration may also be employed by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. It may also be desirable to administer the Therapeutic locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, by injection, by means of a catheter, by means of a suppository, or by means of an implant. In a specific embodiment, administration may be by direct injection at the site (or former site) of a malignant tumor or neoplastic or pre-neoplastic tissue.

In another embodiment of the invention, the Therapeutic may be delivered in a vesicle, in particular a liposome. See *e.g.*, Langer, 1990. *Science* 249:1527-1533. In yet another embodiment, the Therapeutic can be delivered in a controlled release system including, *e.g.*: a delivery pump (see *e.g.*, Saudek, *et al.*, 1989. *New Engl. J. Med.* 321:574 and a semi-permeable polymeric material (see *e.g.*, Howard, *et al.*, 1989. *J. Neurosurg.* 71:105). Additionally, the controlled release system can be placed in proximity of the therapeutic target (*e.g.*, the brain), thus requiring only a fraction of the systemic dose. See, *e.g.*, Goodson, In: *Medical Applications of Controlled Release* 1984. (CRC Press, Boca Raton, FL).

In a specific embodiment of the invention, where the Therapeutic is a nucleic acid encoding a protein, the Therapeutic nucleic acid may be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular (*e.g.*, by use of a retroviral vector, by direct injection, by use of microparticle bombardment, by coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see *e.g.*, Joliot, *et al.*, 1991. *Proc. Natl. Acad. Sci. USA* 88:1864-1868), and the like. Alternatively, a nucleic acid Therapeutic can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

The invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically-effective amount of a Therapeutic, and a pharmaceutically acceptable carrier. As utilized herein, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopoeia or other generally recognized pharmacopoeia for use in animals and, more particularly, in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle

with which the therapeutic is administered and includes, but is not limited to such sterile liquids as water and oils.

The amount of the Therapeutic of the invention which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and may be determined by standard clinical techniques by those of average skill within the art. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the overall seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. However, suitable dosage ranges for intravenous administration of the Therapeutics of the invention are generally about 20-500 micrograms (μg) of active compound per kilogram (Kg) body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 picograms (pg)/kg body weight to 1 mg/kg body weight. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems. Suppositories generally contain active ingredient in the range of 0.5% to 10% by weight; oral formulations preferably contain 10% to 95% active ingredient.

The invention also provides a pharmaceutical pack or kit, comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions and Therapeutics of the invention. Optionally associated with such container(s) may be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

Example 1--Identification of HPS protein-IP complexes

A modified, improved yeast two hybrid system was utilized to identify polypeptides that interact with the HPS polypeptide interactions. Expression vectors were constructed to encode two hybrid proteins. For the "forward" screen, one hybrid consisted of the DNA binding domain of the yeast transcriptional activator Gal4 fused to a portion of the HPS protein. The other hybrid consisted of the Gal4 activator domain fused to "prey" protein

sequences encoded by a mammalian cDNA library. In the "reverse" screen, the portion of the HPS protein was fused to the Gal4 activator domain, and the prey protein sequences of the mammalian cDNA library were fused to the DNA binding domain, but the assay was otherwise identically performed.

5 Each of the aforementioned vectors was then inserted into complementary (a and α) mating types of yeast using methods known within the art. See *e.g.*, Chien, *et al.*, 1991. *Proc. Natl. Acad. Sci. USA* 88:9578-9581. Mating was carried out so as to facilitate the expression of both vector constructs within the same yeast cells, thus allowing the interaction to occur. Interaction between the bait and prey domains led to transcriptional activation of reporter
10 genes containing cis-binding elements for Gal4. The reporter genes encoding the indicator protein β -galactosidase and metabolic markers for uracil and histidine auxotrophy, were included in specific fashion in one or the other of the yeast strains used in the mating. In this manner, yeast were selected for successful mating, expression of both fusion constructs and expression of the HPS protein or HPS protein-IPs. Yeast clones which contained interacting
15 proteins were selected and cultured in individual wells of microtiter plates. The plasmids containing the HPS protein-IP sequences were then isolated and characterized.

