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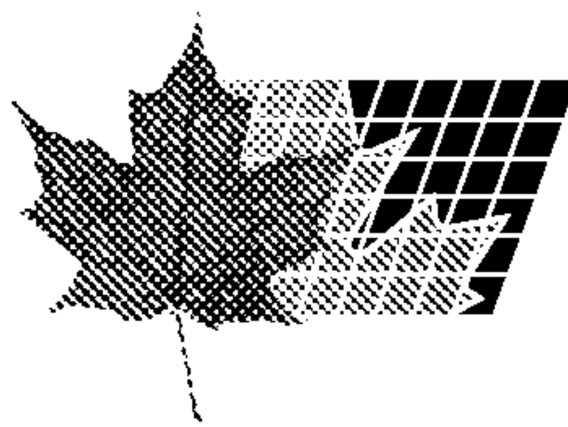

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(72) ZHANG, XIAN-FENG, US
(72) AVRUCH, JOSEPH, US
(71) THE GENERAL HOSPITAL CORPORATION, US

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(54) **PROTEINE FIXATRICE DE RAS (PRE1)**
(54) **RAS-BINDING PROTEIN (PRE1)**

(57) L'invention concerne une protéine fixatrice de Ras désignée par PRE1. PRE1 se trouve naturellement dans différents tissus et types cellulaires de mammifères. L'invention concerne également l'ADN isolé codant PRE1, des vecteurs et des cellules contenant cet ADN, et des anticorps spécifiques de PRE1. L'invention concerne en outre un procédé de criblage in vitro servant à identifier une substance qui module la liaison PRE1-Ras, et une technique in vitro d'identification d'une substance qui module l'expression du gène PRE1.

(57) Disclosed is a Ras-binding protein designated PRE1. PRE1 occurs naturally in various mammalian tissues and cell types. An isolated DNA endocing PRE1, vectors and cells containing the DNA, and PRE1-specific antibodies are also disclosed. Also disclosed is an in vitro screening method for indentifying a substance which modulates PRE1-Ras binding, and an in vitro method for identifying a substance that modulates PRE1 gene expression.

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(71) Applicant *(for all designated States except US)*: THE GENERAL HOSPITAL CORPORATION [US/US]; 55 Fruit Street, Boston, MA 02114 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: ZHANG, Xian-feng [CN/US]; 249 Pearl Street, Cambridge, MA 02139 (US). AVRUCH, Joseph [US/US]; 277 St. Paul Street, Brookline, MA 02445 (US).

(74) Agent: CREASON, Gary, L.; Fish & Richardson, P.C., 225 Franklin Street, Boston, MA 02110-2804 (US).

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(54) Title: RAS-BINDING PROTEIN (PRE1)

(57) Abstract

Disclosed is a Ras-binding protein designated PRE1. PRE1 occurs naturally in various mammalian tissues and cell types. An isolated DNA endocing PRE1, vectors and cells containing the DNA, and PRE1-specific antibodies are also disclosed. Also disclosed is an *in vitro* screening method for indentifying a substance which modulates PRE1-Ras binding, and an *in vitro* method for identifying a substance that modulates PRE1 gene expression.

Ras-Binding Protein (PRE1)

Field of the Invention

The invention relates to recombinant DNA, cell
5 biology and oncology.

Background of the Invention

In humans, mutations in the cellular Ras gene (c-ras) which render Ras constitutively active, have been associated with different types of cancers (Bos et al.,
10 *Cancer Res.* **94**:4682-4689). Ras relays signals from receptor tyrosine kinases, (Fantl et al., 1993, *Annu. Rev. Biochem.*,**62**:453-481), non-tyrosine kinase receptors (Woodrow et al., 1993, *J. Immunol.*, **150**: 3853-3861) and heterotrimeric G protein-coupled receptors (Van Corven et
15 al., 1993, *Proc. Nat'l. Acad. Sci.*, **90**:1257-1261). Ras is located at the inner surface of the plasma membrane. Activation of cell surface receptors promotes the exchange of Ras-bound GDP for GTP, thereby causing a conformational change in Ras. This conformational change
20 activates Ras so that it interacts with downstream targets or effectors (Wittinghofer et al., 1996, *Trends In Biochem. Sci.*, **21**:488-491.

Several candidate Ras effectors have been proposed based on their ability to bind to Ras through its
25 effector loop. Among these are: Raf, PI-3 kinase, members of the Ral-GDS family, Rin-1, AF-6, diacylglycerol kinases, PKC ζ, and MEKK1 (*See, e.g.*, Katz et al., 1997,
Curr. Opin. Genet. Dev., **7**:75-79; Marshall, 1996, *Curr.*
30 *Opin. Cell Biol.*, **8**:197-204). Activation of effectors such as these leads to activation of other downstream signal transduction molecules. This signaling cascade culminates in the modulation of gene expression, and thereby causes changes in cellular function, growth, and
35 division.

Summary of the Invention

A Ras effector protein, designated PRE1, has been identified and characterized. PRE1 protein is expressed in various tissues and cell lines, and PRE1 binds to Ras.

5 The invention features an isolated DNA containing a nucleotide sequence that encodes a PRE1 protein. The PRE1 protein encoded by the DNA shares at least 80% sequence identity with SEQ ID NO:2, and it binds to Ras. The nucleotide sequence can define a DNA molecule whose 10 complement hybridizes under high stringency conditions to a DNA whose nucleotide consists of SEQ ID NO:1. Preferably, the isolated DNA encodes a naturally-occuring mammalian PRE1. In some embodiments, the DNA encodes an amino acid sequence consisting of SEQ ID NO:2. The DNA 15 can contain the nucleotide sequence of SEQ ID NO:1 or the coding region of the nucleotide sequence of SEQ ID NO:1, or degenerate variants of those sequences. The invention also includes a vector containing the above-described DNA, which DNA can be operably linked to one or more 20 expression control sequences. The invention also includes a cell containing such a vector.

The invention features a substantially pure PRE1 protein that includes an amino acid sequence that shares at least 80% sequence identity with SEQ ID NO:2 and binds 25 to Ras. Preferably, the sequence identity is at least 85%, more preferably, it is at least 90%, and most preferably, it is at least 95%. The amino acid sequence of the PRE1 protein can differ from SEQ ID NO:2 solely by conservative amino acid substitutions (i.e., substitution 30 of one amino for another of the same class) or by non-conservative sustitutions, deletions, or insertions located at positions that do not destroy the function of the protein. In some embodiments, the protein has an amino acid sequence consisting of SEQ ID NO:2. Also 35 included in the invention is any naturally-occurring

homolog or isoform of SEQ ID NO:2. The invention includes Ras-binding domain-containing PRE1 protein fragments, e.g., amino acids 266-360 or 188-413, and heterologous fusion proteins containing a Ras-binding
5 domain-containing PRE1 protein fragment.

The invention also features a PRE1-specific antibody, which can be polyclonal or monoclonal. The antibody can be conjugated to a detectable label.

