ESTROGEN-REGULATED MYO-INOSITOL PHOSPHATE SYNTHASE: COMPOSITIONS AND METHODS OF USE

Abstract: The present invention provides nucleic acid and protein sequences for a novel estrogen-regulated myo-inositol phosphate synthase, methods for using these sequences for modulating the effects of estrogen in mammalian cells, methods of detecting estrogen signaling in cells, and methods of identifying compounds capable of acting as an estrogen receptor α agonists or antagonists.

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CROSS-REFERENCE TO RELATED APPLICATION
This application claims benefit of U.S. Provisional Application No. 60/188,306, filed March 20, 2000, which application is incorporated herein by reference for all purposes.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT
Not applicable.

BACKGROUND OF THE INVENTION
The ovarian sex steroid hormone estrogen has a broad spectrum of biological functions including inhibiting the development of atherosclerosis, osteoporosis, and neurological disorders including Alzheimer’s Disease and Parkinson’s Disease. Estrogen has also been associated with breast and other cancers. Despite the importance of estrogen, and estrogen signaling in these and other diseases, the molecular mechanisms of estrogen action are largely unknown, and the ability to modulate estrogen signaling is poorly developed.

It appears that estrogen regulates the expression of many genes by binding to its two known nuclear receptors, ERα and ERβ. These two receptor types are encoded by distinct genes, and have distinct ligand affinity and tissue distribution (see, e.g., Kuiper et al., Endocrinology 1997 138:863-70). The importance of estrogen receptor α has been demonstrated by the generation of a knock-out mouse, called ERKO. Both male and female ERKO mice are sterile, and display a variety of phenotypic effects including decreased bone density, defects in their reproductive tissues, and decreased likelihood of developing oncogene-induced cancer. See, e.g., Couse et al., (1999) Endocr. Rev. 20:358-417; Korach (1994) Science 266:1524-7; Bocchinfuso et al., (1999) Cancer Res. 59:1869-76. Mice lacking estrogen receptor β are fertile, but females display decreased ovarian activity, leading to decreased litter sizes. Krege, et al. (1998) Proc. Natl. Acad. Sci. USA 95:15677-82. Mice lacking both α and β receptors are infertile and display an ovarian phenotype that is distinct from that of either receptor knockout alone. Couse et al., (1999) Science 286:2328-31.

Thus, there is a need in the art for new approaches to understanding and modulating estrogen signaling in animals. The present invention addresses these and other needs.

**SUMMARY OF THE INVENTION**

The present invention provides nucleic acid and protein sequences for a novel estrogen-regulated myo-inositol phosphate synthase, methods for using these sequences for modulating the effects of estrogen in mammalian cells, methods of detecting estrogen signaling in cells, and methods of identifying compounds capable of acting as an estrogen receptor α agonists or antagonists. In one aspect, the present invention provides an isolated nucleic acid encoding an estrogen-regulated myo-inositol phosphate synthase protein, the protein having at least one of the following characteristics: (1) comprising at least about 90% amino acid sequence identity to SEQ ID NO:1; or (2) specifically binding to polyclonal antibodies generated against a polypeptide comprising a sequence of SEQ ID NO:1, but not to polyclonal antibodies generated against a polypeptide comprising the amino acid sequence of SEQ ID NO: 3; wherein the protein does not comprise the amino acid sequence shown as SEQ ID NO:3.

In one embodiment, the nucleic acid encodes a protein comprising the amino acid sequence of SEQ ID NO:1. In another embodiment, the nucleic acid comprises a nucleotide sequence that is at least about 90% identical to SEQ ID NO:2. In another embodiment, the nucleic acid comprises the nucleotide sequence of SEQ ID NO:2. In another embodiment, the nucleic acid hybridizes under moderately stringent
wash conditions to a nucleic acid comprising the nucleotide sequence of SEQ ID NO:2. In another embodiment, the nucleic acid hybridizes under stringent wash conditions to a nucleic acid comprising the nucleotide sequence of SEQ ID NO:2. In another embodiment, the nucleic acid is from a mouse.

In another aspect, the present invention provides an expression cassette comprising the nucleic acid. In another aspect, the present invention provides an isolated eukaryotic cell comprising the expression cassette.

In another aspect, the present invention provides an isolated estrogen-regulated myo-inositol phosphate synthase protein, the protein having at least one of the following characteristics: (1) comprising at least about 70% amino acid sequence identity to SEQ ID NO:1; or (2) specifically binding to polyclonal antibodies generated against a polypeptide comprising a sequence of SEQ ID NO:1, but not to polyclonal antibodies generated against a polypeptide comprising the amino acid sequence of SEQ ID NO: 3; wherein the protein does not comprise the amino acid sequence shown as SEQ ID NO:3.

In one embodiment, the protein comprises the amino acid sequence of SEQ ID NO:1. In another embodiment, the protein is from a mouse.

In another aspect, the present invention provides antibodies that selectively bind to an estrogen-regulated myo-inositol phosphate synthase protein having at least one of the following characteristics: (1) comprising at least about 90% amino acid sequence identity to SEQ ID NO:1; or (2) specifically binding to polyclonal antibodies generated against a polypeptide comprising a sequence of SEQ ID NO:1, but not to polyclonal antibodies generated against a polypeptide comprising the amino acid sequence of SEQ ID NO: 3; wherein the protein does not comprise the amino acid sequence shown as SEQ ID NO:3.

In another aspect, the present invention provides a method of modulating the effects of estrogen receptor α-mediated estrogen signaling in a mammalian cell, the method comprising modulating the level of expression or activity of an estrogen-regulated myo-inositol phosphate synthase protein having at least one of the following characteristics: (1) comprising at least about 70% amino acid sequence identity to SEQ ID NO:1 or 3; or (2) specifically binding to polyclonal antibodies generated against a polypeptide comprising a sequence of SEQ ID NO:1 or 3.

In one embodiment, the level of expression is modulated by introducing a polynucleotide into the cell, whereby the presence or expression of the polynucleotide
modulates the level of expression of the estrogen-regulated myo-inositol phosphate synthase protein in the cell. In another embodiment, the polynucleotide encodes a full length estrogen-regulated myo-inositol phosphate synthase protein, wherein expression of the polynucleotide increases the level of expression of the estrogen-regulated myo-inositol phosphate synthase protein in the cell. In another embodiment, the polynucleotide is an antisense sequence, wherein the presence or expression of the polynucleotide decreases the level of expression of estrogen-regulated myo-inositol phosphate synthase in the cell. In another embodiment, a compound is administered to the cell, whereby the level of the expression or activity of the estrogen-regulated myo-inositol phosphate synthase protein is modulated.

In another embodiment, the cell is present in a mammal. In another embodiment, the level of expression or activity of the estrogen-regulated myo-inositol phosphate synthase protein is increased, whereby the development of atherosclerosis or osteoporosis in the mammal is inhibited. In another embodiment, the level of expression or activity of the estrogen-regulated myo-inositol phosphate synthase protein is decreased, whereby the development of breast or other cancer is inhibited.

In another aspect, the present invention provides a method of detecting the presence of estrogen receptor α-mediated estrogen signaling in a mammalian cell, the method comprising detecting the expression of an estrogen-regulated myo-inositol phosphate synthase protein encoding nucleic acid in the cell, the protein: (1) comprising at least about 70% amino acid sequence identity to SEQ ID NO:1 or 3; or (2) specifically binding to polyclonal antibodies generated against a polypeptide comprising a sequence of SEQ ID NO:1 or 3.

In one embodiment, the presence of estrogen signaling is used in order to determine the tissue-specific distribution of estrogen signaling in a mammal. In another embodiment, the expression of the nucleic acid in the cell is detected by detecting the expression or activity of the encoded estrogen-regulated myo-inositol phosphate synthase protein in the cell. In another embodiment, the expression of the nucleic acid in the cell is detected by detecting the level of estrogen-regulated myo-inositol phosphate synthase protein-encoding mRNA in the cell.

In another aspect, the present invention provides a method of identifying a compound capable of acting as an estrogen-receptor α agonist or antagonist, the method comprising: (1) contacting a cell comprising an estrogen receptor α with the compound;
and (2) detecting the functional effect of the compound on the cell, wherein an increase in the level of estrogen regulated myo-inositol phosphate synthase mRNA, protein, or protein activity in the cell indicates that the compound is capable of acting as an estrogen receptor α agonist, and a decrease in the level of estrogen regulated myo-inositol phosphate synthase mRNA, protein, or protein activity in the cell indicates that the compound is capable of acting as an estrogen receptor α antagonist.

In another embodiment, the mRNA is at least about 70% identical to the sequence shown as SEQ ID NO:2 or 4. In another embodiment, the protein is at least about 70% identical to the sequence shown as SEQ ID NO:1 or 3.

10 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a Northern blot analysis of mMIPS mRNA levels in the livers of untreated normal male mice and estrogen treated ERKO mice.

Figure 2 provides the amino acid sequence of mMIPS.

Figure 3 provides the nucleotide sequence of a cDNA encoding mMIPS.

Figure 4 illustrates the domain structure of MIPS. The domain structure was analyzed with HMM using the translated amino acid sequence.

Figure 5 illustrates the regulation of the MIPS gene by estrogen. Northern hybridization of liver RNA from a wildtype (WT) mouse and a knock-out mouse (ERKO), which lacks the estrogen receptor α, both of which were treated with vehicle (V), 17β-estradiol (E2) and antiestrogen compound (ZK). The mouse inositol 1-phosphate synthase gene was up-regulated by E2 in the WT mouse only, demonstrating that this gene is regulated via the estrogen receptor α.

Figure 6 illustrates the regulation of MIPS expression in ERKO mice, which lack the estrogen receptor α. The mouse inositol 1-phosphate synthase gene was up-regulated by E2 in WT mice only, demonstrating that this gene is regulated via the estrogen receptor α.

Figure 7 illustrates the regulation of MIPS expression in BERKO mice, which lack the estrogen receptor β. The MIPS gene was up-regulated by E2 in BERKO mice only, demonstrating that this gene is regulated via the estrogen receptor α. The MIPS gene was not upregulated when treated with both E2 and ICI, an inhibitor of the α receptor.
DETAILED DESCRIPTION OF THE INVENTION
AND PREFERRED EMBODIMENTS

I. Introduction

The present invention provides nucleic acids and polypeptides for mMIPS, a novel estrogen-regulated myo-inositol phosphate synthase. mMIPS and other mammalian MIPS genes are dramatically upregulated by estrogen, in particular by estrogen acting specifically through the estrogen receptor \( \alpha \) (see, Figures 5-7), indicating that MIPS is a molecular mediator of the in vivo function of estrogen. In fact, it has been discovered that MIPS is upregulated by estrogen in both the liver and brain and, in addition, it has been found to increase the synthesis of IP3 (see, e.g., Novak, et al., J. Neurochem. 72(4):1431-1440 (1999), the teachings of which are incorporated herein by reference). Accordingly, the effects of estrogen can be modulated by modulating the expression or activity of mMIPS or other MIPS proteins, and, in addition, the presence, level, or tissue distribution of estrogen receptor \( \alpha \)-mediated estrogen signaling can be detected by detecting MIPS expression or activity. MIPS can also be used as a marker gene for the investigation and identification of tissue-specific and estrogen receptor-specific ligands, such as estrogen receptor \( \alpha \) agonists and antagonists.

Modulators, recombinant forms, derivatives, variants, or fragments of MIPS can be used to enhance or inhibit estrogen \( \alpha \)-mediated estrogen signaling in cells, and can therefore be useful in the treatment of a wide variety of estrogen-related diseases. For example, estrogen signaling can be enhanced to treat diseases including, but not limited to, osteoporosis, cardiovascular diseases (e.g., atherosclerosis), Alzheimer Disease, or Parkinson's Disease. Alternatively, estrogen signaling can be inhibited to treat, e.g., breast or other cancers.

In numerous embodiments, the present invention provides methods of screening for modulators, e.g., activators, inhibitors, stimulators, enhancers, etc., of MIPS nucleic acids and proteins. Such modulators can affect MIPS activity in any of a number of ways, e.g., by modulating MIPS transcription, translation, phosphorylation, mRNA or protein stability, by altering the binding of MIPS to heterologous proteins or other molecules; or by affecting MIPS protein activity. In preferred embodiments, modulators that increase or decrease MIPS activity or levels are used to treat any of the above-recited estrogen-related diseases or conditions.
In one embodiment, compounds are screened, e.g., using high throughput screening (HTS), to identify those compounds that can bind to and/or modulate the activity of an isolated MIPS polypeptide or fragment thereof. In another embodiment, MIPS proteins are recombinantly expressed in cells, and potential modulators of MIPS are assayed by detecting the presence or activity of the MIPS in the cells.

In numerous embodiments, a MIPS polynucleotide or polypeptide is introduced into a cell, in vivo or ex vivo, and the MIPS activity in the cell is thereby modulated. For example, a polynucleotide encoding a full length MIPS polypeptide is introduced into a population of cells, thereby increasing the level or activity of MIPS in the cells. Alternatively, an antisense, ribozyme, or dominant-negative encoding polynucleotide can be introduced into a population of cells, thereby inhibiting the MIPS levels and/or activity in the cells. Such methods are particularly useful for the treatment of estrogen related diseases and conditions, as discussed infra.

The present invention also provides methods for detecting MIPS nucleic acid and protein expression, allowing investigation into estrogen-mediated signaling and transcription, e.g., through an estrogen receptor α, and allowing the specific identification, in vitro or in vivo, of estrogen responsive cells, in particular cells that are responsive to estrogen through an estrogen receptor α. MIPS also provides useful nucleic acid probes for paternity and forensic investigations. MIPS polypeptides can also be used to generate monoclonal and polyclonal antibodies useful for identifying estrogen responsive cells, particularly cells responding to estrogen via an estrogen receptor α. MIPS expression can be identified using techniques such as reverse transcription and amplification of mRNA, isolation of total RNA or poly A+ RNA, northern blotting, dot blotting, in situ hybridization, RNase protection, S1 digestion, probing DNA microchip arrays, western blots, and the like.

Functionally, MIPS nucleic acids encode myo-inositol phosphate synthases. MIPS expression is strongly regulated by estrogen signaling, particular mediated by estrogen receptor α. Related MIPS genes from other species share at least about 60% nucleotide sequence identity over a region of at least about 50 nucleotides in length, optionally 100, 200, 500, or more nucleotides in length, to SEQ ID NO:2 or 4, or encode polypeptides sharing at least about 60% amino acid sequence identity over an amino acid region at least about 25 amino acids in length, optionally 50 to 100 amino
acids in length to SEQ ID NO:1 or 3. Preferably, the mMIPS polypeptides comprise about 557 amino acids.

The present invention also provides polymorphic variants of the mMIPS protein depicted in SEQ ID NO: 1: variant #1, in which a leucine residue is substituted for an isoleucine residue at amino acid position 21; and variant #2, in which an alanine residue is substituted for a glycine residue at amino acid position 36.

Specific regions of the MIPS nucleotide and amino acid sequences may be used to identify polymorphic variants, interspecies homologs, and alleles of MIPS genes. This identification can be made in vitro, e.g., under stringent hybridization conditions, or PCR and sequencing, or by using the sequence information in a computer system for comparison with other nucleotide sequences. Typically, identification of polymorphic variants and alleles of MIPS is made by comparing an amino acid sequence of about 25 amino acids or more, e.g., 50-100 amino acids. Amino acid identity of approximately at least 60% or above, optionally 65%, 70%, 75%, 80%, 85%, or 90-95% or above typically demonstrates that a protein is a polymorphic variant, interspecies homolog, or allele of MIPS. Sequence comparison can be performed using any of the sequence comparison algorithms discussed below. Antibodies that bind specifically to MIPS polypeptides or a conserved region thereof can also be used to identify alleles, interspecies homologs, and polymorphic variants.

Polymorphic variants, interspecies homologs, and alleles of MIPS are confirmed by examining, e.g., expression or activity of the putative homolog in response to estrogen. Typically, a MIPS polypeptide having an amino acid sequence of SEQ ID NO:1 or 3 is used as a positive control in comparison to the putative MIPS protein to demonstrate the identification of a polymorphic variant or allele of the MIPS gene or protein.