The prey cDNAs were obtained from a human fetal brain cDNA library of 1×10^7 independent isolates (Catalog No. HL4029AH; Clontech, Palo Alto, CA). The library was synthesized from Xho1-dT₁₅-primed fetal brain mRNA (from five male/female 19-22 week
20 fetuses) which was then directionally cloned into either pAD-GAL4 (a yeast Gal4 activation domain cloning vector including the LEU2 gene for selection in yeast deficient in leucine biosynthesis) or pBD-GAL4 (a yeast Gal4 DNA-binding domain cloning vector including the TRP1 gene for selection in yeast deficient in tryptophane biosynthesis).

One forward screen was utilized to test the interaction of prey cDNA products against
25 an array of bait proteins, one of which was encoded by the HPS protein nucleotide sequence [GenBank Accession Number u65676] of nucleotides 210-1292 (hereinafter bait fragment 210-1292). Bait fragment 210-1292 was then amplified from the full-length HPS protein cDNA by PCR amplification by standard techniques. The amplified fragment was ligated into the *Bam*HI and *Eco*RI restriction sites of pGBT9BS (see *e.g.*, Yang, *et al.*, 1995. *Nucl. Acids*
30 *Res.* 23:1152-1156). The sequences were confirmed by nucleic acid sequencing to ascertain that the PCR amplification reproduced an accurate copy of the sequence. This test determined

that as predicted, the sequence encoded an interacting domain identical to the human HPS protein.

Three reverse screens were utilized to test the interaction of prey cDNA products against an array of bait proteins. The fragments 210-1292, 1272-2306, and 1272-2357, respectively, were amplified from the full-length HPS protein cDNA by PCR amplification by standard techniques. The amplified fragments were then cloned into the vector pGAD-GH (Clontech). The sequences were confirmed by nucleic acid sequencing to ascertain that the PCR amplification reproduced an accurate copy of the sequence. This test determined that as predicted, the sequence encoded an interacting domain identical to the human HPS protein.

In the forward screen, the nucleic acid encoding the introduced bait was expressed by lithium acetate/polyethylene glycol transformation (see *e.g.*, Ito, *et al.*, 1983. *J. Bacteriol.* 153:163-168) into the yeast strain YULH (mating type a, *ura3*, *his3*, *lys2*, *Ade2*, *trp1*, *leu2*, *gal4*, *gal80*, *GAL1-URA3*, *GAL1-lacZ*)(forward screen); while the prey sequences were introduced by transformation into the yeast strain N106r (mating type a, *ura3*, *his3*, *ade2*, *trp1*, *leu2*, *gal4*, *gal80*, *cyh^r*, *Lys2::GAL1_{UAS}-IS3_{TATA}-HIS3*, *ura3::GAL1_{UAS}-GAL_{TATA}-lacZ*). For the reverse screens, baits were transformed into N106r and preys into YULH.

The two transformed populations were then mated using standard methods known within the art (see *e.g.*, Sherman, *et al.*, 1991. *Getting Started with Yeast. Vol. 194* (Academic Press, New York, NY)). In brief, cells were grown until reaching a mid-to-late log phase within media which selected for the presence of the appropriate plasmids. The two mating strains (α and a) were then diluted in YAPD media (see *Id.*) filtered onto nitrocellulose membranes and incubated at 30°C for 6-8 hours. The cells were then transferred to media selective for the desired diploids (*i.e.*, yeast possessing reporter genes for β -galactosidase, uracil auxotrophy, and histidine auxotrophy, as well as expressing the vectors encoding the bait and prey). The mating products were plated on synthetic complete (SC) media (see Kaiser, Michaelis & Mitchell. Eds., 1994. *Methods in Yeast Genetics. 1994 Ed.* (Cold Spring Harbor Laboratory Press, New York, NY) lacking adenine and lysine (to select for successful mating), leucine and tryptophan (to select for expression of genes encoded by both the bait and prey plasmids), and uracil and histidine (to select for protein interactions). This medium is hereinafter referred to as SCS medium, for SC Selective medium.