The invention also features a screening method for
10 identifying a substance that modulates binding of PRE1 protein to Ras. The method includes the following steps: (a) providing a sample solution of PRE1 protein; (b) adding to the sample solution a candidate substance; (c) adding to the sample solution a Ras sample; and (d)
15 detecting an increase or decrease in binding of PRE1 protein to Ras in the presence of the candidate substance, compared to the binding of PRE1 to Ras in the absence of the candidate substance.

The invention also features a method of producing
20 PRE1 protein. The method includes the following steps: (a) providing a cell transformed with an isolated DNA comprising a nucleotide sequence that encodes a protein the amino acid sequence of which is SEQ ID NO:2; (b) culturing the cell; and (c) collecting the protein
25 encoded by the nucleotide sequence.

The invention also features a screening method for identifying a substance that modulates PRE1 gene expression. The method includes the following steps: (a) providing a test cell; (b) contacting the test cell
30 with a candidate substance; and (c) detecting an increase or decrease in the level of PRE1 gene expression in the presence of the candidate substance, compared to the level of PRE1 gene expression in the absence of the candidate substance.

The invention also features a method for isolating a PRE1-binding substance, e.g., Ras protein. The method includes the following steps: (a) providing a sample of immobilized PRE1 protein; (b) contacting a mixture 5 containing the substance with said immobilized PRE1 protein; (c) separating unbound components of the mixture from bound components of the mixture; (d) recovering the PRE1-binding substance from said immobilized PRE1 protein.

10 As used herein, "high stringency conditions" means the following: hybridization at 42°C in the presence of 50% formamide; a first wash at 65°C with about 2 × SSC containing 1% SDS; followed by a second wash at 65°C with 0.1 × SSC.

15 As used herein, "isolated DNA" means DNA free of the genes that flank the gene of interest in the genome of the organism in which the gene of interest naturally occurs. The term therefore includes a recombinant DNA incorporated into a vector, into an autonomously 20 replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote. It also includes a separate molecule such as a cDNA, a genomic fragment, a fragment produced by polymerase chain reaction (PCR), or a restriction fragment. It also includes a recombinant 25 nucleotide sequence that is part of a hybrid gene, i.e., a gene encoding a fusion protein. Also included is a recombinant DNA that includes a portion of SEQ ID NO:1 and that encodes an alternative splice variant of PRE1.

As used herein, "operably linked" means 30 incorporated into a genetic construct so that expression control sequences effectively control expression of a gene of interest.

As used herein, "protein" means any peptide-linked chain of amino acids, regardless of length or post-

translational modification, e.g., glycosylation or phosphorylation.

As used herein, "PRE1" means: (1) a protein, the amino acid sequence of which is SEQ ID NO:2, or (2) a protein that shares at least 80% amino acid sequence identity with SEQ ID NO:2 and binds to Ras.

As used herein, "sequence identity" means the percentage of identical subunits at corresponding positions in two sequences when the two sequences are aligned to maximize subunit matching, i.e., taking into account gaps and insertions. When a subunit position in both of the two sequences is occupied by the same monomeric subunit, e.g., if a given position is occupied by an adenine in each of two DNA molecules, then the molecules are identical at that position. For example, if 7 positions in a sequence 10 nucleotides in length are identical to the corresponding positions in a second 10-nucleotide sequence, then the two sequences have 70% sequence identity. Preferably, the length of the compared sequences is at least 60 nucleotides, more preferably at least 75 nucleotides, and most preferably 100 nucleotides. Sequence identity is typically measured using sequence analysis software (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705).

As used herein, "PRE1-specific antibody" means an antibody that binds to a protein, the amino acid sequence of which is SEQ ID NO:2 and displays no substantial binding to other naturally-occuring proteins other than those sharing the same antigenic determinants as PRE1. The term includes polyclonal and monoclonal antibodies.

As used herein, "substantially pure protein" means a protein separated from components that naturally accompany it. Typically, the protein is substantially

pure when it is at least 60%, by weight, free from the proteins and other naturally-occurring organic molecules with which it is naturally associated. Preferably, the purity of the preparation is at least 75%, more 5 preferably at least 90%, and most preferably at least 99%, by weight. A substantially pure PRE1 protein can be obtained, for example, by extraction from a natural source, by expression of a recombinant nucleic acid encoding an PRE1 polypeptide, or by chemical synthesis. 10 Purity can be measured by any appropriate method, e.g., column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis. A chemically-synthesized protein or a recombinant protein produced in a cell type other than the cell type in which it 15 naturally occurs is, by definition, substantially free from components that naturally accompany it. Accordingly, substantially pure proteins include those having sequences derived from eukaryotic organisms but synthesized in *E. coli* or other prokaryotes.

20 As used herein, "fragment", as applied to a protein, means at least about 10 amino acids, usually about 20 contiguous amino acids, preferably at least 40 contiguous amino acids, more preferably at least 50 amino acids, and most preferably at least about 60 to 80 or 25 more contiguous amino acids in length. Such peptides can be generated by methods known to those skilled in the art, including proteolytic cleavage of the protein, *de novo* synthesis of the fragment, or genetic engineering.

As used herein, "test cell" means a cell that 30 expresses a PRE1 gene in the absence of a PRE1 gene repressor. Preferably, the PRE1 gene in the test cell is under the control of a promoter that is naturally associated with a PRE1 gene.

As used herein, "vector" means a replicable nucleic acid construct, e.g., a plasmid or viral nucleic acid.

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 pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods 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 document, including definitions, will control. Unless otherwise indicated, materials, methods, and examples described herein are illustrative only and not intended to be limiting.

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

Brief Description of the Drawing

Fig. 1 is the nucleotide sequence of a murine PRE1 cDNA, and the deduced amino acid sequence.

Detailed Description

PRE1 Structure and Function

A full-length murine PRE1 cDNA has been cloned and sequenced. The PRE1 cDNA clone contains 3018 bp (Fig. 1) and includes a complete open reading frame that encodes a protein 413 amino acids in length. The sequence of the 3018 bp cDNA is shown in Fig. 1. The cDNA sequence around the first ATG matches the Kozak consensus sequence for a translational start site. The open reading frame from this methionine includes 413 amino acids, yielding a

highly basic polypeptide (PI=9.41) with a predicted molecular weight of 46.4 KD. One structural feature of PRE1 is the presence of a cysteine-histidine rich segment (a.a. 118-165, $H-X_{13}-C-X_2-C-X_{10}-C-X_2-C-X_4-H-X_2-C-X_7-C$)
5 typical of a diacylglycerol/phorbol ester (DAG_PE) binding site (Ono et al.,1989, *Proc.Natl.Acad.Sci*, **86**:4868-4871). PRE1's carboxyterminal region shares some homology to the putative Ras-binding domains of other proteins (Ponting et al., 1996, *Trends In Biochem. Sci.*
10 **21**:422-425). PRE1 also has a proline rich region in its aminoterminal region, with five PXXP sequences (aa 17-20 PEPP; 31-34 PPPP; 34-37 PARP; 77-80 PVRP and 105-108 PQDP), which are possible SH3 domain binding sites (Ren et al., 1993, *Science* **259**:1157-1161).