Nucleotide and amino acid sequence information for MIPS may also be used to construct models of MIPS polypeptides in a computer system. These models are subsequently used to identify compounds that can activate or inhibit MIPS proteins. Such compounds that modulate the activity of MIPS genes or proteins can be used to investigate the role of MIPS genes in estrogen mediated signaling, e.g., through the estrogen receptor α.
The present invention also provides assays, preferably high throughput assays, to identify compounds or other molecules that interact with and/or modulate MIPS. In certain assays, a particular domain or fragment of MIPS is used.

The present invention also provides methods to treat diseases or conditions associated with estrogen receptor α-mediated estrogen signaling. For example, MIPS activity and/or expression can be altered in cells of a patient to treat or prevent diseases and conditions including, but not limited to, atherosclerosis, osteoporosis, Alzheimer Disease, Parkinson's Disease, and breast cancer.

Transgenic animals and cells lacking one or more MIPS alleles, or containing altered forms of MIPS are also provided, as are kits for using the herein-disclosed polynucleotides and polypeptides and for practicing the herein-disclosed methods.

II. Definitions

As used herein, the following terms have the meanings ascribed to them below unless specified otherwise.

As used herein, “MIPS” refers to a myo-inositol phosphate synthase as shown in SEQ ID NO:1 or 3, or any derivative, homolog, or fragment thereof, or to any nucleic acid encoding such a protein, derivative, homolog, or fragment thereof. MIPS proteins or derivatives can be expressed in any cell type, including any eukaryotic or prokaryotic cell, or synthesized in vitro. Typically, MIPS nucleic acids encode active myo-inositol phosphate synthases that are capable of catalyzing the primary reaction in the synthesis of inositol. It will be recognized that derivatives, homologs, and fragments of MIPS can readily be used in the present invention. Such MIPS variants can comprise any one or more domains of the polypeptide shown as SEQ ID NO:1 or 3, or multiple copies of any one or more domains, or any number of domains in novel combinations with each other or with other proteins or protein domains. Figure 4 illustrates the domain structure of MIPS.

The term “MIPS” also refers to polymorphic variants, alleles, mutants, and interspecies homologs that: (1) have about 60% amino acid sequence identity, optionally about 75, 80, 85, 90, or 95% amino acid sequence identity to SEQ ID NO:1 or 3 over a window of about 25 amino acids, optionally 50-100 amino acids; (2) specifically bind to antibodies raised against an immunogen comprising an amino acid sequence of SEQ ID NO:1 or 3, and conservatively modified variants thereof; or (3) specifically hybridize
(with a size of at least about 100, optionally at least about 500-1000 nucleotides) under stringent hybridization conditions to a sequence of SEQ ID NO:2 or 4, and conservatively modified variants thereof.

“mMIPS” refers to estrogen receptor α-mediated estrogen regulated MIPS proteins and nucleic acids that are derived from mouse. mMIPS polypeptides and polynucleotides are typically at least about 70%, 80%, 90%, or 95% similar or identical to the sequence shown as SEQ ID NO:1 or SEQ ID NO:2, respectively, or to any derivative, fragment, variant of SEQ ID NO:1 or SEQ ID NO:2.

“Biological sample,” as used herein, refers to a sample of biological tissue or fluid that contains one or more MIPS nucleic acids encoding one or more MIPS proteins. Biological samples may also include sections of tissues such as frozen sections taken for histological purposes. A biological sample is typically obtained from a eukaryotic organism, such as insects, protozoa, birds, fish, reptiles, and preferably a mammal such as rat, mouse, cow, dog, guinea pig, or rabbit, and most preferably a primate such as a chimpanzee or a human.

By “determining the functional effect” is meant assaying for a compound that modulates, e.g., increases or decreases, a parameter that is indirectly or directly under the influence of a MIPS polypeptide, e.g., functional, physical and chemical effects. Such functional effects can be measured by any means known to those skilled in the art, e.g., changes in spectroscopic characteristics (e.g., fluorescence, absorbance, refractive index), hydrodynamic (e.g., shape), chromatographic, or solubility properties, changes in gene expression of MIPS or of any marker genes indicative of MIPS activity, changes in MIPS enzyme activity, and the like.

“Inhibitors,” “activators,” and “modulators” of MIPS genes or proteins are used to refer to inhibitory, activating, or modulating molecules identified using in vitro and in vivo assays for MIPS. Inhibitors are compounds that, e.g., bind to MIPS proteins, partially or totally block MIPS activity, downregulate MIPS expression or stability, or prevent MIPS binding to heterologous molecules. Activators are compounds that, e.g., bind to MIPS, stimulate MIPS activity, increase MIPS expression or stability, or facilitate MIPS binding to any other protein or factor. Modulators may include genetically modified versions of MIPS proteins, e.g., dominant negative or activated forms of MIPS. Such assays for inhibitors and activators are described below and include, e.g., expressing MIPS proteins in cells, applying putative modulator compounds, and then determining the
functional effects on MIPS activity. Samples or assays comprising MIPS polypeptides that are treated with a potential activator, inhibitor, or modulator are compared to control samples without the inhibitor, activator, or modulator to examine the effect of the candidate compound. Control samples (untreated with the compound) are assigned a relative MIPS activity value of 100%. Inhibition of a MIPS polypeptide is achieved when the activity value relative to the control is about 80%, optionally 50% or 25-0%.

Activation of a MIPS polypeptide is achieved when the activity value relative to the control is 110%, optionally 150%, optionally 200-500%, or 1000-3000% higher.

The terms "isolated" "purified" or "biologically pure" refer to material that is substantially or essentially free from components which normally accompany it as found in its native state. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein that is the predominant species present in a preparation is substantially purified. In particular, an isolated MIPS nucleic acid is separated from open reading frames that flank the MIPS gene and encode proteins other than MIPS. The term "purified" denotes that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. Particularly, it means that the nucleic acid or protein is at least 85% pure, optionally at least 95% pure, and optionally at least 99% pure.

"Nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs).

Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzner et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); Rossolini et al., Mol. Cell. Probes
The term nucleic acid is used interchangeably with gene, cDNA, mRNA, oligonucleotide, and polynucleotide. The terms “polypeptide,” “peptide” and “protein” are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer.

The term “amino acid” refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

“Conservatively modified variants” applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refer to those nucleic acids which encode identical or essentially identical amino acid sequences, or, where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids can encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are “silent variations,” which are one species of
conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence.

As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

The following eight groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Glycine (G);
2) Aspartic acid (D), Glutamic acid (E);
3) Asparagine (N), Glutamine (Q);
4) Arginine (R), Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);
6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);
7) Serine (S), Threonine (T); and
8) Cysteine (C), Methionine (M)
(see, e.g., Creighton, Proteins (1984)).

Macromolecular structures such as polypeptide structures can be described in terms of various levels of organization. For a general discussion of this organization, see, e.g., Alberts et al., Molecular Biology of the Cell (3rd ed., 1994) and Cantor and Schimmel, Biophysical Chemistry Part I: The Conformation of Biological Macromolecules (1980). "Primary structure" refers to the amino acid sequence of a particular peptide. "Secondary structure" refers to locally ordered, three dimensional structures within a polypeptide. These structures are commonly known as domains. Domains are portions of a polypeptide that form a compact unit of the polypeptide and
are typically 50 to 350 amino acids long. Typical domains are made up of sections of lesser organization such as stretches of β-sheet and α-helices. “Tertiary structure” refers to the complete three dimensional structure of a polypeptide monomer. “Quaternary structure” refers to the three dimensional structure formed by the noncovalent association of independent tertiary units. Anisotropic terms are also known as energy terms.

A “label” or a “detectable moiety” is a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include ^{32}\text{P}, fluorescent dyes, electron-dense reagents, enzymes (e.g., as commonly used in an ELISA), biotin, digoxigenin, or haptons and proteins which can be made detectable, e.g., by incorporating a radiolabel into the peptide or used to detect antibodies specifically reactive with the peptide.

A “labeled nucleic acid probe or oligonucleotide” is one that is bound, either covalently, through a linker or a chemical bond, or noncovalently, through ionic, van der Waals, electrostatic, or hydrogen bonds to a label such that the presence of the probe may be detected by detecting the presence of the label bound to the probe.

As used herein a “nucleic acid probe or oligonucleotide” is defined as a nucleic acid capable of binding to a target nucleic acid of complementary sequence through one or more types of chemical bonds, usually through complementary base pairing, usually through hydrogen bond formation. As used herein, a probe may include natural (i.e., A, G, C, or T) or modified bases (7-deazaguanosine, inosine, etc.). In addition, the bases in a probe may be joined by a linkage other than a phosphodiester bond, so long as it does not interfere with hybridization. Thus, for example, probes may be peptide nucleic acids in which the constituent bases are joined by peptide bonds rather than phosphodiester linkages. It will be understood by one of skill in the art that probes may bind target sequences lacking complete complementarity with the probe sequence depending upon the stringency of the hybridization conditions. The probes are optionally directly labeled as with isotopes, chromophores, lumiphores, chromogens, or indirectly labeled such as with biotin to which a streptavidin complex may later bind. By assaying for the presence or absence of the probe, one can detect the presence or absence of the select sequence or subsequence.

The term “recombinant” when used with reference, e.g., to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the
alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (nonrecombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all.

The term “heterologous” when used with reference to portions of a nucleic acid indicates that the nucleic acid comprises two or more subsequences that are not found in the same relationship to each other in nature. For instance, the nucleic acid is typically recombinantly produced, having two or more sequences from unrelated genes arranged to make a new functional nucleic acid, e.g., a promoter from one source and a coding region from another source. Similarly, a heterologous protein indicates that the protein comprises two or more subsequences that are not found in the same relationship to each other in nature (e.g., a fusion protein).

A “promoter” is defined as an array of nucleic acid control sequences that direct transcription of a nucleic acid. As used herein, a promoter includes necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription. A “constitutive” promoter is a promoter that is active under most environmental and developmental conditions. An “inducible” promoter is a promoter that is active under environmental or developmental regulation. The term “operably linked” refers to a functional linkage between a nucleic acid expression control sequence (such as a promoter, or array of transcription factor binding sites) and a second nucleic acid sequence, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence.

An “expression vector” is a nucleic acid construct, generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular nucleic acid in a host cell. The expression vector can be part of a plasmid, virus, or nucleic acid fragment. Typically, the expression vector includes a nucleic acid to be transcribed operably linked to a promoter.

The terms “identical” or percent “identity,” in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (i.e., 60% identity, optionally 65%, 70%, 75%, 80%, 85%, 90%, or 95% identity over a specified region), when compared and aligned for maximum
correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Such sequences are then said to be “substantially identical.” This definition also refers to the compliment of a test sequence. Optionally, the identity exists over a region that is at least about 50 amino acids or nucleotides in length, or more preferably over a region that is 75-100 amino acids or nucleotides in length.

The term “similarity,” or percent “similarity,” in the context of two or more polypeptide sequences, refer to two or more sequences or subsequences that have a specified percentage of amino acid residues that are either the same or similar as defined in the 8 conservative amino acid substitutions defined above (i.e., 60%, optionally 65%, 70%, 75%, 80%, 85%, 90%, or 95% similar over a specified region), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Such sequences are then said to be “substantially similar.” Optionally, this similarity exists over a region that is at least about 50 amino acids in length, or more preferably over a region that is at least about 75-100 amino acids in length.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

A “comparison window,” as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for

One example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments to show relationship and percent sequence identity. It also plots a tree or dendogram showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng & Doolittle, *J. Mol. Evol.* 35:351-360 (1987). The method used is similar to the method described by Higgins & Sharp, *CABIOS* 5:151-153 (1989). The program can align up to 300 sequences, each of a maximum length of 5,000 nucleotides or amino acids. The multiple alignment procedure begins with the pairwise alignment of the two most similar sequences, producing a cluster of two aligned sequences. This cluster is then aligned to the next most related sequence or cluster of aligned sequences. Two clusters of sequences are aligned by a simple extension of the pairwise alignment of two individual sequences. The final alignment is achieved by a series of progressive, pairwise alignments. The program is run by designating specific sequences and their amino acid or nucleotide coordinates for regions of sequence comparison and by designating the program parameters. Using PILEUP, a reference sequence is compared to other test sequences to determine the percent sequence identity relationship using the following parameters: default gap weight (3.00), default gap length weight (0.10), and weighted end gaps. PILEUP can be obtained from the GCG sequence analysis software package, *e.g.*, version 7.0 (Devereaux *et al.*, *Nuc. Acids Res.* 12:387-395 (1984)).

Additional examples of algorithms that is suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.*, *Nuc. Acids Res.* 25:3389-3402 (1977) and Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990), respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood
word score threshold (Altschul et al., supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always \( > 0 \)) and N (penalty score for mismatching residues; always \( < 0 \)). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity \( X \) from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters \( W, T \), and \( X \) determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength \( (W) \) of 11, an expectation \( (E) \) or 10, \( M=5, N=-4 \) and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation \( (E) \) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1989)) alignments \( (B) \) of 50, expectation \( (E) \) of 10, \( M=5, N=-4 \), and a comparison of both strands.

The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, Proc. Natl. Acad. Sci. USA 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability \( (P(N)) \), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

An indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the antibodies raised against the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules or their complements hybridize to each other under stringent conditions, as described below. Yet another indication that
two nucleic acid sequences are substantially identical is that the same primers can be used
to amplify the sequence.

The phrase “selectively (or specifically) hybridizes to” refers to the
binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence
under stringent hybridization conditions when that sequence is present in a complex
mixture (e.g., total cellular or library DNA or RNA).

The phrase “stringent hybridization conditions” refers to conditions under
which a probe will hybridize to its target subsequence, typically in a complex mixture of
nucleic acid, but to no other sequences. Stringent conditions are sequence-dependent and
will be different in different circumstances. Longer sequences hybridize specifically at
higher temperatures. An extensive guide to the hybridization of nucleic acids is found in
Tijssen, *Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic
Probes*, “Overview of principles of hybridization and the strategy of nucleic acid assays”
(1993). Generally, stringent conditions are selected to be about 5-10°C lower than the
thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The
T_m is the temperature (under defined ionic strength, pH, and nucleic concentration) at
which 50% of the probes complementary to the target hybridize to the target sequence at
equilibrium (as the target sequences are present in excess, at T_m 50% of the probes are
occupied at equilibrium). Stringent conditions will be those in which the salt
concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium
ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C
for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes
(e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the
addition of destabilizing agents such as formamide. For selective or specific
hybridization, a positive signal is at least two times background, optionally 10 times
background hybridization. Exemplary stringent hybridization conditions can be as
following: 50% formamide, 5x SSC, and 1% SDS, incubating at 42°C, or, 5x SSC, 1%
SDS, incubating at 65°C, with wash in 0.2x SSC, and 0.1% SDS at 65°C. Such washes
can be performed for 5, 15, 30, 60, 120, or more minutes.

Nucleic acids that do not hybridize to each other under stringent conditions
are still substantially identical if the polypeptides which they encode are substantially
identical. This occurs, for example, when a copy of a nucleic acid is created using the
maximum codon degeneracy permitted by the genetic code. In such cases, the nucleic
acids typically hybridize under moderately stringent hybridization conditions. Exemplary
"moderately stringent hybridization conditions" include a hybridization in a buffer of 40% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 1X SSC at 45°C. Such washes can be performed for 5, 15, 30, 60, 120, or more minutes. A positive hybridization is at least twice background. Those of ordinary skill will readily recognize that alternative hybridization and wash conditions can be utilized to provide conditions of similar stringency.

"Antibody" refers to a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively.

Antibodies exist, e.g., as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce F(ab)'2, a dimer of Fab which itself is a light chain joined to V_H-C_H1 by a disulfide bond. The F(ab)'2 may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the F(ab)'2 dimer into an Fab' monomer. The Fab' monomer is essentially Fab with part of the hinge region (see Fundamental Immunology (Paul ed., 3d ed. 1993)). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized de novo either chemically or by using recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized de novo using recombinant DNA methodologies (e.g., single chain Fv) or those identified using phage display libraries (see, e.g., McCafferty et al., Nature 348:552-554 (1990)).
For preparation of monoclonal or polyclonal antibodies, any technique known in the art can be used (see, e.g., Kohler & Milstein, Nature 256:495-497 (1975); Kozbor et al., Immunology Today 4: 72 (1983); Cole et al., pp. 77-96 in Monoclonal Antibodies and Cancer Therapy (1985)). Techniques for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized antibodies. Alternatively, phage display technology can be used to identify antibodies and heteromeric Fab fragments that specifically bind to selected antigens (see, e.g., McCafferty et al., Nature 348:552-554 (1990); Marks et al., Biotechnology 10:779-783 (1992)).