Selected clones were subsequently examined for expression of β -galactosidase to confirm the formation of an HPS protein•HPS protein-IP interaction. Filter-lift assays for β -

galactosidase were then performed as per a modification of the protocol of Breeden & Nasmyth, 1985. *Cold Spring Harbor Quant. Biol.* 50: 643-650. Colonies were patch-plated onto SCS plates, grown overnight, and replica-plated onto Whatman No. 1 filters. The filters were then examined for

5 β -galactosidase activity (*i.e.*, colonies which were "positive" turned a visible blue color).

The cells contained within colonies which were "positive" for protein interaction contained a mixture of DNA-binding and activation-domain plasmids. These cells were regrown as single isolates in the individual wells of 96-well microtitration plates.

Approximately 10 μ l of each selected isolate was lysed, the inserts within the pAD-GAL4 or pGAD-GH for the activation domain plasmids and pBD-GAL4 or pGBT9BS plasmids were 10 PCR amplified using primers specific for the flanking sequences of each vector and approximately 300 nucleotides (of what would be the protein's amino-terminus following translation) was determined using an ABI 377 sequenator. Comparison to known sequences was made using the "BLAST" program publicly-available through the National Center for 15 Biotechnology Information. A summary of the HPS protein and HPS protein-IP interacting domains and identified isolates is shown in Table I, below.

In the forward screening assays, two unique isolates were identified as novel sequences. The determined nucleic acid sequences and corresponding inferred amino acid sequences of the identical ESTs cg49368.b1; cg49367.h11; cg49424.c10 (hereinafter HPIP1) 20 and cg Hs2950_0 (hereinafter HN1 homolog) are shown in Figures 1 and 2, respectively.

In the three reverse screening assays, seven isolates were found to be identical to published proteins.

1. Identified sequences interacting with the HPS protein domain encoded by nucleotides 25 1272-2306 include those identical to:

(i) the 14-3-3 protein (*eta*) sequence (GenBank Accession Number X80536) starting at nucleotide 764 (corresponds to the c-terminal region of the protein which is translated from nucleotides 192-932) and

(ii) nuclear factor NF90 (GenBank Accession Number U10324) starting at 30 nucleotide 1930 (corresponds to the c-terminal region of the protein which is translated from nucleotides 265-2280).

2. Two identified sequence interacting with the HPS protein domain 1272-2357 were demonstrated to be identical to:

(i) the 14-3-3 protein (*eta*) sequence (GenBank Accession Number X80536) starting at nucleotide 764 (corresponds to the c-terminal region of the protein which is translated from nucleotides 192-932) and

(ii) CDK2 sequence (GenBank Accession Number X61622) starting at nucleotide 4 (the protein is translated from nucleotides 1-897).

3. In addition, the four identified sequence interacting with the HPS protein domain 210-1292 were demonstrated to be identical to:

(i) human Hrs (GenBank Accession Number D84064) starting at nucleotides 98 and 100 (the protein translated from nucleotides 61-2394);

(ii) BMK1 alpha sequence (GenBank Accession Number U29725) starting at nucleotide 2431 (corresponds to the c-terminal region of the protein which is translated from nucleotides 222-2672);

(iii) atrophin-1 sequence (GenBank Accession Number U23851) starting at nucleotide 2649 (corresponds to the carboxyl-terminal region of the protein which is translated from nucleotides 74-3628) and

(iv) DGS-1 sequence (GenBank Accession Number L77566) starting at nucleotides 16 and 27 (the protein is translated from nucleotides 1-1698).