15 Ras interacts with downstream effectors to cause changes in cellular function, growth, and division. Mutations in this signaling pathway can lead to abnormal cell proliferation or neoplasia. Substances that block the Ras-effector interaction can thus inhibit abnormal
20 cell proliferation or neoplasia. Screening for substances that modulate Ras-PRE1 interaction is therefore useful for identifying potential cancer therapy agents.

PRE1 may be a catalytic signaling molecule.
25 Preliminary experiments, however, indicate that prokaryotic recombinant PRE1 (a.a. 188-413) does not alter Ras-GTP exchange or Ras-GTPase activity *in vitro*. PRE1 may also be an adapter molecule, serving to link activated Ras with another, presumably catalytic,
30 signaling molecule. The Ras binding domain (a.a. 266-360) in PRE1 is located in its carboxyterminal region, and there are potential SH3 binding sites in the aminoterminal region. PRE1 may link a SH3 domain-containing protein to activated Ras. PRE1 also contains
35 a DAG_PE binding consensus sequence motif also found in a

variety of proteins involved in intracellular signaling including Raf (Rapp et al., 1988, *Cold Spring Harb. Symp. Quant. Biol.*, **53 Pt.1**:173-184), PKC (Ono et al.,1989, *Proc.Natl.Acad.Sci.*, **86**:4868-4871), VAV (Ahmed et al.,
5 1992, *Biochem. J.*, **287**:995-999), n-chimerin (Ahmed et al., 1990, *Biochem. J.*, **272**:767-773) and DAG kinases (Sakane et al., 1990, *Nature*, **344**:345-348). DAG may bind to the DAG_PE site in PRE1 and regulate its function.

Expression Control Sequences and Vectors

10 The PRE1 protein-encoding DNA ("PRE1 DNA") of this invention can be used in a screening method to identify a substance that inhibits cell proliferation or neoplasia in a mammal. For such uses, the PRE1 DNA is typically cloned into an expression vector, i.e., a vector wherein
15 PRE1 DNA is operably linked to expression control sequences. The need for, and identity of, expression control sequences will vary according to the type of cell in which the PRE1 DNA is to be expressed. Generally, expression control sequences include a transcriptional
20 promoter, enhancer, suitable mRNA ribosomal binding sites, and sequences that terminate transcription and translation. Suitable expression control sequences can be selected by one of ordinary skill in the art. Standard methods can be used by the skilled person to
25 construct expression vectors. *See generally,* Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual* (2nd Edition), Cold Spring Harbor Press, Cold Spring Harbor, NY. Vectors useful in this invention include plasmid vectors and viral vectors. Preferred viral vectors are
30 those derived from retroviruses, adenovirus, adeno-associated virus, SV40 virus, or herpes viruses.

PRE1 DNA *In Vitro*

In some embodiments of the invention, PRE1 DNA is introduced into, and expressed in, a prokaryotic cell, e.g., *Escherichia coli*. For expression in a prokaryotic cell, PRE1 DNA can be integrated into a bacterial chromosome or expressed from an extrachromosomal DNA.

In other embodiments of the invention, the PRE1 DNA is introduced into, and expressed in, a eukaryotic cell *in vitro*. Eukaryotic cells useful for expressing PRE1 DNA *in vitro* include, but are not limited to, COS, CHO, and Sf9 cells. Transfection of the eukaryotic cell can be transient or stable. The PRE1 DNA can be, but is not necessarily, integrated into a chromosome of the eukaryotic cell.

PRE1-Specific Antibody

Some embodiments of this invention include a PRE1-specific antibody. Standard protocols for monoclonal and polyclonal antibody production are known and can be carried out by one of ordinary skill in the art to obtain antibodies useful in this invention.

The invention encompasses not only an intact monoclonal or polyclonal antibody, but also an immunologically active antibody fragment. Examples of such a fragment include a Fab or $F(ab_2)$ fragment, an engineered single chain Fv molecule, and a chimeric antibody. Typically, a chimeric antibody includes a variable region of a non-human antibody, e.g., a murine variable region, and a constant region of a human antibody.

Antibody Label

In some embodiments of the invention, the PRE1-specific antibody includes a detectable label. Various types of detectable labels can be linked to, or

incorporated into, an antibody of this invention. Examples of useful label types include radioactive, non-radioactive isotopic, fluorescent, chemiluminescent, paramagnetic, enzyme, or colorimetric.

5 Examples of useful enzyme labels include malate hydrogenase, staphylococcal dehydrogenase, delta-5-steroid isomerase, alcohol dehydrogenase, alpha-glycerol phosphate dehydrogenase, triose phosphate isomerase, peroxidase, alkaline phosphatase, asparaginase, glucose 10 oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, and glucoamylase, acetylcholinesterase. Examples of useful radioisotopic labels include ^{3}H, ^{131}I, ^{125}I, ^{32}P, ^{35}S, and ^{14}C. Examples of useful fluorescent labels include 15 fluorescein, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, and fluorescamine. Examples of useful chemiluminescent label types include luminal, isoluminal, aromatic acridinium ester, imidazole, acridinium salt, oxalate ester, luciferin, luciferase, and aequorin.

20 Suitable labels can be coupled to, or incorporated into antibodies or antibody fragments through standard techniques known to those of ordinary skill in the art. Typical techniques are described by Kennedy et al., (1976) *Clin. Chim. Acta* 70, 1-31; and Schurs et al., 25 (1977) *Clin. Chim. Acta* 81, 1-40. Useful chemical coupling methods include those that use glutaraldehyde, periodate, dimaleimide and m-maleimido-benzyl-N-hydroxy-succinimide ester.

Screening assays

30 The invention can be used to screen candidate substances for the ability to inhibit the interaction of Ras with PRE1.

In an exemplary screening method, the two-hybrid expression system described below is used to screen for

substances capable of inhibiting Ras-PRE1 interaction *in vivo*. The two-hybrid method is a well known yeast-based genetic assay to detect protein-protein interactions *in vivo* (*See, e.g.*, Bartel et al., 1993, In *Cellular Interactions in Development: A Practical Approach*, Oxford University Press, Oxford, pp. 153-179; Chien et al., 1991, *Proc. Natl. Acad. Sci. USA*, **88**:9578-9582; Fields et al., 1989, *Nature*, **340**:245-247; Fritz et al., 1992, *Curr. Biol.*, **2**:403-405; Guarente, L., 1993, *Proc. Natl. Acad. Sci. USA*, **90**:1639-1641). In this system, a GAL4 binding site, linked to a reporter gene such as lacZ, is contacted in the presence and absence of a candidate substance with a GAL4 binding domain linked to a PRE1 fragment and a GAL4 transactivation domain II linked to a Ras fragment. Expression of the reporter gene is monitored, and a decrease in its expression is an indication that the candidate substance inhibits the interaction of Ras with PRE1. One of ordinary skill in the art will recognize that other screening assays are known and can be used to identify candidate substances that inhibit Ras-PRE1 interaction.