A “chimeric antibody” is an antibody molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, e.g., an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity.

An “anti-MIPS” antibody is an antibody or antibody fragment that specifically binds a polypeptide encoded by a MIPS gene, cDNA, or a subsequence thereof.

The term “immunoassay” is an assay that uses an antibody to specifically bind an antigen. The immunoassay is characterized by the use of specific binding properties of a particular antibody to isolate, target, and/or quantify the antigen.

The phrase “specifically (or selectively) binds to an antibody or “specifically (or selectively) immunoreactive with,” when referring to a protein or peptide, refers to a binding reaction that is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times the background and do not substantially bind in a significant amount to other proteins present in the sample. Specific binding to an antibody under such conditions may require an antibody that is selected for its specificity for a particular protein. For example, polyclonal antibodies raised to a MIPS polypeptide from specific species such as rat, mouse, or human can be selected to obtain only those polyclonal antibodies that are specifically immunoreactive with the MIPS protein and not with other proteins, except for.
polymorphic variants and alleles of the MIPS protein. This selection may be achieved by subtracting out antibodies that cross-react with MIPS molecules from other species. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (see, e.g., Harlow & Lane, Antibodies, A Laboratory Manual (1988), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity). Typically a specific or selective reaction will be at least twice background signal or noise and more typically more than 10 to 100 times background.

The phrase “selectively associates with” refers to the ability of a nucleic acid to “selectively hybridize” with another as defined above, or the ability of an antibody to “selectively (or specifically) bind to a protein, as defined above.

By “host cell” is meant a cell that contains an expression vector and supports the replication or expression of the expression vector. Host cells may be prokaryotic cells such as E. coli, or eukaryotic cells such as yeast, insect, amphibian, or mammalian cells such as CHO, HeLa and the like, e.g., cultured cells, explants, and cells in vivo.

III. Manipulation and detection of MIPS nucleic acids

In numerous embodiments of the present invention, nucleic acids encoding a MIPS polypeptide, e.g., mMIPS, including a full-length MIPS protein, or any derivative, variant, homolog, or fragment thereof, will be used. Such nucleic acids are useful for any of a number of applications, including for the production of MIPS protein, for diagnostic assays, for therapeutic applications, for the preparation of MIPS-specific probes, for assays for MIPS binding and/or modulating compounds, to identify and/or isolate MIPS homologs from other species, and other applications.

A. General recombinant DNA methods

Numerous applications of the present invention involve the cloning, synthesis, maintenance, mutagenesis, and other manipulations of nucleic acid sequences that can be performed using routine techniques in the field of recombinant genetics. Basic texts disclosing the general methods of use in this invention include Sambrook et al., Molecular Cloning. A Laboratory Manual (2nd ed. 1989); Kriegler, Gene Transfer and Expression: A Laboratory Manual (1990); and Current Protocols in Molecular Biology (Ausubel et al., eds., 1994)).
For nucleic acids, sizes are given in either kilobases (kb) or base pairs (bp). These are estimates derived from agarose or acrylamide gel electrophoresis, from sequenced nucleic acids, or from published DNA sequences. For proteins, sizes are given in kilodaltons (kDa) or amino acid residue numbers. Protein sizes are estimated from gel electrophoresis, from sequenced proteins, from derived amino acid sequences, or from published protein sequences.


The sequence of the cloned genes and synthetic oligonucleotides can be verified after cloning using, *e.g.*, the chain termination method for sequencing double-stranded templates of Wallace et al., *Gene* 16:21-26 (1981).

### B. Isolating and Detecting MIPS nucleotide sequences

In numerous embodiments of the present invention, MIPS nucleic acids, *e.g.*, mMIPS RNA or DNA, will be isolated and cloned using recombinant methods. Such embodiments are used, *e.g.*, to isolate MIPS polynucleotides for protein expression or during the generation of variants, derivatitives, expression cassettes, or other sequences derived from MIPS, to monitor MIPS gene expression, for the isolation or detection of MIPS sequences in different species, for diagnostic purposes in a patient, *i.e.*, to detect mutations in MIPS, or for genotyping and/or forensic applications.

Often, the nucleic acid sequences encoding MIPS proteins and related nucleic acid sequence homologs are cloned from cDNA and genomic DNA libraries by hybridization with probes, or isolated using amplification techniques with oligonucleotide primers. For example, MIPS sequences are typically isolated from mammalian nucleic acid (genomic or cDNA) libraries by hybridizing with a nucleic acid probe, the sequence of which can be derived from SEQ ID NO: 2 or 4, or can be amplified using primers derived from SEQ ID NO: 2 or 4.

Amplification techniques using primers can also be used to amplify and isolate MIPS sequences from DNA or RNA (*see, e.g.*, Dieffenbach & Dveksler, *PCR*...
Primer: A Laboratory Manual (1995)). Primers can be used, e.g., to amplify either the full length sequence or a probe of from one to several hundred nucleotides, which is then used to screen a mammalian library for full-length MIPS clones.

Nucleic acids encoding MIPS polypeptides can also be isolated from expression libraries using antibodies as probes. Such polyclonal or monoclonal antibodies can be raised using the sequence of SEQ ID NO:1 or 3, or derivatives or fragments thereof.

Polymorphic variants, alleles, and interspecies homologs that are substantially identical to a MIPS gene can be isolated using MIPS nucleic acid probes, and oligonucleotides under stringent hybridization conditions, by screening libraries. Alternatively, expression libraries can be used to clone MIPS polymorphic variants, alleles, and interspecies homologs, by detecting expressed homologs immunologically with antisera or purified antibodies made against a MIPS polypeptide, which also recognize and selectively bind to the MIPS homolog.

More distantly related MIPS homologs can be identified using any of a number of well known techniques, including by hybridizing a MIPS probe with a genomic or cDNA library using moderately stringent conditions, or under low stringency conditions. Also, a distant homolog can be amplified from a nucleic acid library using degenerate primer sets, i.e., primers that incorporate all possible codons encoding a given amino acid sequence, in particular based on a highly conserved amino acid stretch. Such primers are well known by those of skill, and numerous programs are available, e.g., on the Internet, for degenerate primer design.

To make a cDNA library, one should choose a source that is rich in MIPS mRNA, e.g., the liver of estrogen-treated male animals. The mRNA is then made into cDNA using reverse transcriptase, ligated into a recombinant vector, and transfected into a recombinant host for propagation, screening and cloning. Methods for making and screening cDNA libraries are well known (see, e.g., Gubler & Hoffman, Gene 25:263-269 (1983); Sambrook et al., supra; Ausubel et al., supra).

For a genomic library, the DNA is extracted from the tissue or cells and either mechanically sheared or enzymatically digested to yield fragments of about 12-20 kb. The fragments are then separated by gradient centrifugation from undesired sizes and are constructed in bacteriophage lambda vectors. These vectors and phage are packaged in vitro. Recombinant phage are analyzed by plaque hybridization as described in Benton

An alternative method of isolating MIPS nucleic acids and homologs combines the use of synthetic oligonucleotide primers and amplification of an RNA or DNA template (see, U.S. Patent Nos. 4,683,195 and 4,683,202; *PCR Protocols: A Guide to Methods and Applications* (Innis *et al.*, eds, 1990)). Methods such as polymerase chain reaction (PCR) and ligase chain reaction (LCR) can be used to amplify nucleic acid sequences of MIPS genes directly from mRNA, from cDNA, from genomic libraries or cDNA libraries. Degenerate oligonucleotides can be designed to amplify MIPS homologs using the sequences provided herein. Restriction endonuclease sites can be incorporated into the primers. Polymerase chain reaction or other *in vitro* amplification methods may also be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of MIPS-encoding mRNA in physiological samples, for nucleic acid sequencing, or for other purposes. Genes amplified by the PCR reaction can be purified from agarose gels and cloned into an appropriate vector.

Synthetic oligonucleotides can be used to construct recombinant MIPS genes for use as probes or for expression of protein. This method is performed using a series of overlapping oligonucleotides usually 40-120 bp in length, representing both the sense and non-sense strands of the gene. These DNA fragments are then annealed, ligated and cloned. Alternatively, amplification techniques can be used with precise primers to amplify a specific subsequence of the MIPS nucleic acid. The specific subsequence is then ligated into an expression vector.

The nucleic acid encoding a MIPS polypeptide is typically cloned into intermediate vectors before transformation into prokaryotic or eukaryotic cells for replication and/or expression. These intermediate vectors are typically prokaryote vectors, *e.g.*, plasmids, or shuttle vectors. Vectors, cells, and transfection methods are well known to those of skill and are described, *e.g.*, in Ausubel or in Sambrook, both *supra*.

Optionally, nucleic acids will be used that encode chimeric proteins comprising a MIPS polypeptide, or one or more domain thereof, in combination with a heterologous polypeptide or polypeptides. For example, a MIPS polypeptide, or fragment
thereof, can be covalently linked to a heterologous protein such as luciferase, green fluorescent protein (GFP), and β-gal, each of which is well known in the art.

In certain embodiments, MIPS polynucleotides will be detected using hybridization-based methods to determine, e.g., MIPS RNA levels or to detect particular DNA sequences, e.g., for genotyping or for forensic applications. For example, gene expression of MIPS can be analyzed by techniques known in the art, e.g., Northern blotting, reverse transcription and amplification of mRNA, dot blotting, in situ hybridization, RNase protection, probing DNA microchip arrays, and the like. In one embodiment, high density oligonucleotide analysis technology (e.g., GeneChip™) is used to identify homologs and polymorphic variants of MIPS, or to monitor levels of MIPS mRNA. In the case where a homolog is linked to a known disease, they can be used with GeneChip™ as a diagnostic tool in detecting the disease in a biological sample, see, e.g., Gunthand et al., AIDS Res. Hum. Retroviruses 14: 869-876 (1998); Kozal et al., Nat. Med. 2:753-759 (1996); Matson et al., Anal. Biochem. 224:110-106 (1995); Lockhart et al., Nat. Biotechnol. 14:1675-1680 (1996); Gingeras et al., Genome Res. 8:435-448 (1998); Hacia et al., Nucleic Acids Res. 26:3865-3866 (1998).

In certain applications, a MIPS DNA sequence will be detected, e.g., for diagnostic or forensic applications. For example, a MIPS allele can be detected in a mammal using Southern blot hybridization, i.e., by isolating genomic DNA, performing a restriction digest on the isolated DNA, separating the restriction fragments electrophoretically, e.g., in an agarose gel, and transferring the separated DNA to a membrane and probing with a specific, labeled sequence. Southern blotting is well known to those of skill, and is taught in numerous sources, including Ausubel et al. and Sambrook et al.

In other embodiments, e.g., to detect tissue specific or temporal patterns of gene expression, for example to monitor estrogen receptor α-mediated estrogen signaling, a MIPS polynucleotide is detected using in situ hybridization. In in situ hybridization, the target nucleic acid is liberated from its cellular surroundings in such as to be available for hybridization within the cell while preserving the cellular morphology for subsequent interpretation and analysis. The following articles provide an overview of the art of in situ hybridization: Singer et al., Biotechniques 4:230-250 (1986); Haase et al., Methods in Virology, vol. VII, pp. 189-226 (1984); and Nucleic Acid Hybridization: A Practical Approach (Hames et al., eds. 1987).
C. Expression in prokaryotes and eukaryotes

Often, a cloned MIPS sequence will be expressed in a prokaryotic or
eukaryotic cell to obtain expression, i.e., production of the encoded mRNA or protein.
For example, in numerous embodiments, a MIPS polynucleotide will be introduced into a
cell to modulate the level of MIPS activity in the cell, and thereby to modulate the effects
of estrogen receptor α-mediated estrogen signaling within the cell. Such embodiments
are useful, e.g., in the treatment of any of a variety of estrogen-related diseases and
conditions. To obtain high level expression of a cloned gene or nucleic acid, such as a
cDNA encoding a MIPS polypeptide, a MIPS sequence is typically subcloned into an
expression vector that contains a strong promoter to direct transcription, a
transcription/translation terminator, and if for a nucleic acid encoding a protein, a
ribosome binding site for translational initiation. In certain embodiments, a MIPS
promoter is used to direct the expression of, e.g., a MIPS coding sequence. See, e.g.,
SEQ ID NO:5. Suitable bacterial promoters are well known in the art and are described,
e.g., in Sambrook et al. and Ausubel et al. Bacterial expression systems for expressing
the MIPS protein are available in, e.g., E. coli, Bacillus sp., and Salmonella (Palva et al.,
expression systems are commercially available. Eukaryotic expression systems for
mammalian cells, yeast, and insect cells are well known in the art and are also
commercially available. In one embodiment, the eukaryotic expression vector is an
adenoviral vector, an adeno-associated vector, or a retroviral vector.

For therapeutic applications, MIPS nucleic acids are introduced into a cell,
in vitro, in vivo, or ex vivo, using any of a large number of methods including, but not
limited to, infection with viral vectors, liposome-based methods, biolistic particle
acceleration (the gene gun), and naked DNA injection. Such therapeutically useful
nucleic acids include, but are not limited to, coding sequences for full-length MIPS,
coding sequences for a MIPS fragment, domain, derivative, or variant, MIPS antisense
sequences, and MIPS ribozymes. Typically, such sequences will be operably linked to a
promoter (e.g., a MIPS promoter as shown in SEQ ID NO:5), but in numerous
applications a nucleic acid will be administered to a cell that is itself directly
therapeutically effective, e.g., certain antisense or ribozyme molecules.

The promoter used to direct expression of a heterologous nucleic acid
depends on the particular application. The promoter is optionally positioned about the
same distance from the heterologous transcription start site as it is from the transcription start site in its natural setting. As is known in the art, however, some variation in this distance can be accommodated without loss of promoter function.

In addition to the promoter, the expression vector typically contains a transcription unit or expression cassette that contains all the additional elements required for the expression of the MIPS-encoding nucleic acid in host cells. A typical expression cassette thus contains a promoter operably linked to the nucleic acid sequence encoding a MIPS polypeptide, and signals required for efficient polyadenylation of the transcript, ribosome binding sites, and translation termination. The nucleic acid sequence encoding a MIPS polypeptide may be linked to a cleavable signal peptide sequence to promote secretion of the encoded protein by the transfected cell. Such signal peptides would include, among others, the signal peptides from tissue plasminogen activator, insulin, and neuron growth factor, and juvenile hormone esterase of *Heliothis virescens*. Additional elements of the cassette may include enhancers and, if genomic DNA is used as the structural gene, introns with functional splice donor and acceptor sites.

In addition to a promoter sequence, the expression cassette should also contain a transcription termination region downstream of the structural gene to provide for efficient termination. The termination region may be obtained from the same gene as the promoter sequence or may be obtained from different genes.

The particular expression vector used to transport the genetic information into the cell is not particularly critical. Any of the conventional vectors used for expression in eukaryotic or prokaryotic cells may be used. Standard bacterial expression vectors include plasmids such as pBR322 based plasmids, pSKF, pET23D, and fusion expression systems such as GST and LacZ. Epitope tags can also be added to recombinant proteins to provide convenient methods of isolation, e.g., c-myc, HA-tag, 6-His tag, maltose binding protein, VSV-G tag, anti-DYKDDDDK tag, or any such tag, a large number of which are well known to those of skill in the art.

Expression vectors containing regulatory elements from eukaryotic viruses are typically used in eukaryotic expression vectors, e.g., SV40 vectors, papilloma virus vectors, and vectors derived from Epstein-Barr virus. Other exemplary eukaryotic vectors include pMSG, pAV009/A¹, pMTO10/A¹, pMAMneo-5, baculovirus pDSVE, and any other vector allowing expression of proteins under the direction of the CMV promoter, SV40 early promoter, SV40 later promoter, metallothionein promoter, murine
Missing at the time of publication
After the expression vector is introduced into the cells, the transfected cells are cultured under conditions favoring expression of the MIPS polypeptide, which is recovered from the culture using standard techniques identified below. Methods of culturing prokaryotic or eukaryotic cells are well known and are taught, e.g., in Ausubel et al., Sambrook et al., and in Freshney, Culture of Animal Cells, 3d Ed., (1993), A Wiley-Liss Publication.