Table I: Protein Interactions with HPS Protein Identified by Yeast Two-Hybrid Screens

Activating Protein Acc. No.	Activating Region b.p.	Binding Protein Acc. No.	ORF of HPSIP	Binding Region b.p.	Isolates	Screen	Remarks (e.g. putative Function of HPSIP)
HPS U65676	1272-2306 1272-2357	14-3-3 <i>eta</i> X80536	192-932	764-* 764-* (c-term.)	1 1	reverse reverse	Vesicular trafficking and signal transduction
HPS U65676	210-1292	Hrs D84064	61-2394	98-* 100-*	1 1	reverse	Vesicular trafficking and signal transduction
HPS U65676	210-1292	BMK1 alpha U29725	222- 2280	2431-* (c-term.)	2	reverse	signal transduction
HPS U65676	1272-2357	CDK2 X61622	1-897	4-*	1	reverse	Cell cycle regulation
HPS U65676	1272-2306	NF90 U10324	265- 2280	1930-* (c-term.)	1	reverse	Large subunit of the nuclear factor of activated T cells; substrate for DNA-dependent protein kinase
HPS U65676	210-1292	Atrophin 1 U23851	74-3628	2649-* (c-term.)	1	reverse	Causes dentatorubral pallidoluysian atrophy (DRPLA, Smith's disease). Vesicle transport?
HPS U65676	210-1292	DGS-1 L77566	1-1698	16-* 27-*	2 1	reverse	Novel gene from developmental defect DiGeorge syndrome (DGS) critical region (22q11).
HPI1 (cg49367.c1, cg49367.b1, cg49424.e9)	1-*	HPS U65676	1-519	210- 1292	2	forward	?
HN1 homolog (cgHs2950_0)	22-*	HPS U65676	106-564	210- 1292	1	forward	expressed in hematopoietic and brain tissues

** See U.S. Patent Application No. Filed April 3, 1998, or PCT Patent Application, filed March, 1999.

Example 2 Verification of the Specificity of HPS Protein•HPS Protein-IP Interactions

5 To ascertain the overall degree of specificity of the bait:prey interaction, two general assays were first performed. In the first instance, yeast cells were created that express the individual plasmids encoding the binding domain fusions of the above mentioned genes (see Table I). These yeast cells were grown overnight, and examined for growth. No growth was

found for all proteins tested, confirming that they were not "self-activating" proteins, that is, these proteins require interaction with a second protein domain for a functional activation complex.

To recapitulate the detected interactions in the forward screen and further demonstrate their specificity, the isolated bait plasmid for HPS were used to transform yeast strain YULH (mating type a). The interacting domains from HPIP1 and human HN1 homolog protein were transformed into strain N106r (mating type alpha). The transformants were reamplified, and a mating performed to recapitulate the identified HPS•HPSIP interactions. HPS complexed specifically with HPIP1 and human HN1 homolog protein. It did not react non-specifically with the vector.

In the second instance, plasmids containing HPIP1 and human HN1 homolog protein inserts were transformed into strain YULH (mating type a) and mated with yeast strain N106r (mating type alpha) expressing proteins other than HPS protein. Promiscuous binders, that is, inserts able to bind with many other proteins in a non-specific fashion, would interact non-specifically with non-HPS protein domains, and would be discarded as non-specific interactants. None of the interactants showed binding to protein other than HPS protein.

To recapitulate the detected interactions in the reverse screen, and further demonstrate their specificity, the isolated bait plasmid for HPS were used to transform yeast strain N106r (mating type alpha). The interacting domains from 14-3-3 *eta*, Hrs, BMK1 alpha, CDK2, NF90, Atrophin-1, DGS-I were transformed into strain YULH (mating type a). The transformants were reamplified, and a mating performed to recapitulate the identified interactions. HPS complexed specifically 14-3-3 *eta*, Hrs, BMK1 alpha, CDK2, NF90, Atrophin-1, DGS-I. It did not react non-specifically with the vector.

In the second instance, plasmids containing 14-3-3 *eta*, Hrs, BMK1 alpha, CDK2, NF90, Atrophin-1, DGS-I inserts were transformed into strain YULH (mating type a) and mated with yeast strain N106r (mating type alpha) expressing proteins other than HPS. Promiscuous binders, that is, inserts able to bind with many other proteins in a non-specific fashion, would interact non-specifically with non-HPS protein domains, and would be discarded as non-specific interactants. None of the interactants showed binding to protein other than HPS protein.