In another screening method, one of the protein components of the Ras-PRE1 binding complex, such as Ras or a PRE1-binding fragment of Ras or PRE-1 or a Ras-binding fragment of PRE-1, is immobilized. Polypeptides can be immobilized using methods known in the art. Such methods include adsorption onto a plastic microtiter plate or specific binding of a glutathione-S-transferase (GST)-fusion protein to a polymeric bead containing glutathione. For example, GST-PRE1 (a.a. 188-413) can be bound to glutathione-Sepharose™ beads. The immobilized protein (*e.g.*, PRE1) is then contacted with the labeled protein to which it binds (Ras in this example) in the presence and absence of a candidate substance. Unbound protein can be removed by washing. The complex then can

be solubilized and analyzed to determine the amount of bound (labeled) protein. A decrease in binding is an indication that the candidate substance inhibits the interaction of Ras and PRE1.

5 A variation of the above-described screening method can be used to screen for other classes of candidate substances, *e.g.*, those that disrupt previously-formed Ras-PRE1 complexes. In this variation, a complex containing Ras (or a PRE1-binding Ras fragment) 10 bound to PRE1 (or a Ras-binding fragment) is immobilized and contacted with a candidate compound. Detection of disruption of the Ras-PRE1 complex by the candidate substance identifies the candidate substance as a potential modulator of Ras-mediated cellular events.

15 The present invention is further illustrated by the following specific examples. The examples are provided for illustration only and are not to be construed as limiting the scope or content of the invention in any way.

20 Examples

Cloning of PRE1 cDNA

A yeast two hybrid screen was carried out to identify potential new Ras effectors in mammalian cells. A cDNA encoding V12-H-Ras without the last four amino 25 acids was subcloned into vector pAS-CYH-II carboxyterminal to the Gal-4 DNA binding domain. This formed the bait construct pAS-Ras, which was used to transform the yeast strain Y190. Stable transformants were selected on Trp(-) plates and a single yeast colony 30 was picked, grown up in Trp medium and saved.

After verifying that V12-H-Ras was correctly expressed in this yeast clone, 100 μg of cDNA made from an activated mouse T cell library constructed in the GAL-4 DNA activation domain vector pACT was transformed into

one million yeast cells expressing pAS-Ras. The transformants were plated out on 10 large His(-)Leu(-)Trp(-) selection plates. After eight days, 20 large colonies appeared. An X-gal filter assay was performed
5 for all the colonies and all showed strong blue color, indicative of lacZ activity. The colonies were individually picked. Yeast plasmid DNA was isolated from each picked colony and transformed into bacterial strain WA921 by electroporation. Transformants were subjected
10 to selection on on Leu(-) LB plus ampicillin plates. The bait plasmid pAS-Ras was usually removed by this selection, and the pACT-cDNA plasmids were isolated from the bacteria using conventional methods. Of the positive clones, two encoded a 2.5 Kb cDNA representing a new
15 gene, which was designated PRE1.

The 2.5 Kb cDNA encoding PRE1 from the initial two-hybrid screen was isolated, labeled with [α-^{32}P] dCTP and used to screen cDNA libraries from mouse brain (Clontech's mouse brain 5'-stretch plus cDNA library in
20 λ-gt 10 vector, CAT. #ML 3000a). A positive clone containing a 3018 bp insert was isolated.

By low stringency screening of cDNA libraries, at least two more cDNAs encoding polypeptides with regions similar or identical to regions of PRE1 were obtained.
25 They diverged substantially from PRE1 in sequences aminoterminal to the Cys-His rich segment. These may represent alternative splice variants of PRE1 gene products or genes related to PRE1.

PRE1-Specific Antibody

30 A polyclonal antiserum was raised against a carboxyterminal fragment of PRE1 (a.a. 188-413) using conventional methods. A polyclonal antibody preparation was produced by affinity chromatography using the recombinant antigen. AGST-PRE1 (a.a. 188-213) fusion

protein was used to immunize NZW rabbits. The antiserum was first depleted of GST-reacting antibodies by repeated incubation with immobilized GST. The GST depleted antiserum was then affinity purified using immobilized PRE1 (a.a. 188-413) on PVDF membrane.

Northern blot

PRE1 mRNA abundance and complexity in murine tissues was examined by Northern blot. A 210 bp PRE1 cDNA fragment (nt. 90-310) was labelled with ^{32}P-dCTP by the random priming method and used for probing PRE1 mRNA. The mouse multiple tissue blot was purchased from Clontech and hybridized in Stratagene's quick hybridization buffer and washed according to the manufacturer's protocol. A single mRNA containing about 3.1 Kb was detected in most mouse tissues, including: heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testis. Some mRNA size variation was noted. The highest levels were observed in brain, liver and spleen, with barely detectable levels in heart.

Tissue and cell line western blot

Western blots using polyclonal anti-PRE1 (a.a. 188-413) antibody were performed. Sprague-Dawley rats (65g) were starved overnight and anesthetized with pentobarbital. Tissues from the rats were excised in the following order: gastrocnemius, testis, spleen, kidney, liver, lung and heart. Brain was excised from other intact anesthetized animals after decapitation. Cell lines were grown to 80%-90% confluency before harvesting. Both tissues and cell lines were disrupted and extracted in RIPA buffer.

An immunoblot of extracts prepared from different rat tissue was performed. A single immunoreactive band at 46 KD was seen in a brain extract. This was in

agreement with the predicted size of the polypeptide encoded by PRE1 cDNA isolated from the mouse brain library. A similar 46 KD band was also seen in other tissues including lung and testis. In addition, however, prominent immunoreactivity to bands at other molecular weights was seen in most tissues, and some tissues lacked a 46 KD band entirely (e.g., skeletal muscle, heart, spleen and liver). All tissues except brain, showed a major 65 KD band, and two bands around 55 KD were also seen in lung, spleen, testis and liver. The 65 and 55 KD bands may represent isoforms of PRE1, the existence of which is suggested by the partial cDNAs isolated from a variety of cDNA libraries. The anti-PRE1 antibody also immunoblotted a single polypeptide in an extract prepared from *C. elegans*. This band was approximately 74 KD.

The murine brain PRE1 cDNA was tagged at the PRE1 amino terminus with an hemagglutinin (HA) epitope and expressed transiently in COS cells. In an immunoblot with anti-PRE1 antibodies, HA-PRE1 showed the expected size of 46 KD. Extracts prepared from several cell line were subjected to PRE1 immunoblot. Of the cell lines examined, only BC3H1, a vascular smooth muscle-like line derived from a radiation induced murine brain tumor, showed a single band at 46 KD. A band of similar size was seen in several other cell lines including RIE-1 (rat intestinal epithelial), MCF-7 (human breast cancer), HEK 293 (human embryonic kidney) and KB (human oral carcinoma). Immunoreactive polypeptides of 55 KD (RIE-1, MCF-7, HEK 293 and KB) and 65 KD (RIE-1, HEK 293 and KB), were at least as abundant in these cell lines, and some lines showed only bands other than the 46 KD polypeptide (e.g., Huh-7, 40 KD; L6, 55 KD).