IV. **Purification of MIPS polypeptides**

Either naturally occurring or recombinant MIPS polypeptides can be purified for use in functional assays, binding assays, diagnostic assays, and other applications. Optionally, recombinant MIPS polypeptides are purified. Naturally occurring MIPS polypeptides are purified, e.g., from mammalian tissue such as the liver, or any other source of a MIPS homolog. Recombinant MIPS polypeptides are purified from any suitable bacterial or eukaryotic expression system, e.g., CHO cells or insect cells.

MIPS proteins may be purified to substantial purity by standard techniques, including selective precipitation with such substances as ammonium sulfate; column chromatography, immunopurification methods, and others (see, e.g., Scopes, Protein Purification: Principles and Practice (1982); U.S. Patent No. 4,673,641; Ausubel et al., supra; and Sambrook et al., supra).

A number of procedures can be employed when recombinant MIPS polypeptide is being purified. For example, proteins having established molecular adhesion properties can be reversibly fused to the MIPS polypeptide. With the appropriate ligand, a MIPS polypeptide can be selectively adsorbed to a purification column and then freed from the column in a relatively pure form. The fused protein is then removed by enzymatic activity. MIPS proteins can also be purified using immunoaffinity columns.

A. **Purification of MIPS Protein from Recombinant Cells**

Recombinant proteins are expressed by transformed bacteria or eukaryotic cells such as CHO cells or insect cells in large amounts, typically after promoter induction; but expression can be constitutive. Promoter induction with IPTG is one example of an inducible promoter system. Cells are grown according to standard procedures in the art. Fresh or frozen cells are used for isolation of protein.
Proteins expressed in bacteria may form insoluble aggregates ("inclusion bodies"). Several protocols are suitable for purification of MIPS inclusion bodies. For example, purification of inclusion bodies typically involves the extraction, separation and/or purification of inclusion bodies by disruption of bacterial cells, e.g., by incubation in a buffer of 50 mM TRIS/HCL pH 7.5, 50 mM NaCl, 5 mM MgCl₂, 1 mM DTT, 0.1 mM ATP, and 1 mM PMSF. The cell suspension can be lysed using 2-3 passages through a French Press, homogenized using a Polytron (Brinkman Instruments) or sonicated on ice. Alternate methods of lysing bacteria are apparent to those of skill in the art (see, e.g., Sambrook et al., supra; Ausubel et al., supra).

If necessary, the inclusion bodies are solubilized, and the lysed cell suspension is typically centrifuged to remove unwanted insoluble matter. Proteins that formed the inclusion bodies may be renatured by dilution or dialysis with a compatible buffer. Suitable solvents include, but are not limited to, urea (from about 4 M to about 8 M), formamide (at least about 80%, volume/volume basis), and guanidine hydrochloride (from about 4 M to about 8 M). Some solvents which are capable of solubilizing aggregate-forming proteins, for example SDS (sodium dodecyl sulfate) and 70% formic acid, are inappropriate for use in this procedure due to the possibility of irreversible denaturation of the proteins, accompanied by a lack of immunogenicity and/or activity. Although guanidine hydrochloride and similar agents are denaturants, this denaturation is not irreversible and renaturation may occur upon removal (by dialysis, for example) or dilution of the denaturant, allowing re-formation of immunologically and/or biologically active protein. Other suitable buffers are known to those skilled in the art. MIPS polypeptides are separated from other bacterial proteins by standard separation techniques, e.g., with Ni-NTA agarose resin.

Alternatively, it is possible to purify MIPS polypeptides from bacteria periplasm. After lysis of the bacteria, when a MIPS protein is exported into the periplasm of the bacteria, the periplasmic fraction of the bacteria can be isolated by cold osmotic shock in addition to other methods known to skill in the art. To isolate recombinant proteins from the periplasm, the bacterial cells are centrifuged to form a pellet. The pellet is resuspended in a buffer containing 20% sucrose. To lyse the cells, the bacteria are centrifuged and the pellet is resuspended in ice-cold 5 mM MgSO₄ and kept in an ice bath for approximately 10 minutes. The cell suspension is centrifuged and the supernatant decanted and saved. The recombinant proteins present in the supernatant can be
separated from the host proteins by standard separation techniques well known to those of skill in the art.

B. Standard protein separation techniques for purifying MIPS polypeptides

1. Solubility fractionation

Often as an initial step, particularly if the protein mixture is complex, an initial salt fractionation can separate many of the unwanted host cell proteins (or proteins derived from the cell culture media) from the recombinant protein of interest. The preferred salt is ammonium sulfate. Ammonium sulfate precipitates proteins by effectively reducing the amount of water in the protein mixture. Proteins then precipitate on the basis of their solubility. The more hydrophobic a protein is, the more likely it is to precipitate at lower ammonium sulfate concentrations. A typical protocol includes adding saturated ammonium sulfate to a protein solution so that the resultant ammonium sulfate concentration is between 20-30%. This concentration will precipitate the most hydrophobic of proteins. The precipitate is then discarded (unless the protein of interest is hydrophobic) and ammonium sulfate is added to the supernatant to a concentration known to precipitate the protein of interest. The precipitate is then solubilized in buffer and the excess salt removed if necessary, either through dialysis or diafiltration. Other methods that rely on solubility of proteins, such as cold ethanol precipitation, are well known to those of skill in the art and can be used to fractionate complex protein mixtures.

2. Size differential filtration

The molecular weight of a MIPS protein can be used to isolate it from proteins of greater and lesser size using ultrafiltration through membranes of different pore size (for example, Amicon or Millipore membranes). As a first step, the protein mixture is ultrafiltered through a membrane with a pore size that has a lower molecular weight cut-off than the molecular weight of the protein of interest. The retentate of the ultrafiltration is then ultrafiltered against a membrane with a molecular cut off greater than the molecular weight of the protein of interest. The recombinant protein will pass through the membrane into the filtrate. The filtrate can then be chromatographed as described below.
3. Column chromatography

MIPS proteins can also be separated from other proteins on the basis of its size, net surface charge, hydrophobicity, and affinity for heterologous molecules. In addition, antibodies raised against proteins can be conjugated to column matrices and the proteins immunopurified. All of these methods are well known in the art. It will be apparent to one of skill that chromatographic techniques can be performed at any scale and using equipment from many different manufacturers (e.g., Pharmacia Biotech).

V. Antibodies to MIPS Proteins

In numerous embodiments of the present invention, antibodies that specifically bind to MIPS polypeptides, e.g., mMIPS polypeptides, are used. Such antibodies have numerous applications, including for the modulation of MIPS activity, and for immunoassays to detect MIPS, and variants, derivatives, fragments, etc. of MIPS. Immunoassays can be used to qualitatively or quantitatively analyze the MIPS polypeptide. A general overview of the applicable technology can be found in Harlow & Lane, Antibodies: A Laboratory Manual (1988).

Methods of producing polyclonal and monoclonal antibodies that react specifically with MIPS polypeptides are known to those of skill in the art (see, e.g., Coligan, Current Protocols in Immunology (1991); Harlow & Lane, supra; Goding, Monoclonal Antibodies: Principles and Practice (2d ed. 1986); and Kohler & Milstein, Nature 256:495-497 (1975)). Such techniques include antibody preparation by selection of antibodies from libraries of recombinant antibodies in phage or similar vectors, as well as preparation of polyclonal and monoclonal antibodies by immunizing rabbits or mice (see, e.g., Huse et al., Science 246:1275-1281 (1989); Ward et al., Nature 341:544-546 (1989)).

A number of MIPS-comprising immunogens may be used to produce antibodies specifically reactive with a MIPS polypeptide. For example, a recombinant MIPS protein, or an antigenic fragment thereof, is isolated as described herein. Recombinant protein can be expressed in eukaryotic or prokaryotic cells as described above, and purified as generally described above. Recombinant protein is the preferred immunogen for the production of monoclonal or polyclonal antibodies. Alternatively, a synthetic peptide derived from the sequences disclosed herein and conjugated to a carrier protein can be used an immunogen. Naturally occurring protein may also be used either in pure or impure form. The product is then injected into an animal capable of producing
antibodies. Either monoclonal or polyclonal antibodies may be generated, for subsequent use in immunoassays to measure the protein.

Methods of production of polyclonal antibodies are known to those of skill in the art. An inbred strain of mice (e.g., BALB/C mice) or rabbits is immunized with the protein using a standard adjuvant, such as Freund’s adjuvant, and a standard immunization protocol. The animal’s immune response to the immunogen preparation is monitored by taking test bleeds and determining the titer of reactivity to the MIPS polypeptide. When appropriately high titers of antibody to the immunogen are obtained, blood is collected from the animal and antisera are prepared. Further fractionation of the antisera to enrich for antibodies reactive to the protein can be done if desired (see, Harlow & Lane, supra).

Monoclonal antibodies may be obtained by various techniques familiar to those skilled in the art. Briefly, spleen cells from an animal immunized with a desired antigen are immortalized, commonly by fusion with a myeloma cell (see Kohler & Milstein, Eur. J. Immunol. 6:511-519 (1976)). Alternative methods of immortalization include transformation with Epstein Barr Virus, oncogenes, or retroviruses, or other methods well known in the art. Colonies arising from single immortalized cells are screened for production of antibodies of the desired specificity and affinity for the antigen, and yield of the monoclonal antibodies produced by such cells may be enhanced by various techniques, including injection into the peritoneal cavity of a vertebrate host. Alternatively, one may isolate DNA sequences which encode a monoclonal antibody or a binding fragment thereof by screening a DNA library from human B cells according to the general protocol outlined by Huse et al., Science 246:1275-1281 (1989).

Monoclonal antibodies and polyclonal sera are collected and titered against the immunogen protein in an immunoassay, for example, a solid phase immunoassay with the immunogen immobilized on a solid support. Typically, polyclonal antisera with a titer of $10^4$ or greater are selected and tested for their cross reactivity against non-MIPS proteins, or even related proteins from other organisms, using a competitive binding immunoassay. Specific polyclonal antisera and monoclonal antibodies will usually bind with a $K_d$ of at least about 0.1 mM, more usually at least about 1 $\mu$M, optionally at least about 0.1 $\mu$M or better, and optionally 0.01 $\mu$M or better.

Using MIPS-specific antibodies, individual MIPS proteins can be detected by a variety of immunoassay methods. For a review of immunological and immunoassay procedures, see Basic and Clinical Immunology (Stites & Terr eds., 7th ed. 1991).
Moreover, the immunoassays of the present invention can be performed in any of several configurations, which are reviewed extensively in *Enzyme Immunoassay* (Maggio, ed., 1980); and Harlow & Lane, *supra*.

A. **Immunological Binding Assays**

MIPS proteins can be detected and/or quantified using any of a number of well recognized immunological binding assays (*see, e.g.*, U.S. Patent Nos. 4,366,241; 4,376,110; 4,517,288; and 4,837,168). For a review of the general immunoassays, see also *Methods in Cell Biology: Antibodies in Cell Biology*, volume 37 (Asai, ed. 1993); *Basic and Clinical Immunology* (Stites & Terr, eds., 7th ed. 1991). Immunological binding assays (or immunoassays) typically use an antibody that specifically binds to a protein or antigen of choice (in this case a MIPS protein or an antigenic subsequence thereof). The antibody (*e.g.*, anti-MIPS) may be produced by any of a number of means well known to those of skill in the art and as described above.

Immunoassays also often use a labeling agent to specifically bind to and label the complex formed by the antibody and antigen. The labeling agent may itself be one of the moieties comprising the antibody/antigen complex. Thus, the labeling agent may be a labeled MIPS polypeptide or a labeled anti-MIPS antibody. Alternatively, the labeling agent may be a third moiety, such a secondary antibody, that specifically binds to the antibody/MIPS complex (a secondary antibody is typically specific to antibodies of the species from which the first antibody is derived). Other proteins capable of specifically binding immunoglobulin constant regions, such as protein A or protein G, may also be used as the label agent. These proteins exhibit a strong nonimmunogenic reactivity with immunoglobulin constant regions from a variety of species (*see, e.g.*, Kronval *et al.*, *J. Immunol.* 111:1401-1406 (1973); Akerstrom *et al.*, *J. Immunol.* 135:2589-2542 (1985)). The labeling agent can be modified with a detectable moiety, such as biotin, to which another molecule can specifically bind, such as streptavidin. A variety of detectable moieties are well known to those skilled in the art.

Throughout the assays, incubation and/or washing steps may be required after each combination of reagents. Incubation steps can vary from about 5 seconds to several hours, optionally from about 5 minutes to about 24 hours. However, the incubation time will depend upon the assay format, antigen, volume of solution, concentrations, and the like. Usually, the assays will be carried out at ambient
temperature, although they can be conducted over a range of temperatures, such as 10°C to 40°C.

1. Noncompetitive assay formats

Immuoassays for detecting a MIPS protein in a sample may be either competitive or noncompetitive. Noncompetitive immunoassays are assays in which the amount of antigen is directly measured. In one preferred “sandwich” assay, for example, the anti-MIPS antibodies can be bound directly to a solid substrate on which they are immobilized. These immobilized antibodies then capture the MIPS protein present in the test sample. The MIPS protein is thus immobilized is then bound by a labeling agent, such as a second MIPS antibody bearing a label. Alternatively, the second antibody may lack a label, but it may, in turn, be bound by a labeled third antibody specific to antibodies of the species from which the second antibody is derived. The second or third antibody is typically modified with a detectable moiety, such as biotin, to which another molecule specifically binds, e.g., streptavidin, to provide a detectable moiety.

2. Competitive assay formats

In competitive assays, the amount of MIPS protein present in the sample is measured indirectly by measuring the amount of a known, added (exogenous) MIPS protein displaced (competed away) from an anti-MIPS antibody by the unknown MIPS protein present in a sample. In one competitive assay, a known amount of MIPS protein is added to a sample and the sample is then contacted with an antibody that specifically binds to the MIPS protein. The amount of exogenous MIPS protein bound to the antibody is inversely proportional to the concentration of MIPS protein present in the sample. In a particularly preferred embodiment, the antibody is immobilized on a solid substrate. The amount of MIPS protein bound to the antibody may be determined either by measuring the amount of MIPS protein present in a MIPS/antibody complex, or alternatively by measuring the amount of remaining uncleaved protein. The amount of MIPS protein may be detected by providing a labeled MIPS molecule.

A hapten inhibition assay is another preferred competitive assay. In this assay the known MIPS protein is immobilized on a solid substrate. A known amount of anti-MIPS antibody is added to the sample, and the sample is then contacted with the immobilized MIPS. The amount of anti-MIPS antibody bound to the known immobilized MIPS protein is inversely proportional to the amount of MIPS protein present in the
sample. Again, the amount of immobilized antibody may be detected by detecting either
the immobilized fraction of antibody or the fraction of the antibody that remains in
solution. Detection may be direct where the antibody is labeled or indirect by the
subsequent addition of a labeled moiety that specifically binds to the antibody as
described above.

3. Cross-reactivity determinations

Immunoassays in the competitive binding format can also be used for
crossreactivity determinations. For example, a protein at least partially encoded by SEQ
ID NO:2 or 4 can be immobilized to a solid support. Proteins (e.g., MIPS proteins and
homologs) are added to the assay that compete for binding of the antisera to the
immobilized antigen. The ability of the added proteins to compete for binding of the
antisera to the immobilized protein is compared to the ability of the MIPS polypeptide
encoded by SEQ ID NO:2 or 4 to compete with itself. The percent cross-reactivity for the
above proteins is calculated, using standard calculations. Those antisera with less than
10% cross-reactivity with each of the added proteins listed above are selected and pooled.
The cross-reacting antibodies are optionally removed from the pooled antisera by
immunoabsorption with the added considered proteins, e.g., distantly related homologs.