(c) Analysis of Novel Sequences

The general procedure for the identity searches of the sequences encoding human EST were performed using publicly-available EST databases such as the National Center for Biotechnology Information (N.C.B.I.) BlastN 2.0 program. See Altschul, *et al.*, 1990. *J. Mol. Biol.* 215:403-410. The BlastN 2.0 program translates the DNA sequence in all six reading frames and compares the translated protein sequence with those within protein databases. The statistical significance is estimated under the assumption that the equivalent of one entire reading frame in the query sequence codes for protein and that significant alignments will involve only coding reading frames. Only those sequences which produce high-scoring segment pairs are shown in the BlastN 2.0 program results.

Additionally, the sequence was analyzed for open reading frames (ORFs) using proprietary software which translates the DNA sequence in all six reading frames of the (assembled) DNA sequence using the standard genetic code. The interacting EST was obtained from directionally-cloned libraries, and thus the direction of translation of the assembled EST is known to be in the 5' to 3' orientation. Within the translations obtained, all ORFs found in frames 1-3 were analyzed. ORFs which included: (i) amino acid sequences greater than 50 amino acids which followed an initiator codon or (ii) an ORF with no initiator methionine at the 5'-terminus were determined to be possible protein products, and were compared to sequences in protein data bases using the BlastP program.

(i) HPIP1

Three identical clones (cg49368.b1, cg49368.h11, and cg49424.c10) were identified as HPS interactants in this invention. The identified prey sequence of 619 nucleotides (herein referred to as HPIP1) was 98 % identical to soares melanocyte EST AA42564 (starting at nucleotide 287) and 97 % identical to EST 384114 (starting at nucleotide 149). However, since a 5' extension with either of these ESTs would change the ORF of the putative HPIP1 protein, HPIP1 was not be extended in either direction. The interaction of HPIP1 with HPS protein starts at nucleotide 1.

An open reading frame (ORF) of 173 amino acids (nucleotides 1-519) could be translated and the resulting protein was designated HPIP1. A BlastP search with the HPIP1 amino acid sequence showed the 62 % similarities to the c-terminus of the 1007 amino acid EG0003.5 protein of *Drosophila melanogaster* (TREMBLNEW-ACC E1331653). Since the

ORF has no methionine start codon, it is likely that it represents the caHPSoxy-terminal part of a longer, novel protein. The HPIP1 nucleotide and amino acid sequences are illustrated in Figure 1 (SEQ ID NOS:1 and 2, respectively).

5 (ii) HN1 Homolog Protein

The identified prey sequence of 729 nucleotides (cgHs2950_0; herein referred to as HN1 homolog protein) was 82% identical to mouse HN1 (Hn1) mRNA. See Tang, *et al.*, 1997, *Mammalian Genome* 8:695-696. The interaction of HN1 homolog protein with HPS protein starts at nucleotide 22.

10 An open reading frame (ORF) of 153 amino acids (nucleotides 106-564) could be translated and the resulting protein was designated HN1 homolog. A BlastP search with the amino acid sequence showed 83% similarities and 79% identities to mouse hematological and neurological expressed sequence 1 (HN1, 154 amino acids; SPTREMBL-ACC:P97825; see Tang, *et al.*, 1997, *Mammalian Genome* 8:695-696). Thus, this HPS protein interactant
15 represents a human homolog of the mouse HN1 protein. The amino acid in position 126 could be Met (ATG), Leu (TTG), Val (GTG), or Leu (CTG).

Mouse Hn1 is expressed in many *fetal* and adult tissues. The highest levels of expression are found in hemopoietic cells, including day 10 yolk sac blood islands-derived circulating erythroblasts, day 13 *fetal* liver, adult bone marrow and spleen. The expression is
20 also very high in day 17 *fetal* brain, while the expression in adult brain is considerably lower (see Tang *et al.*, 1997, *Mammalian Genome* 8:695-696). The HN1 homolog protein nucleotide and amino acid sequences are illustrated in Figure 2 (SEQ ID NOS:3 and 4, respectively).