Ras/PRE binding *in vitro*

A GST-PRE1 (a.a. 188-413) fusion protein (corresponding to the PRE1 polypeptide encoded by the initial cDNA isolate) was expressed and purified from
5 *E. coli*. Purified, prokaryotic recombinant [c-H-Ras] (2.5 mg/ml) was loaded with [GTP-γ-S] (2 mM) or [GDP-β-S] (2 mM) at 37°C for 15 minutes in the buffer containing 50 mM Tris-HCl, pH 7.5, 7.5 mM EDTA, 2.5 mM $MgCl_2$, 0.5 mg/ml bovine serum albumin and 1 mM DTT. Various amounts of
10 GTP-γ-S or GDP-β-S loaded Ras proteins were mixed with purified prokaryotic recombinant GST-PRE (188-413) or GST as a control. After incubation at 30°C for 20 minutes, 0.4 ml of 7.5% (V/V) glutathione-Sepharose™ beads suspended in the binding buffer (50 mM Tris-HCl, pH 7.5,
15 150 mM NaCl, 1% Triton X-100, 0.2% BSA and 2 mM DTT), was added to recover GST or GST fusion proteins and any associated proteins. After tumbling at 4°C for 30 minutes, the beads were washed five times with the binding buffer and the bound polypeptides were eluted
20 with SDS sample buffer.

The proteins were separated by SDS-PAGE, transferred to PVDF membrane and probed with the monoclonal anti-Ras Pan-Ras-2 antibodies (Oncogene Science). The bands were visualized using the ECL
25 reagents (Amersham). This assay showed that GST-PRE1 (a.a. 188-413), but not GST, binds to Ras, and considerably more Ras-GTP-γ-S bound than Ras-GDP-β-S. These results showed the direct binding of PRE1 and Ras proteins, and established the binding of the two proteins
30 was GTP-dependent.

Ras-PRE1 association in COS-7 cells

The following experiment was carried out using the COS-7 transient expression system. This system was used because it was previously shown that in serum-starved

COS-7 cells, Ras is mostly in the GDP bound form, and addition of epidermal growth factor (EGF) or tetradecanoyl phorbol acetate (TPA) rapidly increases Ras-GTP charging (McCollam et al., 1997, *J. Biol. Chem.*, 5 **270**:15954-15947).

COS-7 cells were plated at a density of 1.2 million per 10 cm dish and transfected 24 hours later with 7 μg of pMT2-HA-c-H-Ras (Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*, (2nd Edition), 10 Cold Spring Harbor Press, Cold Spring Harbor, NY) or empty vector and 12 μg of pEBG-GST-PRE1 (Nagata et al., 1990, *Nucleic Acids Research*, **18**:5322) using the DEAE-Dextran method. Forty-eight hours later, cells were starved for 24 hours and subsequently were stimulated 15 with of EGF (100 ng/ml) or TPA (0.1 μM) for various times. Cells were extracted in lysis buffer (30 mM HEPES, pH 7.4, 1% Triton X-100, 20 mM β-glycerophosphate, 2 mM $NaPP_i$, 1 mM orthovanadate, 20 mM NaF, 20 mM KCl, 2 mM EGTA, 3 mM EDTA, 7.5 mM $MgCl_2$, 14 mM β-ME and a cocktail 20 of protease inhibitors). Lysates were freeze-thawed once and spun at 17000 X g for 20 minutes. Supernatants were incubated with monoclonal anti-HA antibodies (12CA5) and protein A-G Sepharose™ beads for 3-4 hours at 4°C and then washed extensively with lysis buffer. The washed 25 beads were eluted in SDS sample buffer. The extracted proteins were subjected to SDS-PAGE, transferred on PVDF membranes and probed using affinity-purified anti-GST polyclonal antibodies or monoclonal anti-HA antibodies. Bound antibodies were visualized using ECL. This assay 30 showed that GST-PRE1 was specifically bound by HA-c-H-Ras, but only after the cells were treated with EGF or TPA. The expression of HA-c-H-Ras and of GST-PRE1 was uniform throughout. Thus, PRE1 was not detectably associated with Ras in serum-starved COS cells, however, 35 within 5 minutes after stimulation by EGF (or TPA), PRE1

associated specifically with Ras. This association diminished by 15 minutes after EGF addition, and was largely reversed by 40 minutes. This may have reflected the downregulation of Ras activation after EGF treatment.

5 Ras-PRE association in KB cells

In situ association between endogenous Ras and endogenous PRE1, under conditions where the levels of the two polypeptides were not increased artificially by transient overexpression, was examined. The human oral 10 carcinoma cell line, KB, expressed both a readily detectable level of PRE1 as well as a substantial number of EGF receptors. KB cells were grown to 80% confluency, starved of serum for 24 hours and subsequently stimulated with EGF (100 ng/ml) for various times. Triton X-100 15 soluble cell lysates were subjected to immunoprecipitation using the monoclonal anti-Ras antibody, Y13-238, which is known to enable isolation of Ras-effector complexes. The Ras immunoprecipitates were washed extensively with the lysis buffer, eluted in SDS 20 sample buffer and subjected to SDS-PAGE, transferred to PVDF membrane and immunoblotted with the affinity purified polyclonal anti-PRE1 antibodies. These experiments showed that, although equal amounts of endogenous Ras was recovered in all samples, the Ras 25 immunoprecipitates contain immunoreactive PRE1 only after treatment of the cells with EGF. The time course of Ras-PRE1 association after EGF treatment in KB cells was more sustained than that observed in COS-7 cells. This may have reflected a different time course of 30 downregulation of Ras activation in those cells. The 46 KD immunoreactive PRE1 polypeptide was recovered with c-Ras, but not the equally abundant 55 KD protein.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: The General Hospital Corporation

(ii) TITLE OF THE INVENTION: RAS-BINDING PROTEIN

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Fish & Richardson, P.C.
(B) STREET: 225 Franklin Street
(C) CITY: Boston
(D) STATE: MA
(E) COUNTRY: US
(F) ZIP: 02110-2804

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: Windows95
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: 01-OCT-1998
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:08/942,572
(B) FILING DATE: 01-OCT-1997

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Fraser, Janis K.
(B) REGISTRATION NUMBER: 34,819
(C) REFERENCE/DOCKET NUMBER: 08472/737001

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 617/542-5070
(B) TELEFAX: 617/542-8906
(C) TELEX: 200154

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3018 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: Coding Sequence
(B) LOCATION: 31...1269

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

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                                 Met Ala Ser Pro Ala Ile Gly Gln
                                  1               5

CGT CCC TAC CCG CTG CTC CTA GAT CCC GAG CCG CCG CGG TAT CTG CAG  102
Arg Pro Tyr Pro Leu Leu Leu Asp Pro Glu Pro Pro Arg Tyr Leu Gln
     10                  15                  20
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AGT CTG GGT GGC ACC GAG CCG CCA CCT CCC GCC CGG CCG CGC CGC TGC      150
Ser Leu Gly Gly Thr Glu Pro Pro Pro Pro Ala Arg Pro Arg Arg Cys
 25                  30                  35                  40