The immunoabsorbed and pooled antisera are then used in a competitive
binding immunoassay as described above to compare a second protein, thought to be
perhaps an allele or polymorphic variant of a MIPS protein, to the immunogen protein
(i.e., MIPS protein encoded by SEQ ID NO:2 or 4). In order to make this comparison, the
two proteins are each assayed at a wide range of concentrations and the amount of each
protein required to inhibit 50% of the binding of the antisera to the immobilized protein is
determined. If the amount of the second protein required to inhibit 50% of binding is less
than 10 times the amount of the protein encoded by SEQ ID NO:2 or 4 that is required to
inhibit 50% of binding, then the second protein is said to specifically bind to the
polyclonal antibodies generated to a MIPS immunogen.

Polyclonal antibodies that specifically bind to a MIPS protein from a
particular species can be made by subtracting out cross-reactive antibodies using MIPS
homologs. For example, antibodies specific to mouse MIPS (SEQ ID NO: 1) can be
made by subtracting out antibodies that are cross-reactive with human MIPS (SEQ ID
NO: 3). In an analogous fashion, antibodies specific to a particular MIPS protein can be
obtained in an organism with multiple MIPS genes.
4. Other assay formats

Western blot (immunoblot) analysis is used to detect and quantify the presence of MIPS protein in a sample. The technique generally comprises separating sample proteins by gel electrophoresis on the basis of molecular weight, transferring the separated proteins to a suitable solid support, (such as a nitrocellulose filter, a nylon filter, or derivatized nylon filter), and incubating the sample with the antibodies that specifically bind the MIPS protein. The anti-MIPS polypeptide antibodies specifically bind to the MIPS polypeptide on the solid support. These antibodies may be directly labeled or alternatively may be subsequently detected using labeled antibodies (e.g., labeled sheep anti-mouse antibodies) that specifically bind to the anti-MIPS antibodies.

Other assay formats include liposome immunoassays (LIA), which use liposomes designed to bind specific molecules (e.g., antibodies) and release encapsulated reagents or markers. The released chemicals are then detected according to standard techniques (see, Monroe et al., Amer. Clin. Prod. Rev. 5:34-41 (1986)).

5. Reduction of nonspecific binding

One of skill in the art will appreciate that it is often desirable to minimize nonspecific binding in immunoassays. Particularly, where the assay involves an antigen or antibody immobilized on a solid substrate it is desirable to minimize the amount of nonspecific binding to the substrate. Means of reducing such nonspecific binding are well known to those of skill in the art. Typically, this technique involves coating the substrate with a proteinaceous composition. In particular, protein compositions such as bovine serum albumin (BSA), nonfat powdered milk, and gelatin are widely used with powdered milk being most preferred.

6. Labels

The particular label or detectable group used in the assay is not a critical aspect of the invention, as long as it does not significantly interfere with the specific binding of the antibody used in the assay. The detectable group can be any material having a detectable physical or chemical property. Such detectable labels have been well-developed in the field of immunoassays and, in general, most any label useful in such methods can be applied to the present invention. Thus, a label is any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful labels in the present invention include magnetic beads
(e.g., DYNABEADS™), fluorescent dyes (e.g., fluorescein isothiocyanate, Texas red, rhodamine, and the like), radiolabels (e.g., ^3H, ^125I, ^35S, ^14C, or ^32P), enzymes (e.g., horse \textit{radish peroxidase}, alkaline phosphatase and others commonly used in an ELISA), and colorimetric labels such as colloidal gold or colored glass or plastic beads (e.g., polystyrene, polypropylene, latex, etc.).

The label may be coupled directly or indirectly to the desired component of the assay according to methods well known in the art. As indicated above, a wide variety of labels may be used, with the choice of label depending on sensitivity required, ease of conjugation with the compound, stability requirements, available instrumentation, and disposal provisions.

Nonradioactive labels are often attached by indirect means. Generally, a ligand molecule (e.g., biotin) is covalently bound to the molecule. The ligand then binds to another molecules (e.g., streptavidin) molecule, which is either inherently detectable or covalently bound to a signal system, such as a detectable enzyme, a fluorescent compound, or a chemiluminescent compound. The ligands and their targets can be used in any suitable combination with antibodies that recognize a MIPS protein, or secondary antibodies that recognize anti-MIPS.

The molecules can also be conjugated directly to signal generating compounds, e.g., by conjugation with an enzyme or fluorophore. Enzymes of interest as labels will primarily be hydrolases, particularly phosphatases, esterases and glycosidases, or oxidases, particularly peroxidases. Fluorescent compounds include fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, etc. Chemiluminescent compounds include luciferin, and 2,3-dihydrophthalazinediones, e.g., luminol. For a review of various labeling or signal producing systems that may be used, see, e.g., U.S. Patent No. 4,391,904.

Means of detecting labels are well known to those of skill in the art. Thus, for example, where the label is a radioactive label, means for detection include a scintillation counter or photographic film as in autoradiography. Where the label is a fluorescent label, it may be detected by exciting the fluorochrome with the appropriate wavelength of light and detecting the resulting fluorescence. The fluorescence may be detected visually, by means of photographic film, by the use of electronic detectors such as charge coupled devices (CCDs) or photomultipliers and the like. Similarly, enzymatic labels may be detected by providing the appropriate substrates for the enzyme and detecting the resulting reaction product. Finally simple colorimetric labels may be
detected simply by observing the color associated with the label. Thus, in various
dipstick assays, conjugated gold often appears pink, while various conjugated beads
appear the color of the bead.

Some assay formats do not require the use of labeled components. For
instance, agglutination assays can be used to detect the presence of the target antibodies.
In this case, antigen-coated particles are agglutinated by samples comprising the target
antibodies. In this format, none of the components need be labeled and the presence of
the target antibody is detected by simple visual inspection.

**VI. Modulating MIPS Activity in Cells**

**A. Assays for Modulators of MIPS Proteins**

In numerous embodiments of this invention, the level of MIPS activity will
be modulated in a cell by administering to the cell, *in vivo* or *in vitro*, any of a large
number of MIPS-modulating molecules, *e.g.*, polypeptides, antibodies, amino acids,
nucleotides, lipids, carbohydrates, or any organic or inorganic molecule. Such MIPS
modulators are particularly useful in the treatment of any of a number of estrogen-related
diseases and conditions including osteoporosis, atherosclerosis and other cardiovascular
conditions, Alzheimer Disease, Parkinson’s Disease, and breast and other types of cancer.

To identify molecules capable of modulating MIPS, assays will be
performed to detect the effect of various compounds on MIPS activity in a cell. Such
assays can involve the identification of compounds that interact with MIPS proteins,
either physically or genetically, and can thus rely on any of a number of standard methods
to detect physical or genetic interactions between compounds. Such assays can also
involve the identification of compounds that affect MIPS expression, activity or other
properties, such as its phosphorylation or ability to bind other proteins. Such assays can
also involve the detection of MIPS activity in a cell, either *in vitro* or *in vivo*, and can thus
involve, *e.g.*, the detection of inositol or inositol phosphate using any standard assay, by
detecting MIPS enzyme activity using standard methods (*see, e.g.*, Novak *et al.*, (1999) *J
or, as MIPS is involved in signaling events in many types of cells, by detecting the level
or activity of any protein or factor that is dependent on MIPS-mediated signaling. Such
cell-based assays can be performed in any type of cell, *e.g.*, a cell that naturally expresses
MIPS, or a cultured cell that produces MIPS due to recombinant expression.
B. **Assays for MIPS-Interacting Compounds**

In certain embodiments, assays will be performed to identify molecules that physically or genetically interact with MIPS proteins. Such molecules can be any type of molecule, including polypeptides, polynucleotides, amino acids, nucleotides, carbohydrates, lipids, or any other organic or inorganic molecule. Such molecules may represent molecules that normally interact with MIPS to effect, e.g., estrogen receptor α-mediated signal transduction, or may be synthetic or other molecules that are capable of interacting with MIPS and that can potentially be used to modulate MIPS activity in cells, or used as lead compounds to identify classes of molecules that can interact with and/or modulate MIPS. Such assays may represent physical binding assays, such as affinity chromatography, immunoprecipitation, two-hybrid screens, or other binding assays, or may represent genetic assays as described infra.

In any of the binding or functional assays described herein, *in vivo* or *in vitro*, any MIPS protein, or any derivative, variation, homolog, or fragment of a MIPS protein, can be used. Preferably, the MIPS protein is at least about 70% similar or identical to SEQ ID NO:1 or 3. In numerous embodiments, a fragment of a MIPS protein is used. Such fragments can be used alone, in combination with other MIPS fragments, or in combination with sequences from heterologous proteins, e.g., the fragments can be fused to a heterologous polypeptide, thereby forming a chimeric polypeptide.

1. **Assays for physical interactions**

Compounds that interact with MIPS proteins can be isolated based on an ability to specifically bind to a MIPS protein or to a fragment thereof. In numerous embodiments, the MIPS protein or protein fragment will be attached to a solid support. In one embodiment, affinity columns are made using the MIPS polypeptide, and physically-interacting molecules are identified. It will be apparent to one of skill that chromatographic techniques can be performed at any scale and using equipment from many different manufacturers (e.g., Pharmacia Biotech). In addition, molecules that interact with MIPS proteins *in vivo* can be identified by co-immunoprecipitation or other methods, i.e., immunoprecipitating MIPS proteins using anti-MIPS antibodies from a cell or cell extract, and identifying compounds, e.g., proteins, that are precipitated along with the MIPS protein. Such methods are well known to those of skill in the art and are taught, *e.g.*, in Ausubel *et al.*, Sambrook *et al.*, Harlow & Lane, all supra.
Two-hybrid screens can also be used to identify polypeptides that interact
in vivo with a MIPS polypeptide or a fragment thereof (Fields et al., Nature 340:245-246
(1989)). Such screens comprise two discrete, modular domains of a transcription factor
protein, e.g., a DNA binding domain and a transcriptional activation domain, which are
produced in a cell as two separate polypeptides, each of which also comprises one of two
potentially binding polypeptides. If the two potentially binding polypeptides in fact
interact in vivo, then the DNA binding and the transcriptional activating domain of the
transcription factor are united, thereby producing expression of a target gene in the cell.
The target gene typically encodes an easily detectable gene product, e.g., β-galactosidase,
GFP, or luciferase, which can be detected using standard methods. In the present
invention, a MIPS polypeptide is fused to one of the two domains of the transcription
factor, and the potential MIPS-binding polypeptides (e.g., encoded by a cDNA library)
are fused to the other domain. Such methods are well known to those of skill in the art,
and are taught, e.g., in Ausubel et al., supra.

C. Assays for MIPS Protein Activity

MIPS genes and their alleles and polymorphic variants encode myo-
inositol phosphate synthases that promote estrogen receptor α-mediated receptor signal
transduction. Accordingly, the activity of MIPS polypeptides can be assessed using a
variety of in vitro and in vivo assays to determine functional, chemical, and physical
effects, e.g., directly measuring the MIPS enzyme activity using in vitro assays, e.g., as
FEMS Microbiol. Lett. 53:339-343, measuring the binding of MIPS to heterologous
proteins or substrates, measuring MIPS protein and/or RNA levels, or measuring other
aspects of MIPS polypeptides, e.g., phosphorylation levels, transcription levels, and the
like. Such assays can be used to test for both activators and inhibitors of MIPS proteins.
Modulators can also be genetically altered versions of MIPS proteins, e.g., dominant
negative forms of MIPS or of proteins that interact with MIPS. Such modulators of
activity are useful for many diagnostic and therapeutic applications.

The MIPS protein of the assay will typically be a recombinant or naturally
occurring polypeptide with a sequence of SEQ ID NO:1 or 3 or conservatively modified
variants thereof. Alternatively, the MIPS protein of the assay will be derived from a
eukaryote and include an amino acid subsequence having amino acid sequence similarity
or identity to SEQ ID NO:1 or 3. Generally, the amino acid sequence similarity or
identity will be at least 60%, optionally at least 70% to 85%, optionally at least 90-95%. Optionally, the polypeptide of the assays will comprise a domain or fragment of a MIPS protein. In certain embodiments, a domain or fragment of a MIPS protein is bound to a solid substrate and used, e.g., to isolate any molecules that can bind to and/or modulate their activity. In certain embodiments, a domain or fragment of a MIPS polypeptide is fused to a heterologous polypeptide, thereby forming a chimeric polypeptide. Such chimeric polypeptides are also useful, e.g., in assays to identify modulators of MIPS.

Samples or assays that are treated with a potential MIPS protein inhibitor or activator are compared to control samples without the test compound, to examine the extent of modulation. Control samples (untreated with activators or inhibitors) are assigned a relative MIPS activity value of 100. Inhibition of a MIPS protein is achieved when the MIPS activity value relative to the control is about 90%, optionally 50%, optionally 25-0%. Activation of a MIPS protein is achieved when the MIPS activity value relative to the control is 110%, optionally 150%, 200-500%, or 1000-2000%.

The effects of the test compounds upon the function of the polypeptides can be measured by examining any of the parameters described above. Any suitable physiological change that affects MIPS activity can be used to assess the influence of a test compound on the polypeptides of this invention. When the functional consequences are determined using intact cells or animals, one can also measure a variety of effects such as changes in intracellular myo-inositol phosphate synthase enzyme activity, inositol or inositol phosphate levels. In intact animals, downstream effects of estrogen signaling including, but not limited to, breast or other cancers, osteoporosis, atherosclerosis, Alzheimer’s Disease, Parkinson’s Disease, can also be examined. In numerous embodiments, cells or animals used in such embodiments are estrogen receptor α-specific, i.e. they contain mutations in genes encoding estrogen receptor β and/or other estrogen receptors. In other embodiments, cells or animals that are mutant for an estrogen receptor α-encoding gene is used as a negative control.

D. Identifying estrogen receptor agonists and antagonists

In another embodiment, transcription levels can be measured to assess the effects of a test compound on estrogen receptor α-specific signal transduction. A host cell containing an estrogen receptor α is contacted with a test compound for a sufficient time to effect any interactions, and then the level of MIPS gene expression is measured.
The amount of time to effect such interactions may be empirically determined, such as by running a time course and measuring the level of transcription as a function of time.

Such assays can be performed using any suitable eukaryotic cells, including, e.g., mammalian cells, insect cells, plant or yeast cells using standard methods. A cell type will be selected that naturally expresses an estrogen receptor, e.g., an estrogen receptor α, or which is induced to express an estrogen receptor by, for example, introducing a heterologous polynucleotide encoding the receptor, operably linked to a promoter, into the cell.

The amount of transcription may be measured using any method known to those of skill in the art to be suitable. For example, mRNA expression of the protein of interest may be detected using, e.g., Northern blots, reverse transcriptase-polymerase chain reaction (RT-PCR), or any standard method, using, e.g., probes or primers designed from SEQ ID NO: 2 or 4. Alternatively, the expression of the MIPS can be detected by detecting the level of the polypeptide products using immunoassays or other assays to detect MIPS enzyme activity. Such assays can use natural forms of MIPS or can use a MIPS fusion protein, e.g., MIPS transcript fused to a reporter sequence, e.g., a sequence encoding chloramphenicol acetyltransferase, luciferase, β-galactosidase, GFP, or alkaline phosphatase. Alternatively, one of these marker sequences can be operably linked to an MIPS promoter. (See, e.g., Mistili & Spector, Nature Biotechnology 15:961-964 (1997))

The amount of transcription is then compared to the amount of transcription in either the same cell in the absence of the test compound, or it may be compared with the amount of transcription in a substantially identical cell that lacks the protein of interest. A substantially identical cell may be derived from the same cells from which the recombinant cell was prepared but which had not been modified by introduction of heterologous DNA. Any difference in the amount of transcription indicates that the test compound has in some manner altered the activity of the protein of interest.

A compound that causes an increase in the amount of MIPS expression, as detected by any of the herein-described methods, is a candidate for an estrogen receptor α agonist that would be useful, e.g., in the inhibition of osteoporosis, atherosclerosis, Alzheimer’s Disease, or Parkinson’s Disease, and a compound that causes a decrease in the amount of MIPS expression is a candidate for an estrogen-receptor α antagonist, which would be useful in the inhibition of, e.g., breast cancer. Candidate agonists or
antagonists can be further characterized by any of a number of methods, including, e.g.,
directly examining their interactions with estrogen receptor α's, examining their ability to
alter MIPS expression in cells that do not express estrogen receptors, examining the
estrogen receptor specificity of the candidate (i.e., estrogen receptor α or β), etc.