The invention is not to be limited in scope by the specific embodiments disclosed herein. Indeed, various modifications of the invention, in addition to those described herein,
25 will become readily apparent to those individuals skilled in the relevant arts from the foregoing descriptions and accompanying figures. Such modifications are intended to fall within the scope of the appended claims. In addition, various publications are cited herein and their disclosures are hereby incorporated by reference in their entirety.

What is claimed is:

1. A purified complex of an HPSIP-binding domain of an HPS polypeptide and an HPS-binding domain of an HPSIP polypeptide, wherein said HPSIP polypeptide is selected from the group consisting of 14-3-3 *eta*, Hrs, BMK1 alpha kinase, CDK2, Nuclear factor NF90, Atrophin-1, DGS-1, HPIP1 and human HN1 homolog protein.
2. The complex of claim 1, wherein said HPS-binding domain is present in a polypeptide comprising the amino acid sequence of a polypeptide selected from the group consisting of 14-3-3 *eta*, Hrs, BMK1 alpha kinase, CDK2, Nuclear factor NF90, Atrophin-1, DGS-1, HPIP1 and human HN1 homolog protein.
3. The complex of claim 1, wherein said HPSIP-binding domain is present in an polypeptide comprising the amino acid sequence of an HPS polypeptide.
4. The complex of claim 3, wherein said HPS polypeptide comprises the amino acid sequence of a human HPS polypeptide.
5. The complex of claim 1, wherein said HPS-binding domain is present in a polypeptide comprising the amino acid sequence of a human HPSIP polypeptide.
6. The complex of claim 1, wherein said HPS polypeptide comprises the amino acid sequence of the polypeptide encoded by nucleotides 210-1292, 1272-2306, or 1272-2357 of the nucleotide sequence disclosed in GenBank Accession No. U65676.
7. The complex of claim 1, wherein said HPSIP polypeptide comprises the amino acid sequence of the HPSIP polypeptide encoded by the open reading frame listed in Table I, column 4..
8. The complex of claim 1, wherein the polypeptide comprising the HPSIP-binding domain is a fragment of an HPS polypeptide.

9. The complex of claim 1, wherein the polypeptide comprising the HPS-binding domain is a fragment of an HPSIP polypeptide.
10. The complex of claim 1, wherein the polypeptide comprising the HPSIP binding domain is labeled.
11. The complex of claim 1, wherein the polypeptide comprising the HPS-binding domain is labeled.
12. A chimeric polypeptide comprising six or more amino acids of an HPS polypeptide covalently linked to six or more amino acids of an HPSIP polypeptide, wherein said HPSIP polypeptide is selected from the group consisting of 14-3-3 *eta*, Hrs, BMK1 alpha kinase, CDK2, Nuclear factor NF90, Atrophin-1, DGS-1, HPIP1 and human HN1 homolog protein.
13. The chimeric polypeptide of claim 12, wherein the amino acids of the HPS polypeptide comprise an HPSIP-binding domain.
14. The chimeric polypeptide of claim 12, wherein the amino acids of the HPSIP polypeptide comprise an HPS-binding domain.
15. The chimeric polypeptide of claim 13, wherein the amino acids of the HPSIP polypeptide comprise an HPS-binding domain.
16. An antibody which specifically binds the complex of claim 1.
17. The antibody of claim 16, wherein the antibody binds with greater affinity to the complex of claim 1 than it binds to an HPSIP-binding domain of an HPS polypeptide that is not part of said complex, or an HPS-binding domain of an HPSIP polypeptide that is not part of said complex.
18. A nucleic acid encoding the chimeric polypeptide of claim 12.