ATC CCC ACG GCC CTG ATC CCC GCG GCC GGG GCG TCA GAG GAT CGC GGT      198
Ile Pro Thr Ala Leu Ile Pro Ala Ala Gly Ala Ser Glu Asp Arg Gly
                 45                  50                  55

GGC AGG AGG AGT GGC CGG AGG GAC CCC GAA CCC ACG CCC CGA GAC TGC      246
Gly Arg Arg Ser Gly Arg Arg Asp Pro Glu Pro Thr Pro Arg Asp Cys
             60                  65                  70

CGA CAC GCT CGC CCT GTC CGG CCC GGT CTG CAG CCG AGA CTG CGG CTG      294
Arg His Ala Arg Pro Val Arg Pro Gly Leu Gln Pro Arg Leu Arg Leu
         75                  80                  85

CGA CCT GGG TCA CAC CGA CCC CGC GAC GTG AGG AGC ATC TTC GAG CAG      342
Arg Pro Gly Ser His Arg Pro Arg Asp Val Arg Ser Ile Phe Glu Gln
     90                  95                 100

CCG CAG GAT CCC CGC GTC TTG GCC GAG AGA GGC GAG GGG CAC CGT TTC      390
Pro Gln Asp Pro Arg Val Leu Ala Glu Arg Gly Glu Gly His Arg Phe
105                 110                 115                 120

GTG GAA CTG GCG CTG CGG GGC GGT CCG GGC TGG TGT GAC CTG TGC GGA      438
Val Glu Leu Ala Leu Arg Gly Gly Pro Gly Trp Cys Asp Leu Cys Gly
                125                 130                 135

CGA GAG GTG CTG CGG CAG GCG CTG CGC TGC GCT AAT TGT AAA TTC ACC      486
Arg Glu Val Leu Arg Gln Ala Leu Arg Cys Ala Asn Cys Lys Phe Thr
            140                 145                 150

TGC CAC TCG GAG TGC CGC AGC CTG ATC CAG TTG GAC TGC AGA CAG AAG      534
Cys His Ser Glu Cys Arg Ser Leu Ile Gln Leu Asp Cys Arg Gln Lys
        155                 160                 165

GGG GGC CCT GCC CTG GAT AGA CGC TCT CCA GGA AGC ACC CTC ACC CCA      582
Gly Gly Pro Ala Leu Asp Arg Arg Ser Pro Gly Ser Thr Leu Thr Pro
    170                 175                 180

ACC TTG AAC CAG AAT GTC TGT AAG GCA GTG GAG GAG ACA CAG CAC CCG      630
Thr Leu Asn Gln Asn Val Cys Lys Ala Val Glu Glu Thr Gln His Pro
185                 190                 195                 200

CCC ACG ATA CAG GAG ATC AAG CAG AAG ATT GAC AGC TAT AAC AGC AGG      678
Pro Thr Ile Gln Glu Ile Lys Gln Lys Ile Asp Ser Tyr Asn Ser Arg
                205                 210                 215

GAG AAG CAC TGC CTG GGC ATG AAG CTG AGT GAA GAT GGC ACC TAC ACA      726
Glu Lys His Cys Leu Gly Met Lys Leu Ser Glu Asp Gly Thr Tyr Thr
            220                 225                 230

GGT TTC ATC AAA GTG CAT TTG AAG CTC CGA CGG CCA GTG ACG GTG CCC      774
Gly Phe Ile Lys Val His Leu Lys Leu Arg Arg Pro Val Thr Val Pro
        235                 240                 245

GCT GGA TCC GGC CCC AGT CCA TCT ATG GAT GCC ATT AAG GAA GTG AAC      822
Ala Gly Ser Gly Pro Ser Pro Ser Met Asp Ala Ile Lys Glu Val Asn
    250                 255                 260

CCT GCA GCC ACC ACA GAC AAG CGG ACT TCC TTC TAC CTG CCA CTC GAT      870
Pro Ala Ala Thr Thr Asp Lys Arg Thr Ser Phe Tyr Leu Pro Leu Asp
265                 270                 275                 280

GCC ATC AAG CAG CTA CAT ATC AGC AGC ACC ACC ACG GTT AGT GAG GTC      918
Ala Ile Lys Gln Leu His Ile Ser Ser Thr Thr Thr Val Ser Glu Val
                285                 290                 295
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ATC CAG GGG CTG CTC AAG AAG TTC ATG GTT GTG GAC AAC CCA CAG AAG      966
Ile Gln Gly Leu Leu Lys Lys Phe Met Val Val Asp Asn Pro Gln Lys
        300                 305                 310

TTT GCA CTT TTT AAG CGG ATA CAC AAA GAT GGA CAA GTG CTC TTC CAG     1014
Phe Ala Leu Phe Lys Arg Ile His Lys Asp Gly Gln Val Leu Phe Gln
    315                 320                 325

AAA CTC TCC ATT GCT GAC TAT CCT CTC TAC CTT CGT CTG CTC GCT GGG     1062
Lys Leu Ser Ile Ala Asp Tyr Pro Leu Tyr Leu Arg Leu Leu Ala Gly
330                 335                 340

CCT GAC ACC GAT GTT CTC AGC TTT GTG CTA AAG GAG AAT GAA ACT GGA     1110
Pro Asp Thr Asp Val Leu Ser Phe Val Leu Lys Glu Asn Glu Thr Gly
345                 350                 355                 360

GAG GTG GAG TGG GAT GCC TTT TCC ATT CCT GAA CTC CAG AAC TTT TTA     1158
Glu Val Glu Trp Asp Ala Phe Ser Ile Pro Glu Leu Gln Asn Phe Leu
                365                 370                 375

ACT ATC CTG GAA AAA GAG GAG CAG GAC AAG ATC CAT CAA CTG CAA AAG     1206
Thr Ile Leu Glu Lys Glu Glu Gln Asp Lys Ile His Gln Leu Gln Lys
            380                 385                 390

AAG TAC AAC AAA TTC CGT CAG AAA CTG GAA GAG GCA TTA CGA GAG TCC     1254
Lys Tyr Asn Lys Phe Arg Gln Lys Leu Glu Glu Ala Leu Arg Glu Ser
        395                 400                 405