In preferred embodiments, the detection of the level of MIPS expression in
the presence or absence of the test compound is tested simultaneously for a large number
of test compounds, e.g., using high throughput screening.

E. Modulators and Binding Compounds

The compounds tested as modulators of a MIPS protein can be any small
chemical compound, including any organic or inorganic molecules, or a biological entity,
such as a protein, sugar, nucleic acid or lipid. Alternatively, modulators can be
genetically altered versions of a MIPS gene. Typically, test compounds will be small
chemical molecules and peptides. Essentially any chemical compound can be used as a
potential modulator or binding compound in the assays of the invention, although most
often compounds can be dissolved in aqueous or organic (especially DMSO-based)
solutions are used. The assays are designed to screen large chemical libraries by
automating the assay steps and providing compounds from any convenient source to
assays, which are typically run in parallel (e.g., in microtiter formats on microtiter plates
in robotic assays). It will be appreciated that there are many suppliers of chemical
compounds, including Sigma (St. Louis, MO), Aldrich (St. Louis, MO), Sigma-Aldrich
(St. Louis, MO), Fluka Chemika-Biochemica Analytika (Buchs, Switzerland) and the
like.

In one preferred embodiment, high throughput screening methods involve
providing a combinatorial chemical or peptide library containing a large number of
potential therapeutic compounds (potential modulator or binding compounds). Such
"combinatorial chemical libraries" are then screened in one or more assays, as described
herein, to identify those library members (particular chemical species or subclasses) that
display a desired characteristic activity. The compounds thus identified can serve as
conventional "lead compounds" or can themselves be used as potential or actual
therapeutics.

A combinatorial chemical library is a collection of diverse chemical
compounds generated by either chemical synthesis or biological synthesis, by combining
a number of chemical "building blocks" such as reagents. For example, a linear
combinatorial chemical library such as a polypeptide library is formed by combining a set of chemical building blocks (amino acids) in every possible way for a given compound length (i.e., the number of amino acids in a polypeptide compound). Millions of chemical compounds can be synthesized through such combinatorial mixing of chemical building blocks.


Devices for the preparation of combinatorial libraries are commercially available (see, e.g., 357 MPS, 390 MPS, Advanced Chem Tech, Louisville KY, Symphony, Rainin, Woburn, MA, 433A Applied Biosystems, Foster City, CA, 9050 Plus, Millipore, Bedford, MA). In addition, numerous combinatorial libraries are themselves
commercially available (see, e.g., ComGenex, Princeton, N.J., Tripos, Inc., St. Louis, MO, 3D Pharmaceuticals, Exton, PA, Martek Biosciences, Columbia, MD, etc.).

1. Solid state and soluble high throughput assays

In one embodiment, the invention provides soluble assays using molecules such as an N-terminal or C-terminal domain of a MIPS protein either alone or covalently linked to a heterologous protein to create a chimeric molecule. In another embodiment, the invention provides solid phase based in vitro assays in a high throughput format, where a domain, chimeric molecule, MIPS protein, or cell or tissue expressing a MIPS protein is attached to a solid phase substrate.

In the high throughput assays of the invention, it is possible to screen up to several thousand different modulators in a single day. In particular, each well of a microtiter plate can be used to run a separate assay against a selected potential modulator, or, if concentration or incubation time effects are to be observed, every 5-10 wells can test a single modulator. Thus, a single standard microtiter plate can assay about 100 (e.g., 96) modulators. If 1536 well plates are used, then a single plate can easily assay from about 100 to about 1500 different compounds. It is possible to assay several different plates per day; assay screens for up to about 6,000-20,000 different compounds is possible using the integrated systems of the invention. More recently, microfluidic approaches to reagent manipulation have been developed.

The molecule of interest can be bound to the solid state component, directly or indirectly, via covalent or non covalent linkage, e.g., via a tag. The tag can be any of a variety of components. In general, a molecule which binds the tag (a tag binder) is fixed to a solid support, and the tagged molecule of interest is attached to the solid support by interaction of the tag and the tag binder.

A number of tags and tag binders can be used, based upon known molecular interactions well described in the literature. For example, where a tag has a natural binder, for example, biotin, protein A, or protein G, it can be used in conjunction with appropriate tag binders (avidin, streptavidin, neutravidin, the Fc region of an immunoglobulin, etc.) Antibodies to molecules with natural binders such as biotin are also widely available and appropriate tag binders; see, SIGMA Immunochemicals 1998 catalogue SIGMA, St. Louis MO).

Similarly, any haptenic or antigenic compound can be used in combination with an appropriate antibody to form a tag/tag binder pair. Thousands of specific
antibodies are commercially available and many additional antibodies are described in the literature. For example, in one common configuration, the tag is a first antibody and the tag binder is a second antibody which recognizes the first antibody.

Synthetic polymers, such as polyurethanes, polyesters, polycarbonates, polyureas, polyamides, polyethyleneimines, polyarylene sulfides, polysiloxanes, polyimides, and polyacetates can also form an appropriate tag or tag binder. Many other tag/tag binder pairs are also useful in assay systems described herein, as would be apparent to one of skill upon review of this disclosure.

Common linkers such as peptides, polyethers, and the like can also serve as tags, and include polypeptide sequences, such as poly-gly sequences of between about 5 and 200 amino acids. Such flexible linkers are known to persons of skill in the art. For example, poly(ethelyne glycol) linkers are available from Shearwater Polymers, Inc. Huntsville, Alabama. These linkers optionally have amide linkages, sulfhydryl linkages, or heterofunctional linkages.

Tag binders are fixed to solid substrates using any of a variety of methods currently available. Solid substrates are commonly derivatized or functionalized by exposing all or a portion of the substrate to a chemical reagent which fixes a chemical group to the surface which is reactive with a portion of the tag binder. For example, groups which are suitable for attachment to a longer chain portion would include amines, hydroxyl, thiol, and carboxyl groups. Aminoalkylsilanes and hydroxyalkylsilanes can be used to functionalize a variety of surfaces, such as glass surfaces. The construction of such solid phase biopolymer arrays is well described in the literature. See, e.g., Merrifield, *J. Am. Chem. Soc.* 85:2149-2154 (1963) (describing solid phase synthesis of, e.g., peptides); Geysen *et al.*, *J. Immun. Meth.* 102:259-274 (1987) (describing synthesis of solid phase components on pins); Frank & Doring, *Tetrahedron* 44:60316040 (1988) (describing synthesis of various peptide sequences on cellulose disks); Fodor *et al.*, *Science*, 251:767-777 (1991); Sheldon *et al.*, *Clinical Chemistry* 39(4):718-719 (1993); and Kozal *et al.*, *Nature Medicine* 2(7):753759 (1996) (all describing arrays of biopolymers fixed to solid substrates). Nonchemical approaches for fixing tag binders to substrates include other common methods, such as heat, cross-linking by UV radiation, and the like.
2. Computer-based assays

Yet another assay for compounds that modulate MIPS protein activity involves computer assisted drug design, in which a computer system is used to generate a three-dimensional structure of a MIPS protein based on the structural information encoded by its amino acid sequence. The input amino acid sequence interacts directly and actively with a pre-established algorithm in a computer program to yield secondary, tertiary, and quaternary structural models of the protein. The models of the protein structure are then examined to identify regions of the structure that have the ability to bind. These regions are then used to identify compounds that bind to the protein.

The three-dimensional structural model of the protein is generated by entering protein amino acid sequences of at least 10 amino acid residues or corresponding nucleic acid sequences encoding a MIPS polypeptide into the computer system. The nucleotide sequence encoding the polypeptide preferably comprises SEQ ID NO:2 or SEQ ID NO:4, and conservatively modified versions thereof. The amino acid sequence, preferably comprising SEQ ID NO:1 or 3, or conservatively modifies versions thereof, represents the primary sequence or subsequence of the protein, which encodes the structural information of the protein. At least 10 residues of the amino acid sequence (or a nucleotide sequence encoding 10 amino acids) are entered into the computer system from computer keyboards, computer readable substrates that include, but are not limited to, electronic storage media (e.g., magnetic diskettes, tapes, cartridges, and chips), optical media (e.g., CD ROM), information distributed by internet sites, and by RAM. The three-dimensional structural model of the protein is then generated by the interaction of the amino acid sequence and the computer system, using software known to those of skill in the art.

The amino acid sequence represents a primary structure that encodes the information necessary to form the secondary, tertiary and quaternary structure of the protein of interest. The software looks at certain parameters encoded by the primary sequence to generate the structural model. These parameters are referred to as “energy terms,” and primarily include electrostatic potentials, hydrophobic potentials, solvent accessible surfaces, and hydrogen bonding. Secondary energy terms include van der Waals potentials. Biological molecules form the structures that minimize the energy terms in a cumulative fashion. The computer program is therefore using these terms...
encoded by the primary structure or amino acid sequence to create the secondary structural model.

The tertiary structure of the protein encoded by the secondary structure is then formed on the basis of the energy terms of the secondary structure. The user at this point can enter additional variables such as whether the protein is membrane bound or soluble, its location in the body, and its cellular location, e.g., cytoplasmic, surface, or nuclear. These variables along with the energy terms of the secondary structure are used to form the model of the tertiary structure. In modeling the tertiary structure, the computer program matches hydrophobic faces of secondary structure with like, and hydrophilic faces of secondary structure with like.

Once the structure has been generated, potential modulator binding regions are identified by the computer system. Three-dimensional structures for potential modulators are generated by entering amino acid or nucleotide sequences or chemical formulas of compounds, as described above. The three-dimensional structure of the potential modulator is then compared to that of the MIPS protein to identify compounds that bind to the protein. Binding affinity between the protein and compound is determined using energy terms to determine which compounds have an enhanced probability of binding to the protein.

Computer systems are also used to screen for mutations, polymorphic variants, alleles and interspecies homologs of MIPS genes. Such mutations can be associated with disease states or genetic traits. As described above, GeneChip™ and related technology can also be used to screen for mutations, polymorphic variants, alleles and interspecies homologs. Once the variants are identified, diagnostic assays can be used to identify patients having such mutated genes. Identification of the mutated MIPS genes involves receiving input of a first nucleic acid sequence of SEQ ID NO:2 or 4, or a first amino acid sequence of SEQ ID NO:1 or 3, and conservatively modified versions thereof. The sequence is entered into the computer system as described above. The first nucleic acid or amino acid sequence is then compared to a second nucleic acid or amino acid sequence that has substantial identity to the first sequence. The second sequence is entered into the computer system in the manner described above. Once the first and second sequences are compared, nucleotide or amino acid differences between the sequences are identified. Such sequences can represent allelic differences in various MIPS genes, and mutations associated with disease states and genetic traits.
VII. Modulating MIPS activity/expression to treat diseases or conditions

In numerous embodiments of this invention, a compound, e.g., nucleic acid, polypeptide, or other molecule is administered to a patient, in vivo or ex vivo, to effect a change in MIPS activity or expression in the patient. Such compounds can be nucleic acids encoding full length MIPS polypeptides, e.g., as shown as SEQ ID NO: 1 or 3, or any derivative, fragment, or variant thereof, operably linked to a promoter. Suitable nucleic acids also include inhibitory sequences such as antisense or ribozyme sequences, which can be delivered in, e.g., an expression vector operably linked to a promoter, or can be delivered directly. Also, any nucleic acid that encodes a polypeptide that modulates the expression of MIPS can be used. In general, nucleic acids can be delivered to cells using any of a large number of vectors or methods, e.g., retroviral, adenoviral, or adeno-associated virus vectors, liposomal formulations, naked DNA injection, and others. All of these methods are well known to those of skill in the art.

Proteins can also be delivered to a patient to modulate MIPS activity. In preferred embodiments, a polyclonal or monoclonal antibody that specifically binds to MIPS will be delivered. In addition, any polypeptide that interacts with and/or modulates MIPS activity can be used, e.g., a polypeptide that is identified using the presently described assays, or any dominant negative form of MIPS or a MIPS-interacting protein. In addition, polypeptides that affect MIPS expression, as detected using the methods described herein, can be used.

Further, any compound that is found to or designed to interact with and/or modulate the activity of MIPS can be used. For example, any compound that is found, using the methods described herein, to bind to or modulate the activity of MIPS can be used.

Any of the above-described molecules can be used to increase or decrease the expression or activity of MIPS, or to otherwise affect the properties and/or behavior of MIPS polypeptides or polynucleotides, e.g., stability, phosphorylation, kinase activity, interactions with other proteins, etc. The present compounds can thus be used to treat any of a number of diseases, including, but not limited to osteoporosis, atherosclerosis and other cardiovascular diseases, Alzheimer’s Disease, Parkinson’s Disease, and breast and other cancers.
A. Administration and Pharmaceutical Compositions

Administration of any of the present molecules can be achieved by any of the routes normally used for introducing a modulator compound into ultimate contact with the tissue to be treated. The modulators are administered in any suitable manner, optionally with pharmaceutically acceptable carriers. Suitable methods of administering such modulators are available and well known to those of skill in the art, and, although more than one route can be used to administer a particular composition, a particular route can often provide a more immediate and more effective reaction than another route.

Pharmaceutically acceptable carriers are determined in part by the particular composition being administered, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of pharmaceutical compositions of the present invention (see, e.g., Remington's Pharmaceutical Sciences, 17th ed. 1985).

The MIPS modulators, alone or in combination with other suitable components, can be made into aerosol formulations (i.e., they can be "nebulized") to be administered via inhalation. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

Formulations suitable for administration include aqueous and nonaqueous solutions, isotonic sterile solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic, and aqueous and nonaqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. In the practice of this invention, compositions can be administered, for example, orally, nasally, topically, intravenously, intraperitoneally, intravesically or intrathecally. The formulations of compounds can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials. Solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described. The modulators can also be administered as part of prepared food or drug.

The dose administered to a patient, in the context of the present invention should be sufficient to effect a beneficial response in the subject over time. The dose will be determined by the efficacy of the particular modulators employed and the condition of the subject, as well as the body weight or surface area of the area to be treated. The size of the dose also will be determined by the existence, nature, and extent of any adverse
side-effects that accompany the administration of a particular compound or vector in a particular subject.

In determining the effective amount of the modulator to be administered, a physician may evaluate circulating plasma levels of the modulator, modulator toxicities, and the production of anti-modulator antibodies. In general, the dose equivalent of a modulator is from about 1 ng/kg to 10 mg/kg for a typical subject.

For administration, modulators of the present invention can be administered at a rate determined by the LD-50 of the modulator, and the side-effects of the compound at various concentrations, as applied to the mass and overall health of the subject. Administration can be accomplished via single or divided doses.

VIII. Transgenic Animals

Transgenic and chimeric non-human mammals, e.g., mice, and methods for generating them are described below. The mammals are useful, *inter alia*, for testing the function of MIPS *in vivo*, to generate models for the study of estrogen-receptor α-related diseases and conditions, and for the development of potential treatments for MIPS related diseases and conditions.

Transgenic and chimeric non-human mammals are generated that contain cells that lack at least one functional endogenous allele for MIPS, e.g., mMIPS. A "chimeric animal" includes some cells that lack the functional MIPS gene of interest and other cells that do not have the inactivated gene. A "transgenic animal," in contrast, is made up of cells that have all incorporated the specific modification which renders the MIPS gene inactive or otherwise altered. While a transgenic animal is typically always capable of transmitting the mutant MIPS gene to its progeny, the ability of a chimeric animal to transmit the mutation depends upon whether the inactivated gene is present in the animal's germ cells. The modifications that inactivate or otherwise alter the MIPS gene can include, for example, insertions, deletions, or substitutions of one or more nucleotides. The modifications can interfere with transcription of the gene itself, with translation and/or stability of the resulting mRNA, or can cause the gene to encode an inactive or otherwise altered MIPS polypeptide, e.g., a MIPS polypeptide with modified binding properties or enzyme activity.