19. An isolated nucleic acid comprising a nucleic acid sequence encoding a polypeptide selected from the group consisting of an HPIP1 polypeptide and a human HN1 homolog polypeptide.
20. The nucleic acid of claim 19, wherein said nucleic acid encodes an HPS binding domain of said polypeptide.
21. The nucleic acid of claim 19, wherein said nucleic acid comprises the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3.
22. A vector comprising the nucleic acid of claim 18 or claim 19.
23. A cell comprising the vector of claim 21.
24. A purified polypeptide comprising the amino acid sequence of a polypeptide selected from the group consisting of HPIP1 and human HN1 homolog protein.
25. The polypeptide of claim 24, wherein said polypeptide comprises the amino acid sequence of a human HPIP1 or human HN1 homolog polypeptide.
26. The polypeptide of claim 24, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:4.
27. The polypeptide of claim 24, wherein said polypeptide comprises an HPS-binding domain.
28. A pharmaceutical composition comprising a therapeutically- or prophylactically-effective amount of a Therapeutic and a pharmaceutically acceptable carrier, wherein said Therapeutic is selected from the group consisting of
the complex of claim 1;
the chimeric polypeptide of claim 12;

the antibody of claim 16, or a fragment or derivative of said antibody;
the nucleic acid of claim 18 or 19; and
the polypeptide of claim 23.

29. A method of producing a HPS-HPSIP polypeptide complex of claim 1, said method comprising
providing a cell containing a nucleic acid encoding an HPSIP binding domain of an HPS polypeptide and a nucleic acid encoding an HPS binding domain of an HPSIP polypeptide;
culturing the cell under conditions that allow for expression of said polypeptides such that a complex forms between the HPSIP binding domain of the HPS polypeptide and the HPS binding domain of the HPSIP polypeptide; and
recovering said complex,
thereby producing said HPS-HPSIP complex.

30. A method of diagnosing or screening for the presence of, or a predisposition for, a disorder characterized by an aberrant level of a complex of an HPS protein and an HPSIP protein in a sample, the method comprising
providing a test sample from said subject;
measuring the level of said complex in said test sample; and
comparing the level of said complex in said test sample to the level of said complex in a reference sample that does not include cells characteristic of an HPS-associated disorder, wherein an alteration in the level of the sample indicates the presence or predisposition to said disorder in said subject.

31. A kit comprising in one or more containers a substance selected from the group consisting of a complex of an HPS and an HPSIP, an antibody against said complex, nucleic acid probes capable of hybridizing to RNA of HPS and RNA of said HPSIP, or pairs of nucleic acid primers capable of priming amplification of at least a portion of a gene for HPS and a gene for said HPSIP, in which said HPSIP is selected from the group consisting of 14-3-3 eta, Hrs, BMK1 alpha kinase, CDK2, nuclear factor NF90, atrophin-1, DGS-1, HPIP1 and human HN1 homolog.

32. A method of treating or preventing a disease or disorder involving aberrant levels of a complex of claim 1 in a subject, the method comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a molecule or molecules that modulate the function of said complex.

FIGURE 1

1/3

1
GGCACGAGACTCAGTATAAATCCAAATAACCATTCTTGGTTAATT
G T R L S I N P N N H S W L I
46
ATCCAGGCAGATATTTACTTTGCAACGAATCAGTATTCAGCAGCT
I Q A D I Y F A T N Q Y S A A
91
CTTCACTATTACCTCCAGGCAGGAGCTGTGTGTTCTGACTTCTTT
L H Y Y L Q A G A V C S D F F
136
AACAAGGCTGTGCCCCCTGATGTTTATACAGACCAGGTAATAAAA
N K A V P P D V Y T D Q V I K
181
CGAATGATAAAATGTTGTTCTTTGCTGAATTGCCACACACAGGTG
R M I K C C S L L N C H T Q V
226
GCTATTTTATGTCAGTTCCTCAGAGAAATTGACTACAAAACAGCG
A I L C Q F L R E I D Y K T A
271
TTTAAATCTCTGCAAGAACAAAACAGTCATGATGCTATGGACTCC
F K S L Q E Q N S H D A M D S
316
TACTACGACTACATATGGGATGTTACCATTTTGAATACTTGACT
Y Y D Y I W D V T I L E Y L T
361
TATCTTCATCATAAAAGAGGAGAAACAGATAAAAGACAAATTGCA
Y L H H K R G E T D K R Q I A
406
ATCAAAGCCATCGGCCAGACAGAGTTGAATGCAAGCAATCCAGAA
I K A I G Q T E L N A S N P E
451
GAAGTGTTACAGCTGGCAGCGCAGAGAAGGAAAAAAAAAGTTTCTC
E V L Q L A A Q R R K K K F L
496
CAAGCAATGGCAAACTTTACTTTTAAGCAGTTAAATTTTTTTAA
Q A M A K L Y F
541
CTTTTATTTTTTAAACAATGGGCTAAAAATAAACAGTATTAAAG
586
GGTAAGTTTATATAATACAAAAAAAAAAAAAAAAA