CAA GGG AAG CCG GGG TAACCAGCCG ACTTCCTGTC CTCTCAGTGC CCTCCAATTT     1309
Gln Gly Lys Pro Gly
    410

ATTTTATTGT TAATTATTTT GCAACAAAGA GTTACTGTTA AGACACCTCT GGTGGTTCCA  1369
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ATCAGAACTC CTGTGGAATG ACCAGTGTTT CCCTGCTCAG TCTGCTGGCC TCTCAGAAAC  1549
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TGCCTCTTCT TCTCTAGTAC TAGACACTCA GGGTCAGCCT GAGATTTCAA GAGGCTACAG  1849
CCTGACCAGG CCGTCTTCTT ATTACCCAGC AGGCTGTGTG CATGCAAACC CAAAGACATA  1909
TATGCACATC TGTGTGGTAT TTCAGCATGT CTCTGTCCAA TGTTTGATAT GTTAACATTT  1969
GAATTTAATG CTGTCCTCCT TATGGGTTTC TACCAAAGAG AAACCAGCCA CTTATCAATT  2029
TTAGTTTCTT GCTGAGCTGC CAGAAAGTAT TACAGAGAAG CACATCCAAG CTGTCTGTGG  2089
CCTACGCCTG CAGGGGGTGG GGGGCCTGAA TCTCCTTGGC CTTCAGTTCC ACCTCCACCT  2149
CTGGCTTTAG GGTCTCCAGC TGTTGCCTGA GTAGTAGCTT TGATTACAGC GGTAAAGTCC  2209
TCCAACTTGG AGTCCTTTCT GGTGGGAAGC ATGGTCTGCT CGCAGCACAG CACTGAGCAG  2269
ACCCGTGGGC CTGACTTCCC TGGTGACTTC AGTGCCTTTT TGTTTGCAGA GAAAAGAGTG  2329
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ATAGAGCCAA GGACACAGCC TCAGAGCACA GGGTGATTGA TGATCAGCCT CTTTCCCATC  2449
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TCAGATTATG ACTCCAACTC TTGAGTAGTT CAGACTTAAA ACCAATCAGC CAGAGTAGCC  2569
AGGACTGCAA AGACACTCAA TACAGATGGA GAAAAACTTG TCCCTTTAAA AGAGGGCCAG  2629
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TTCCTCGGGT TGTTTTAAGC CCAAGCTTCT CCGTGTAGCC TAAAAAGTTC CCTACCAGCC  2749
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GCATCGTTTG CAGAGCTGCA AAAAGCAACA TGAGCTAGCG ACTCTGAGGT TGTGCACGCC  2869
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CCCCCGTCCT GCTGTGTACT ACAGGGGCAC GTGGAATCAT TCCCTTCATC CTGCATGTCT  2989
GTAGCGTTAG GAGAAGGCAT GGCTCCTGC                                    3018
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 413 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

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

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

We claim:

1. An isolated DNA comprising a nucleotide sequence that encodes a naturally-occuring protein that: (a) shares at least 80% sequence identity with SEQ ID NO:2 and (b) binds to Ras.

2. The DNA of claim 1, wherein said nucleotide sequence defines a DNA molecule whose complement hybridizes under high stringency conditions to a DNA whose nucleotide sequence consists of SEQ ID NO:1.

3. The DNA of claim 1, wherein said protein has an amino acid sequence consisting of SEQ ID NO:2.

4. The DNA of claim 3, wherein said nucleotide sequence is the coding region of the nucleotide sequence of SEQ ID NO:1.

5. A vector comprising the DNA of claim 1.

6. The vector of claim 5, wherein said DNA is operably linked to one or more expression control sequences.

7. A cell comprising the DNA of claim 1.

8. A substantially pure protein that: (a) comprises an amino acid sequence that shares at least 80% sequence identity with SEQ ID NO:2 and (b) binds to Ras.

9. A substantially pure protein comprising amino acid residues 266-360 of SEQ ID NO:2.

10. A substantially pure protein comprising amino acid residues 188-413 of SEQ ID NO:2.

11. A substantially pure protein whose amino acid sequence is SEQ ID NO:2.

12. The protein of claim 9, wherein the protein is a fused heterologous polypeptide.

13. The protein of claim 12, wherein said fused heterologous polypeptide comprises a glutathione-S-transferase fragment.

14. An antibody that binds specifically to the protein of claim 8.

15. The antibody of claim 10, further comprising a detectable label.

16. A screening method for identifying a substance that modulates binding of a PRE1 protein to a Ras protein, said method comprising the steps of:

(a) providing a sample solution of a PRE1 protein;

(b) adding to said sample solution a candidate substance;

(c) adding to said sample solution a Ras protein; and

(d) detecting an increase or decrease in binding of said PRE1 protein to said Ras protein in the presence of said candidate substance, compared to the binding of the PRE1 protein to the Ras protein in the absence of said candidate substance, as an indication that said candidate substance modulates binding of said PRE1 protein to said Ras protein.

17. A method of producing a PRE1 protein, said method comprising the steps of:

(a) providing a cell transformed with an isolated DNA comprising a nucleotide sequence that encodes a PRE1 protein;

(b) culturing said cell; and

(c) collecting said PRE1 protein encoded by said nucleotide sequence.

18. A screening method for identifying a substance that modulates PRE1 gene expression, said method comprising the steps of :

(a) providing a test cell;

(b) contacting said test cell with a candidate substance; and

(c) detecting an increase or decrease in the level of PRE1 gene expression in the presence of said candidate substance, compared to the level of PRE1 gene expression in the absence of said candidate substance, as an indication that said candidate substance modulates PRE1 gene expression.

19. A method for isolating a PRE1-binding substance, said method comprising the steps of:

(a) providing a sample of an immobilized PRE1 protein;

(b) contacting a mixture containing said PRE1-binding substance with said immobilized PRE1 protein;

(c) separating unbound components of said mixture from bound components of said mixture;

(d) recovering said PRE1-binding substance from said immobilized PRE1 protein.