The claimed methods are useful for producing transgenic and chimeric animals of most vertebrate species. Such species include, but are not limited to, nonhuman mammals, including rodents such as mice and rats, rabbits, ovines such as
sheep and goats, porcines such as pigs, and bovines such as cattle and buffalo. Methods of obtaining transgenic animals are described in, for example, Puhler, A., Ed., Genetic Engineering of Animals, VCH Publ., 1993; Murphy and Carter, Eds., Transgenesis Techniques: Principles and Protocols (Methods in Molecular Biology, Vol. 18), 1993; and Pinkert, CA, Ed., Transgenic Animal Technology: A Laboratory Handbook, Academic Press, 1994.


Typically, a modified MIPS gene, e.g., mMIPS, is introduced, e.g., by homologous recombination, into embryonic stem cells (ES), which are obtained from preimplantation embryos and cultured in vitro. See, e.g., Hooper, ML, Embryonal Stem Cells: Introducing Planned Changes into the Animal Germline (Modern Genetics, v. 1), Int'l. Publ. Distrib., Inc., 1993; Bradley et al. (1984) Nature 309, 255-258. Subsequently, the transformed ES cell is combined with a blastocyst from a non-human animal, e.g., a mouse. The ES cells colonize the embryo and in some embryos form the germ line of the resulting chimeric animal. See, Jaenisch (1988) Science 240: 1468-1474. Alternatively, ES cells or somatic cells that can reconstitute an organism ("somatic repopulating cells") can be used as a source of nuclei for transplantation into an enucleated fertilized oocyte giving rise to a transgenic mammal. See, e.g., Wilmut et al. (1997) Nature 385: 810-813.

Other methods for obtaining a transgenic or chimeric animal having a mutant MIPS gene in its genome is to contact fertilized oocytes with a vector that includes a polynucleotide that encodes a modified, e.g., inactive, MIPS polypeptide. In some animals, such as mice, fertilization is typically performed in vivo and fertilized ova are surgically removed. In other animals, particularly bovines, it is preferably to remove ova from live or slaughterhouse animals and fertilize the ova in vitro. See DeBoer et al., WO 91/08216. In vitro fertilization permits the modifications to be introduced into substantially synchronous cells.

Fertilized oocytes are typically cultured in vitro until a pre-implantation embryo is obtained containing about 16-150 cells. The 16-32 cell stage of an embryo is described as a morula, whereas pre-implantation embryos containing more than 32 cells are termed blastocysts. These embryos show the development of a blastocoel cavity, typically at the 64 cell stage. The presence of a desired MIPS mutation in the cells of the embryo can be detected by methods known to those of skill in the art, e.g., Southern

Pre-implantation embryos are transferred to an appropriate female resulting in the birth of a transgenic or chimeric animal, depending upon the stage of development when the transgene is integrated. Chimeric mammals can be bred to form true germline transgenic animals. Chimeric mice and germline transgenic mice can also be ordered from commercial sources (e.g., Deltagen, San Carlos, CA).

Other methods for introducing mutations into mammalian cells or animals include recombinase systems, which can be employed to delete all or a portion of a locus of interest. Examples of recombinase systems include, the cre/lox system of bacteriophage P1 (see, e.g., Gu et al. (1994) *Science* 265: 103-106; Terry et al. (1997) *Transgenic Res.* 6: 349-356) and the FLP/FRT site specific integration system (see, e.g., Dymecki (1996) *Proc. Natl. Acad. Sci. USA* 93: 6191-6196). In these systems, sites recognized by the particular recombinase are typically introduced into the genome at a position flanking the portion of the gene that is to be deleted. Introduction of the recombinase into the cells then catalyzes recombination which deletes from the genome the polynucleotide sequence that is flanked by the recombination sites. If desired, one can obtain animals in which only certain cell types lack the MIPS gene of interest, e.g., by using a tissue specific promoter to drive the expression of the recombinase. See, e.g., Tsien et al. (1996) *Cell* 87: 1317-26; Brocard et al. (1996) *Proc. Natl. Acad. Sci. USA* 93: 10887-10890; Wang et al. (1996) *Proc. Natl. Acad. Sci. USA* 93: 3932-6; Meyers et al. (1998) *Nat. Genet.* 18: 136-41).

The presence of any mutation in a MIPS gene in a cell or animal can be detected using any method described herein, e.g., Southern blot, PCR, or DNA sequencing. See, e.g., Ausubel et al., supra.
IX. Kits

MIPS genes and their homologs are useful tools for a number of applications, including, but not limited to, identifying estrogen-responsive cells, especially those responsive through an estrogen receptor $\alpha$, for forensics and paternity determinations, and for treating any of a large number of estrogen-associated diseases. MIPS specific reagents that specifically hybridize to MIPS nucleic acids, such as MIPS probes and primers, and MIPS specific reagents that specifically bind to or modulate the activity of a MIPS protein, *e.g.*, MIPS antibodies or other compounds can thus be provided in a kit for the practice of any of the applications described herein.

Nucleic acid assays for the presence of DNA and RNA for a MIPS polynucleotide in a sample include numerous techniques known to those skilled in the art, such as Southern analysis, Northern analysis, dot blots, RNase protection, S1 analysis, amplification techniques such as PCR and LCR, and *in situ* hybridization. In *in situ* hybridization, for example, the target nucleic acid is liberated from its cellular surroundings in such as to be available for hybridization within the cell while preserving the cellular morphology for subsequent interpretation and analysis. The following articles provide an overview of the art of *in situ* hybridization: Singer *et al.*, *Biotechniques* 4:230-250 (1986); Haase *et al.*, *Methods in Virology*, vol. VII, pp. 189-226 (1984); and *Nucleic Acid Hybridization: A Practical Approach* (Hames *et al*., eds. 1987). In addition, an MIPS protein can be detected with the various immunoassay techniques described above. The test sample is typically compared to both a positive control (*e.g.*, a sample expressing a recombinant MIPS protein) and a negative control.

The present invention also provides kits for screening for modulators of MIPS proteins or nucleic acids. Such kits can be prepared from readily available materials and reagents. For example, such kits can comprise any one or more of the following materials: MIPS nucleic acids or proteins, reaction tubes, and instructions for testing MIPS activity. Optionally, the kit contains a biologically active MIPS protein. A wide variety of kits and components can be prepared according to the present invention, depending upon the intended user of the kit and the particular needs of the user.

X. Examples

To identify sequences that are specifically expressed in response to estrogen signaling through the estrogen receptor $\alpha$, subtraction cloning was used to isolate expressed sequence tags (EST) representing mRNA that is more abundant in the
liver of normal male mice treated with estrogen than in the liver of estrogen receptor alpha knockout (ERKO) male mice treated with estrogen. Using these mRNAs, cDNA clones representing the full-length mRNA molecules were obtained.

Three variants of the cDNA sequence were obtained that differ from each other by the presence or absence of three sequence regions termed A, B and C. These regions may represent alternatively spliced exons or un-spliced introns. The sequence of variant one, which lacks regions A, B and C, is shown as SEQ ID NO:2.

Variant one encodes a putative protein composed of 557 amino acids that is shown as SEQ ID NO:1. This protein begins with a methionine codon (ATG) at nucleotide 38 and terminates with a stop codon (TAA) at nucleotide 1712. This methionine codon lies within a sequence context that closely matches that of the consensus sequence for translation initiation (Kozak (1989) J. Cell Biol. 108:229-241). This protein sequence has 59% sequence identity and 70% sequence similarity to the myo inositol-phosphate synthase from the plant Arales Lemnaceae. Similar sequence similarity at the amino acid level exists with myo inositol-phosphate synthases from several other plant species. A lower level of homology was observed between the protein encoded by variant 1 and the yeast myo inositol-phosphate synthase (51% identity and 62% similarity).

Variant two is identical to variant one except for the presence of region A. Region A is composed of 89 nucleotides and is inserted between nucleotides 452 and 453 in the sequence shown as SEQ ID NO:2. The presence of region A in variant two creates a stop codon that is in frame with the initiating methionine codon at nucleotide 38. Variant two would therefore encode a predicted protein of 148 amino acids that is composed of the amino terminal 139 amino acids of the protein shown as SEQ ID NO:1. This truncated form would not be expected to possess the same enzymatic function as the myo inositol-phosphate synthase shown in Figure 2.

Variant three is identical to the sequence shown as SEQ ID NO:2 except for the presence of regions A, B, C, and the absence of nucleotides 1 to 320. Region A was already described above. Region B consists of 151 nucleotides inserted between nucleotides 644 and 645 of the sequence shown as SEQ ID NO:2. Region C consists of 95 nucleotides inserted between nucleotides 794 and 795 of SEQ ID NO:2. Variant three therefore lacks the initiating methionine codon at nucleotide 38 in SEQ ID NO:2 and so does not encode the mMIPS protein shown in Figure 2. Instead, variant three is predicted to encode a protein of 110 amino acids that begins at a methionine codon within region A.
This methionine codon within region A lies in a sequence context that matches the consensus sequence for translation initiation (Kozak (1989) J. Cell Biol. 108:229-241). However, there are 4 other methionine codons closer to the 5' end of the DNA sequence of variant 2, suggesting that this protein may not be produced. The central 64 amino acids of this potential protein are identical to amino acids 139 to 203 of SEQ ID NO:1. The first 22 amino acids and the last 24 amino acids of the potential 110 amino acid protein encoded by variant three are divergent from the mMIPS protein encoded by variant one.

Northern blot analysis with a hybridization probe derived from the mMIPS cDNA (Figure 1) showed that the mMIPS mRNA is undetectable in the liver of untreated normal male mice and estrogen treated ERKO mice. However, the mMIPS mRNA was strongly expressed in the livers of estrogen treated normal male mice. Furthermore, Northern blot analysis also revealed that mMIPS mRNA is undetectable in the livers of ovarectamized and untreated male mice, and is highly induced in both by estrogen treatment. These experiments demonstrate that expression of mMIPS mRNA in the liver requires the presence of a functional estrogen receptor alpha, and indicates that transcription of the mMIPS gene in the liver is activated by estrogen specifically via the alpha but not the beta-receptor. This is the first demonstration of estrogen receptor subtype specific modulation of gene expression.

mMIPS is strongly expressed in the developing mouse embryo as early as day 7, with expression going down by day 11, and then continuing out to day 17. Tissue distribution of the mMIPS mRNA in mouse was studied by reverse transcriptase-polymerase chain reaction (RT-PCR).

It is understood that the embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.
WHAT IS CLAIMED IS:

1. An isolated nucleic acid encoding an estrogen-regulated myo-
inositol phosphate synthase protein, said protein having at least one of the following
characteristics:
   (1) comprising at least about 90% amino acid sequence identity to SEQ
ID NO: 1; or
   (2) specifically binding to polyclonal antibodies generated against a
polypeptide comprising the amino acid sequence of SEQ ID NO: 1, but not to polyclonal
antibodies generated against a polypeptide comprising the amino acid sequence of SEQ
ID NO: 3;
   wherein the protein does not comprise the amino acid sequence shown as
SEQ ID NO: 3.

2. The nucleic acid of claim 1, wherein said nucleic acid encodes a
protein comprising the amino acid sequence of SEQ ID NO: 1.

3. The nucleic acid of claim 1, wherein said nucleic acid comprises a
nucleotide sequence that is at least about 90% identical to SEQ ID NO: 2.

4. The nucleic acid of claim 3, wherein said nucleic acid comprises
the nucleotide sequence of SEQ ID NO: 2.

5. The nucleic acid of claim 1, wherein said nucleic acid hybridizes
under moderately stringent wash conditions to a nucleic acid comprising the nucleotide
sequence of SEQ ID NO: 2.

6. The nucleic acid of claim 5, wherein said nucleic acid hybridizes
under stringent wash conditions to a nucleic acid comprising the nucleotide sequence of
SEQ ID NO: 2.

7. The nucleic acid of claim 1, wherein said nucleic acid is from a
mouse.

8. An expression cassette comprising the nucleic acid of claim 1.
9. An isolated eukaryotic cell comprising the expression cassette of claim 8.

10. An isolated estrogen-regulated myo-inositol phosphate synthase protein, said protein having at least one of the following characteristics:
(1) comprising at least about 90% amino acid sequence identity to SEQ ID NO: 1; or
(2) specifically binding to polyclonal antibodies generated against a polypeptide comprising the amino acid sequence of SEQ ID NO: 1, but not to polyclonal antibodies generated against a polypeptide comprising the amino acid sequence of SEQ ID NO: 3;
wherein said protein does not comprise an amino acid sequence of SEQ ID NO: 4.

11. The protein of claim 10, wherein said protein comprises the amino acid sequence of SEQ ID NO: 1.

12. The protein of claim 10, wherein said protein is from a mouse.


14. A method of modulating estrogen receptor α-specific estrogen signaling in a mammalian cell, said method comprising modulating the level of expression or activity of an estrogen-regulated myo-inositol phosphate synthase protein in said cell, said estrogen regulated myo-inositol phosphate synthase comprising at least about 70% amino acid sequence identity to SEQ ID NO: 1 or 3.

15. The method of claim 14, wherein said level of expression of said estrogen-regulated myo-inositol phosphate synthase protein is modulated by introducing a polynucleotide into said cell, whereby the presence or expression of said polynucleotide modulates said level of expression of said estrogen-regulated myo-inositol phosphate synthase protein.

16. The method of claim 15, wherein said polynucleotide encodes a full-length estrogen-regulated myo-inositol phosphate synthase protein, and wherein
expression of said polynucleotide increases said level of expression of said estrogen-regulated myo-inositol phosphate synthase protein in said cell.

17. The method of claim 15, wherein said polynucleotide is an antisense sequence, and wherein the presence or expression of said polynucleotide decreases said level of expression of said estrogen-regulated myo-inositol phosphate synthase protein.

18. The method of claim 14, wherein a compound is administered to said cell, whereby said level of said expression or activity of said estrogen-regulated myo-inositol phosphate synthase protein is modulated.

19. The method of claim 14, wherein said cell is present in a mammal.

20. The method of claim 19, wherein said level of expression or activity of said estrogen-regulated myo-inositol phosphate synthase protein is increased, whereby the development of atherosclerosis or osteoporosis in said mammal is inhibited.

21. A method of detecting the presence of estrogen receptor α-specific estrogen signaling in a mammalian cell, said method comprising detecting the expression of an estrogen-regulated myo-inositol phosphate synthase nucleic acid in said cell, said nucleic acid comprising at least about 70% nucleotide sequence identity to SEQ ID NO: 2 or 4.

22. The method of claim 21, wherein said presence of estrogen signaling in said cell is detected in order to determine the responsiveness of said cell to estrogen.

23. The method of claim 21, wherein said presence of estrogen signaling in said cell is detected in order to determine the tissue-specific distribution of estrogen signaling in a mammal.

24. The method of claim 21, wherein said expression of said nucleic acid in said cell is detected by detecting the expression or activity of an estrogen regulated myo-inositol phosphate synthase protein in said cell.
25. The method of claim 21, wherein said expression of said nucleic acid in said cell is detected by detecting the level of estrogen-regulated myo-inositol phosphate synthase mRNA in said cell.

26. A method of identifying a compound capable of acting as an estrogen-receptor α agonist or antagonist, the method comprising:
   (1) contacting a cell comprising an estrogen receptor α with said compound; and
   (2) detecting the functional effect of said compound on said cell, wherein an increase in the level of estrogen-regulated myo-inositol phosphate synthase mRNA, protein, or protein activity in said cell indicates that said compound is capable of acting as an estrogen receptor α agonist, wherein an decrease in the level of estrogen-regulated myo-inositol phosphate synthase mRNA, protein, or protein activity in said cell indicates that said compound is capable of acting as an estrogen receptor α antagonist.