FIGURE 2

2/3

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1 CTCCTGCAGCGGTGGTCCGGCTGTTGGGTGTGGAGTTTCCCAGCGC
46 CCCTCGGGTCCGACCCCTTTGAGCGTTCTGCTCCGGCGCCACTACC

91 TCGCTCCTCGGCGCCATGACCACAACCACCACCTTCAAGGGAGTC
    MetThrThrThrThrThrPheLysGlyVal

136 GACCCCAACAGCAGGAATAGCTCCCGAGTTTTGCGGCCTCCAGGT
    AspProAsnSerArgAsnSerSerArgValLeuArgProProGly

181 GGTGGATCCAATTTTTCATTAGGTTTTGATGAACCAACAGAACAA
    GlyGlySerAsnPheSerLeuGlyPheAspGluProThrGluGln

226 CCTGTGAGGAAGAACAAAATGGCCTCTAATATCTTTGGGACACCT
    ProValArgLysAsnLysMetAlaSerAsnIlePheGlyThrPro

271 GAAGAAAATCAAGCTTCTTGGGCCAAGTCAGCAGGTGCCAAGTCT
    GluGluAsnGlnAlaSerTrpAlaLysSerAlaGlyAlaLysSer

316 AGTGGTGGCAGGGAAGACTTGGAGTCATCTGGACTGCAGAGAAGG
    SerGlyGlyArgGluAspLeuGluSerSerGlyLeuGlnArgArg

361 AACTCCTCTGAAGCAAGCTCCGGAGACTTCTTAGATCTGAAGGGA
    AsnSerSerGluAlaSerSerGlyAspPheLeuAspLeuLysGly

406 GAAGGTGATATTCATGAAAATGTGGACACAGACTTGCCAGGCAGC
    GluGlyAspIleHisGluAsnValAspThrAspLeuProGlySer

451 CTGGGGCAGAGTGAAGAGAAGCCCGTGCCTNTGCGCCTGTGCCCA
    LeuGlyGlnSerGluGluLysProValProXaaArgLeuCysPro

496 GCCCAGTGCCCCGGCCCCAGTGCCATCCAGAAGAAATCCCCTGGC
    AlaArgCysProGlyProSerAlaIleGlnLysLysSerProGly

541 GGCAAGTCCAGCCTCGTCTTGGGTTAGCTCTGACTGTCCTGAACG
    GlyLysSerSerLeuValLeuGly

586 CTGTCGTTCTGTCTGTTTCCTCCATGCTTGTGAACTGCACAACCTT
631 GAGCCTGACTGTACATCTCTTGGATTTGTTTCATTAAAAAAGAAGC
676 ACTTTANGTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
721 AAAAAACAA
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FIGURE 3

3/3

	HPS (forward)	HPS (reverse)
14-3-3 eta	-	+
Hrs	-	+
BMK1 alpha	-	+
CDK2	-	+
Nuclear factor NF90	-	+
Atrophin-1	-	+
DGS-I	-	+
HPIP-1 (cg49368.b1, cg49367.h11, cg49424.c10)	+	-
HN1 homolog (cgHs2950_0)	+	-
Retinoblastoma	-	-
p27(Kip1)	-	-
RGL-2	-	-
Vector control	-	-