20. The method of claim 15, wherein said PRE1-binding substance is Ras protein.

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GTAGCTGCGCCGCTGACTGAGGCCTTGGCCATGGCTTCCCCGGCCATCGGGCAACGTCCC   60
                                   M A S P A I G Q R P
TACCCGCTGCTCCTAgATCCCGAGCCGCCGCGGTATCTGCAGAGTCTGGGTGGCACCGAG  120
Y P L L L D P E P P R Y L Q S L G G T E
CCGCCACCTCCCGCCCGGCCGCGCCGCTGCATCCCCACGGCCCTGATCCCCGCGGCCGGG  180
P P P P A R P R R C I P T A L I P A A G
GCGTCAGAGGATCGCGGTGGCAGGAGGAGTGGCCGGAGGGACCCCGAACCCACGCCCCGA  240
A S E D R G G R R S G R R D P E P T P R
GACTGCCGACACGCTCGCCCTGTCCGGCCCGGTCTGCAGCCGAGACTGCGGCTGCGACCT  300
D C R H A R P V R P G L Q P R L R L R P
GGGTCACACCGACCCCGCGACGTGAGGAGCATCTTCGAGCAGCCGCAGGATCCCCGCGTC  360
G S H R P R D V R S I F E Q P Q D P R V
TTGGCCGAGAGAGGCGAGGGGCACCGTTTCGTGGAACTGGCGCTGCGGGGCGGTCCGGGC  420
L A E R G E G H R F V E L A L R G G P G
TGGTGTGACCTGTGCGGACGAGAGGTGCTGCGGCAGGCGCTGCGCTGCGCTAATTGTAAA  480
W C D L C G R E V L R Q A L R C A N C K
TTCACCTGCCACTCGGAGTGCCGCAGCCTGATCCAGTTGGACTGCAGACAGAAGGGGGGC  540
F T C H S E C R S L I Q L D C R Q K G G
CCTGCCCTGGATAGACGCTCTCCAGGAAGCACCCTCACCCCAACCTTGAACCAGAATGTC  600
P A L D R R S P G S T L T P T L N Q N V
TGTAAGGCAGTGGAGGAGACACAGCACCCGCCCACGATACAGGAGATCAAGCAGAAGATT  660
C K A V E E T Q H P P T I Q E I K Q K I
GACAGCTATAACAGCAGGGAGAAGCACTGCCTGGGCATGAAGCTGAGTGAAGATGGCACC  720
D S Y N S R E K H C L G M K L S E D G T
TACACAGGTTTCATCAAAGTGCATTTGAAGCTCCGACGGCCAGTGACGGTGCCCGCTGGA  780
Y T G F I K V H L K L R R P V T V P A G
TCCGGCCCCAGTCCATCTATGGATGCCATTAAGGAAGTGAACCCTGCAGCCACCACAGAC  840
S G P S P S M D A I K E V N P A A T T D
AAGCGGACTTCCTTCTACCTGCCACTCGATGCCATCAAGCAGCTACATATCAGCAGCACC  900
K R T S F Y L P L D A I K Q L H I S S T
ACCACGGTTAGTGAGGTCATCCAGGGGCTGCTCAAGAAGTTCATGGTTGTGGACAACCCA  960
T T V S E V I Q G L L K K F M V V D N P
CAGAAGTTTGCACTTTTTAAGCGGATACACAAAGATGGACAAGTGCTCTTCCAGAAACTC 1020
Q K F A L F K R I H K D G Q V L F Q K L
TCCATTGCTGACTATCCTCTCTACCTTCGTCTGCTCGCTGGGCCTGACACCGATGTTCTC 1080
S I A D Y P L Y L R L L A G P D T D V L
AGCTTTGTGCTAAAGGAGAATGAAACTGGAGAGGTGGAGTGGGATGCCTTTTCCATTCCT 1140
S F V L K E N E T G E V E W D A F S I P
GAACTCCAGAACTTTTTAACTATCCTGGAAAAAGAGGAGCAGGACAAGATCCATCAACTG 1200
E L Q N F L T I L E K E E Q D K I H Q L
CAAAAGAAGTACAACAAATTCCGTCAGAAACTGGAAGAGGCATTACGAGAGTCCCAAGGG 1260
Q K K Y N K F R Q K L E E A L R E S Q G
AAGCCGGGGTAACCAGCCGACTTCCTGTCCTCTCAGTGCCCTCCAATTTATTTTATTGTT 1320
K P G *
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FIG. 1A

AATTATTTTGCAACAAAGAGTTACTGTTAAGACACCTCTGGTGGTTCCACCAGTCGCCTG 1380

CCCAGCAGTTAACAGATGTGGCACAAAGTCTCTTCCACGCAGTGTCTATGCAGGGTTCCG 1440

ATTCCTGCTAACCCACCACACCATGGCTCTGGAGAGCTTCCCGCCTGGGATCAGAACTCC 1500

TGTGGAATGACCAGTGTTTCCCTGCTCAGTCTGCTGGCCTCTCAGAAACCAAATAGTTGC 1560

CTCTCTGGTCACCAAACTCCAATCAATCACCAGCCGGCAAAAGGAAAGAAAGGTTTCAGA 1620

GCCTGTGTGTTCTTTCTCTGGATTTACTCTTCAGTTCCTCTTTTGGTTTGTTTGGTTGGT 1680

TTTTTTTGGCCACGTATAGTATATTTAAGGATCAAATGTGGCATATTCATTCTAGCTAAG 1740

TCCTTGAAAGCAGGAAAATGCTCATGAAAGGACTGTCCTTGCCCCAAGGTGCCTCTTCTT 1800

CTCTAGTACTAGACACTCAGGGTCAGCCTGAGATTTCAAGAGGCTACAGCCTGACCAGGC 1860

CGTCTTCTTATTACCCAGCAGGCTGTGTGCATGCAAACCCAAAGACATATATGCACATCT 1920

GTGTGGTATTTCAGCATGTCTCTGTCCAATGTTTGATATGTTAACATTTGAATTTAATGC 1980

TGTCCTCCTTATGGGTTTCTACCAAAGAGAAACCAGCCACTTATCAATTTTAGTTTCTTG 2040

CTGAGCTGCCAGAAAGTATTACAGAGAAGCACATCCAAGCTGTCTGTGGCCTACGCCTGC 2100

AGGGGGTGGGGGGCCTGAATCTCCTTGGCCTTCAGTTCCACCTCCACCTCTGGCTTTAGG 2160

GTCTCCAGCTGTTGCCTGAGTAGTAGCTTTGATTACAGCGGTAAAGTCCTCCAACTTGGA 2220

GTCCTTTCTGGTGGGAAGCATGGTCTGCTCGCAGCACAGCACTGAGCAGACCCGTGGGCC 2280

TGACTTCCCTGGTGACTTCAGTGCCTTTTTGTTTGCAGAGAAAAGAGTGGGGCACTTTGC 2340

TTGAAGCTCTCTGCTGGCTTGCCCCTGGCAGGAAGTGGACAATGGTGCTATAGAGCCAAG 2400

GACACAGCCTCAGAGCACAGGGTGATTGATGATCAGCCTCTTTCCCATCAAGCTTCCCGG 2460

TCAGGCTTTGACTTTGAAGATGCGAGGTTACTAGACTGCATTGACAGCATCAGATTATGA 2520

CTCCAACTCTTGAGTAGTTCAGACTTAAAACCAATCAGCCAGAGTAGCCAGGACTGCAAA 2580

GACACTCAATACAGATGGAGAAAAACTTGTCCCTTTAAAAGAGGGCCAGTGTTTCAATTG 2640

AGCCTCCAGAGGAGACCACTTTCATGTTGTGCTTGCCTTTCCATACCCTTTCCTCGGGTT 2700

GTTTTAAGCCCAAGCTTCTCCGTGTAGCCTAAAAAGTTCCCTACCAGCCCAGCTGAAGCC 2760

ACACTGCTCCCGTCCCAGAAGAACGCCAAATCCTTGTCATTCAAACTGTGCATCGTTTGC 2820

AGAGCTGCAAAAAGCAACATGAGCTAGCGACTCTGAGGTTGTGCACGCCATCAGCCCCTT 2880

GGCTGCCTGAGGTCTCATGCCCAGCCTTACACCTCTCTCCCTTAAGAAGCCCCCGTCCTG 2940

CTGTGTACTACAGGGGCACGTGGAATCATTCCCTTCATCCTGCATGTCTGTAGCGTTAGG 3000

AGAAGGCATGGCTCCTGC 3018

FIG. 1B