27. The method of claim 26, wherein said estrogen-regulated myo-inositol phosphate synthase mRNA is at least about 70% identical to SEQ ID NO: 2 or 4, and wherein said estrogen-regulated myo-inositol phosphate synthase protein is at least about 70% identical to SEQ ID NO: 1 or 3.
Figure 2. Predicted protein sequence of mMIPS

1  MEPAAEILVD SPDVYSPET IEARYEYRTT RVSREGGVLR VQPRATRFTF
51  RTARQVPRLG VMLVGWGGNN GSTLTAAVLA NRLRALTWPTR TGRKEANYYG
101  SLTQAGTVNL GLOENGREVF VPFSAIILP MV APNDLVFDGW DISSLNLLEA
151  MRRAQVLDG LQEQLWPHME SLRPRPSVYI PEFIAANQTA RADNLIPGTR
201  AQQLEQIRKD IRDFRSSAGL DKVIVLWTAN TERFCEVVPG RNDTAENLLH
251  TQLGLVESP STLFAVASIL EDCAFNGSP QNTLVPAGALE LASQRHVFVG
301  GDDFKSGQTK VKSVLVDFLI GSGLKTMSIV SYNHLGNNDG QNLSAPLQFR
351  SKEVTKSSV DDMVHSNHVL YAPGERPDHC VVIKVYPYVG DSKRALDEYT
401  SELMLGGTNT LVHLNTCEDS LLAAPIMLDDL ALLTELQVRV SFCTDSDEPP
451  QGFHTVLSLL SFLFKAPLVP PGSPVNAVF RQRSCIENIF RACVGLPPQN
501  HMLEHKMER PGPGIKPGEV VATSPLLCKK EPTPATNGCT GDANGHPQAP
551  TPKLSTA*
Figure 3. cDNA sequence of mMIPS (variant 1)

1  CTGCCGCAGT CTGATCCAAC TCTGGGCCGC TGCCACGGAT GGAGCCCCGC
51  GCGGAGATCC TCGTTGGATAG CCCGGACGTG GTCTACAGCC CCGAAACCAT
101  CGAGGCGGCGC TACGAGTACC GGCAAACGGC CGTCAGGGGC GAGGGCGGCG
151  TGCTGGGCGT GCAGCCCCAG GCTACGGTTC TCACCTTCCG CACCGCAGGG
201  CAGGTGCCCC GACTCGGGGT CATGTTGGTC GGCTGGGGGC GGAACAACGG
251  CTCCACGCTC ACTGCTGCTG TTCTGGCCAA TGCGCTGCGC CTAAACCTGGC
301  CCACGGCGAC AGGTCGCAAG GAGGCAAACCT ATTATGGATC GTTGACCCAG
351  GCGGCGGCGCG TGAATCGGGG TCTGGATAG AAGGCCTGGG AGGTTGGTTG
401  GCCCTTCAGT GGGCGTACTG CCATGGTGGC CCCAACGAC CGTGGGTTTG
451  ATGGCTGGGA TTTCTGGTCG CTGAACCTGG CGAGGGCAGT CGGGCGGGCG
501  CAGGTCTCTGG ACTGCGGTCT GCAGGAACAG CTGTGGCCCC ACATGGAAGG
551  CCTCGTCGCG CGGCCCCTCAG TCTACATTCG TGATTTACG CTGGCCAACC
601  AGACAGCACG TGCCGACAAC CTCATCCCTG GCACACGTGC CCCACAGGGG
651  GAGCAAAATCC GAAAGGACAT TAGAATTTC CGATCCAGTG GGGGATGGA
701  TAAAGTCTACG GTCGGTGAGA CCGCCATAC CGAGGGCGTT CGGAGGTTG
751  TCCCGAGTGC CAATGACAACA GCAGAAAACC TGCTACATAC TATCAGCTT
801  GGCCTGAGGA TGTCACCGTC CACACATTTT GCTGGGCCA GCAATCTGGA
851  GGACTCGGCC TACTCTCTATG GATCCCCACA GAACACACTG GTACCGGGTG
901  CCCTGGAACGT GGCTCGGCAG CGCCTGTGTT TGTTAGGGTG TGATGACTTC
951  AAGTCAGGGC AGACTAAAGGT CAAGTCTCTC TGCTGGGACT CTCTCATCAG
1001  CTCTGGGCTC ACAAGCTATG CCATCGTGAG CTATACCAAC CTGGGCAACA
1051  ACGACGGCGA GAACCTGCTG GCACCGCTGC AGTCCCGCTC CAAGGAGGTTG
1101  ACAAGAGGCA GTGTGGGGGA CGACATGGTT CACAGCAACC ATGTGCTCTA
1151   CGCGCCTGGA GAGCGGCCAG ACCACTGTGT GGTGATCAAA TATGTGCCCT
1201   ATGTTGCGCA CAGCAAGCGT GCCCTGGAGC AGTACACCTC CGAGCTGTATG
1251   CTGGGTGGGA CAAACACCTT GGTGCCCAT AATACCTGCG AGGATTCGGT
1301   CCTGCGCCCGG CCCATCATGC TGGACCTAGC GCTGCTCACA GAGCTGTGTC
1351   AGCCGCGTGAG CTTCTGCACA GACTCGGACC CCGAGCCTCA GGGCTTCCAC
1401   ACAGTGCTGT GCCGTCTTAG CTTCTGTATT AAGGCCGCCG TTGTGCCCCC
1451   GGGCAGCGCT GTAGTGAAATG CCCTCTTCG CCAAGCGACG TGTATCGAGA
1501   ATATTTTCAG GGCTTGCGTG GGGCTCCCGC CCCAGAACCA CATGCTATTA
1551   GAGCACAAGA TGGAGCGTCC AGGCCAGGCC ATCAAGCCAG GAGAGGTGTG
1601   GGCCACCAGC CCACGCCTTT GCAAGAAAAGA GCCACACCTT GCCACCAATG
1651   GCTGCACCGG CGATGCCAAT GGGCAACCGC AGGCACCAAC ACACAAAGCTG
1701   TCCACTGCTT AAATCAAGTG ACCGGACCAG ACCCTCTAC CCCCAATGCC
1751   TCCAACCATA GTACTTCAGC CTTGGCCAAA GACAAATAAG CAAGTCTCAA
1801   GCTAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAA
FIGURE 4
Regulation of mIPS Genes by Estrogen.
Northern hybridization of liver RNA from WT and ERKO mice treated with vehicle (V), 17β-estradiol (E2), and antiestrogen ZK compound. The mouse inositol 1-phosphate synthase genes was up-regulated by E2 in WT mouse.
Regulation of MITPS Expression in BERKO Mice

FIGURE 7
SEQUENCE LISTING

SEQ ID NO:1

amino acid sequence of mMIPS

1
MEPAAEILVD SPDVYSPET IEARYEYRTT RVSREGGVLRL QPRATRFTF
51
RTARQVPRLG VMLVGWGNN GSTLTAALVA NRLRTLWPTR TGRKEANYYG
101
SLTQAGTVNL GLDENGREVF VPFSALLPMV APNDLVFDGW DISLLNLAEAA
151
MRRAQVLDCCG LQEBLWHPHME SLRPSPSYI PEPIAANQTA RADNLIPGTR
201
AQOLEQIRKD IRDFRSSAGL DKVIVLWTAN TERFCEVVPQ RANDTAENLLLH
251
TQLGLGVEGPS STLFAVASIL EDCAFMLNPS QNLVPQAGLE LASQHVFVGF
301
GDFFKSGQTK VKSULVDFFLI GSSLKMTSV SYNHGLQNDG QNLSAPLQFR
351
SKEVTSSSVV DDMVSHSNHVL YAPGERPDHC VVIKYIPYVG DSKRALDEYT
401
SELMLGGTN T LVLHNTCEDS LLAAPIMDDL ALLTELQRV SFCTDSDEPE
451
QGFHTVLSLL SFLFKAPLVP PGSPVNLALF QRQSCIENIF RACVGLPPQN
501
HMLLEHKMER PGPGKIPGEV VATSLPCKK EPTPATNGCT GDANGHPQAP
551
TPKLSTA*

SEQ ID NO:2

cDNA sequence of mMIPS, variant 1

1
CTGCCGCAGT CTGATCCAAC TCTGCGCGGC TGCCACGAT GAGGCCCGCC
51
GCGAGATTCC TCGTGGTAG TCCCGACCTG GCTACAGGCG CCGAACCAAT
101
CGAGGCAGCG TACAGCTACC GGAACACCGG CGTCAGCCCG GAGGGCCGGG
151
TGCTGCGGTT GCAGCCACAG GCTACGCGGT TCACCTTGCG CACCGCCCGG
201
CAGGTCGCCCA GACTGCCGTT CATGTGGCTG GCTGGGCGGC GGAACAACCG
251
CTCCAGCCCG ACTGCTGCGT TTCTGCGCGA TCGTGGCCCG CTAACTCCGG
301
CACCGCCCGAG AGTGCAGCAAG GAGGCAACT ATATCGATGC GGGACCCAG
351
GCGGGCAGCC TGAACCTTGGG TCTGGATGAG AACCGCCCGGG AGGTGGTTTGT
401
GCGCTCCAGT GCACCTGCAG CCACTGGGGG CCCAACACGAC CTGGTGTCCG
451
ATGGCTGGAA TATCTGCAGT CGAAGCCGGC CGAGGCGCAT GCGGCACGCG
501
CAGTGCTCCA GACTGCCGCG TGAGCAACAG CTCGCGCGCC ACATGGAGAG
551
CTCGCGCTCC CGGCCCTCGA TCTAcATTCC TGAGTCTACG GCTGGCACC
SEQ ID NO:3

30  amino acid sequence of human MIPS

MEAAAQFFVESPDVYGPAIEAQUEYRTTRVSVREGGVLKHPSTRFTFRTARQVPRLG
VMLVGVGNNGSTLTAVALANRLRSLPRTSGKEANYYYYGLSFTQGTVSGLDAEGQBYFV
VPFSALPVMVPNDLVFWDGISLNLAEAMRRAKVLVWGLQEQLWHAMEALRPRSPVYI
SEQUENCE ID NO: 4

nucleotide sequence of human MIPS

CCGCCTGTCCGCCCGCCGTCCCTGAGCTGACTCTGCGCCGCCTGGCCGATGGAGGCCG
CCGCCAGTTCTTCGTGAGAGCCCGGACGTTGCTACGGCCCCGAGCCATCGAGGCG
AATACGAGTACGGACGACCCTCCGTCAGCGGCAGGGTGGCCGTTCTCAAGGTCACCCCA
CTCCACGCCTTCACCCCTGACCAGCCGGCCGAGGGTGGCTCGCTCTGCTGTTCTG
TCGCTGAGGGCAGGAAACCGTGCTCAACTCAGCCGCGCTGCTGCGCAATGACTGC
GTTCGTCTGGGCCCACGGCAGCAGGCCGCCAGGGGAGGCGCCACTACGCTGGCTGACTC
AGGCAGGGCACCCTGAGCCCTGAGGCTGGACGCGAGGCGGGTGCAGGGTCTGGCTCAGAGC
AACGTGCGCCCACTGAGGGCCCTCGGCCGCCGCTCATTGCTGCTGAAGAGATGGCTGG
CAGCGGAACCTGGCGGCCGATGGCGCCGCGCAGGAAGGTGGCTGGAGCTAGGGGTCTGGCTGCTGAGG
TAGTGCTGCTGGACGCGCAGCTTCCTGCTGAGAGGGTGGCTGCTTCTCAATGGTCTGCGCAGAACCC
TGGCTGCCCAACCGACGAGGGCGCCGAGGCCAACCATTACCTACCTCCAGGTGCCGTCGTGCGCAGACG
TGAGAGCAGATCCGAGCAGATCCGAGACTTCCTGCCGCGGCTGACAAAGTCTA
TCAAGGACCATGCTCCATCGTGAGTTCAACCAACATCGGGCAACACGATGGGGGAAACCATAT
CGGCCCAATTGCAATCTCGCTGCAACCATTGGCTGCTGGAGGTGCTGCGCCTCTCGGCTCTCTC
TCGCGCTGGCCAGACATCCTGGAGGGCTGGCTCCTCTCCTCAATGGTCTGCGCAGAACCC
TGGTGCCGCCAGCTCTGGAGCTGGCTGGACGACCGGGTGGTTTTTGTCGGCGGAGATGACTG
TCAGAGCAGCAGCCGACATAAGCTCACTGGCTGCTGAGAGGGTGGCCTTCTTCTCAGTCTGCGGCC
CACCCATCATGCTGGACCTAGCGTGCCTGAGCCGACCGCTGACGTTCGGCA
CTGACATGGACCCCGAGCGCCAGCCTTCCCACCCCGTGCTGCCTCTGCTACTTCCCTCT
TCAAGCGCCACTAGTGGCCCGCCGCCGACCGCGGTGCTCAATTGCGCTTTTCCGCCAGCGCA
GCTGCTAGAAACATGCTTCAAGCCTGGGTGCGCTGGCCCGCCACAACAGAAACATGCTCC
TGAAACACAAATAGGAGCAGCGCCAGGGCGAGCCTCAAGCAGTTGAGCCCTGGCTGCAA
CCTACCCTATGTTGAAACAGAAAGGACCAGTGACTCCGTCGCAACCAATGCGTGCAACGGT
ATGCCAATGGGCATCTGCAAGAGAGCAGCGCCCAATGCGCACCACACCTGAGGCCGCCGTCA
CAGTTTCTCGCTCTTCTCCTCCCGCTGCCCGCCACAGCCTACCTTGAGGCCGCCCAACA
ATAAAGGCGCTGCGACTCACGCCCTGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

SEQ ID NO:5

sequence of human MIPS promoter

GGCGAGGAAGGTGGAGAGACTGAGATTCTGAGCTTTTCCGATGCCTTAGCTCAAGAGCGG
GGGCAGGGCAAGGGCCCCCCATAACTTGAGGGCTGAGGGAGTTTCTGTTTCTGAGGCCCAAC
TGCTCTCATTTGCGATAATAGTTGATTAAATCTCTTGAGCGACTGGAGTTGAGCTCAATGT
CTAACCAAGAAGGAGAACAACAAACCAAAATACCTTCTTTCAACTCCTCTATCCCGAGGCCC
AGGTCTGGCCCAAAGACCCAAAATCCTCCCAACGTAGCAGAAATTTTCTATGCTGCTAC
GGTGCTCGATGCTTGGGCTTTGAGTTTTTTCTTCTCAGTGAACCCATCCTCTCTCATGCTCAAA
ACCTTGGCCAGCAGCCCCCTCATACTTGGAGCCAGCTGCTTGCAGGGGCCTGGCCTGATTG
CCACCCACTGGGGCCGGGTTGCTCCAGACTCCACAACTTCTTCACTTGAGACFTGGGCGCTCC
CAGGGTGAGGCTTGGAGCCAGAACAGTCTTAAGCCTCTCATCCTCCAAGTGCCCCGCTGTCTCT
TGCAAAACGTCTTTCCAACATACACTCCTTTTGGCCAGCTCAGTGGTGGAATCTTTCCGGCC
CCAGGGCCTGTTGGGAGGAGCTGCGCCCGGCCTGCCGGCTGGTCCCA
CGTCTCCTATCAGCGCGCATAGGGGGGCGCCCTGCGTACGGCCGCTATTCTCTCCCTCCCTG
CCCGCGCTGCGGTCGTCCCCTCTCTCTTCCAGCGAAGAGCCCTCCCGCCTGCCG
CCACAGCCTGCGCTGCTGCTGGGAAAAACAGGCCAACAGGCCGCACCTCTTT
GTGAGCCCGCTGGGAGAAGAGTGGACTTTTGGCTCCCGAGTAGGTAGGCTGAGGTA
GCCAGGGATGGAGCTGCTGCTGCTGCCCCAGAGGGGAGGTAGTGTCAGTGTGT
GGCTCGAGAGATCTGCTTTGAGTTTCTGCGTCTGCTCTCTTCTCTCTGGCTGAGGTCGTTCT
CTGACCAGAGCCCCTCTACTCCTCCCCCAGGGGGCAGCTCTCTTCGCCCCGACECTCACC
CTGCTGTTGCTGGCTTGGCTGCTGTAGGCTTTTCTGCTCGTTAAGGGGGGAGATGGCAG
CCATGAGCCCGCTGGGGCTGCAGCGAGCGAGGTGAACCTATACGCCGCCGGCTGCTGGA
AGCCTGGCCACTTCCGCCAGGCGGGGACCCGATGGCCACGGTGCTGTGTTGTCCGGCC
TCGAACCGGGTCTCCAGACGTGTTGGGGCCTGCCCTGCCAGCGGAGAAGGCGGGGAC
GGGGGGGGGAGGGGGCGCAGCGGCTTTCCTGGAGGTCGCGCAGCCGAGGGCCGGCCGC
TGTCGCCGCCGCTGCTGAGTCGACTCTGCACCGGGTG