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(54) Title: ADIPOCYTE COMPLEMENT RELATED PROTEIN ZACRP11

(57) Abstract: The present invention relates to polynucleotide and polypeptide molecules for zacrp11, a novel member of the family of proteins bearing a collagen-like domain and a C1q domain. Novel zacrp11 polypeptides, polynucleotides encoding the polypeptides, and related compositions and methods are disclosed. Also disclosed are antibodies to the zacrp11 protein or fragments thereof.
ADIPOCYTE COMPLEMENT RELATED PROTEIN ZACRP11

BACKGROUND OF THE INVENTION

Cell-cell and cell-extracellular matrix interactions allow for exchange of information between, and coordination among, various cells of a multi-cellular organism and are fundamental for most biological processes. These interactions play a role in everything from fertilization to death. Such interactions are essential during development and differentiation and are critical for the function and protection of the organism. For example, interaction between the cell and its environment is necessary to initiate and mediate tissue remodeling. Tissue remodeling may be initiated, for example, in response to many factors including physical injury, cytotoxic injury, metabolic stress or developmental stimuli. Modulation between pathology and healing (or metabolic optimization) may be done, in part, by the interaction of stimulated cells with the extracellular matrix as well as the local solvent.

The adipocyte complement related protein family plays a role in the interaction of cells with their environment, and appear to act at the interface of the extracellular matrix and the cell. These proteins include, Acrp30 (Scherer et al., J. Biol. Chem. 270:26746-49, 1995), apM1 (Maeda et al., Biochem. Biophys. Res. Comm., 221:286-9, 1996), GBP28 (Nakano et al., J. Biochem. 120:803-12, 1996), zsig39 (Sheppard and Humes, WIPO Published Patent No: WO99/10492), zsig37 (Sheppard, WIPO Published Patent No: WO99/04000), ZCRP30R1 (Smith et al., WIPO Published Patent No. WO99/56619), ACRP30R1L (Hensley et al., WIPO Published Patent No: WO99/59618), ACRP30R2 (Hensley et al., WIPO Published Patent No: WO99/64629), PRO 353 and PRO 344 (Wood et al., WIPO Published Patent No. WO99/28462), zacrp2 (Piddington et al., WO 00/63376), zacrp3 (Piddington et al., WO 00/63377), zacrp4 (Piddington et al., WO 01/02565), zacrp5 (Piddington et al., WO 00/73444), zacrp6 (Piddington et al., WO 00/73466), and zacrp12 (Piddington et al., WO 00/****).
These proteins all share a collagen-like domain comprising perfect Gly-Xaa-Pro and imperfect Gly-Xaa-Xaa collagen repeats, and a C1q domain. Complement factor C1q consists of six copies of three related polypeptides (A, B and C chains), with each polypeptide being about 225 amino acids long with a near amino-terminal collagen domain and a carboxy-terminal globular region. Six triple helical regions are formed by the collagen domains of the six A, six B and six C chains, forming a central region and six stalks. A globular head portion is formed by association of the globular carboxy terminal domain of an A, a B and a C chain. C1q is composed of six globular heads linked via six collagen-like stalks to a central fibril region. Sellar et al., Biochem. J. 274: 481-90, 1991. This configuration is often referred to as a bouquet of flowers. Acrp30 has a similar bouquet structure formed from a single type of polypeptide chain. The C1q globular domain of ACRP30 has been determined to have a 10 beta strand “jelly roll” topology (Shapiro and Scherer, Curr. Biol. 8:335-8, 1998). The structural elements such as folding topologies, conserved residues and similar trimer interfaces and intron positions are homologous to the tumor necrosis factor family suggesting a link between the TNF and C1q families.

Proteins that play a role in cellular interaction, such as transcription factors and hormones are useful diagnostic and therapeutic agents. Proteins that mediate specific interactions, such a remodeling, would be particularly useful. The present invention provides such polypeptides for these and other uses that should be apparent to those skilled in the art from the teachings herein.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a novel adipocyte complement related protein, designated “zacrp11”. The present invention also provides “zacrp11” variant polypeptides and “zacrp11” fusion proteins, as well as nucleic acid molecules encoding such polypeptides and proteins, and methods for using these nucleic acid molecules and amino acid sequences.
Within one aspect, the present invention provides a polypeptide selected from the group consisting of: (a) a polypeptide comprising amino acid residues 61-111 of SEQ ID NO:2; (b) a polypeptide comprising amino acid residues 21-111 of SEQ ID NO:2; (c) a polypeptide comprising amino acid residues 1-111 of SEQ ID NO:2; (d) a polypeptide comprising amino acid residues 112-219 of SEQ ID NO:2; (e) a polypeptide comprising amino acid residues 112-268 of SEQ ID NO:2; (f) a polypeptide comprising amino acid residues 61-219 of SEQ ID NO:2; (g) a polypeptide comprising amino acid residues 61-268 of SEQ ID NO:2; (h) a polypeptide comprising amino acid residues 21-219 of SEQ ID NO:2; (i) a polypeptide comprising amino acid residues 21-268 of SEQ ID NO:2; (j) a polypeptide comprising amino acid residues 1-219 of SEQ ID NO:2; and (k) a polypeptide comprising amino acid residues 1-268 of SEQ ID NO:2. In one embodiment, the polypeptide described above further comprises a moiety selected from the group consisting of: affinity tags, toxins, radionucleotides, enzymes, and fluorophores.

Within a second aspect, the present invention provides a fusion protein comprising a first portion and a second portion joined by a peptide bond, the first portion consisting of a polypeptide selected from the group consisting of: (a) a polypeptide comprising amino acid residues 61-111 of SEQ ID NO:2; (b) a polypeptide comprising amino acid residues 21-111 of SEQ ID NO:2; (c) a polypeptide comprising amino acid residues 1-111 of SEQ ID NO:2; (d) a polypeptide comprising amino acid residues 112-219 of SEQ ID NO:2; (e) a polypeptide comprising amino acid residues 112-268 of SEQ ID NO:2; (f) a polypeptide comprising amino acid residues 61-219 of SEQ ID NO:2; (g) a polypeptide comprising amino acid residues 61-268 of SEQ ID NO:2; (h) a polypeptide comprising amino acid residues 21-219 of SEQ ID NO:2; (i) a polypeptide comprising amino acid residues 21-268 of SEQ ID NO:2; (j) a polypeptide comprising amino acid residues 1-219 of SEQ ID NO:2; and (k) a polypeptide comprising amino acid residues 1-268 of SEQ ID NO:2; and the second portion comprising another polypeptide. In one embodiment, the fusion protein is as described above, wherein the second portion is a collagen-like domain or a C1Q domain from an adipocyte complement related protein. In another embodiment, the polypeptide described above is selected from the group consisting of: (a) a polypeptide consisting of
amino acid residues 61-111 of SEQ ID NO:2; (b) a polypeptide consisting of amino acid residues 21-111 of SEQ ID NO:2; (c) a polypeptide consisting of amino acid residues 1-111 of SEQ ID NO:2; (d) a polypeptide consisting of amino acid residues 112-219 of SEQ ID NO:2; (e) a polypeptide consisting of amino acid residues 112-268 of SEQ ID NO:2; (f) a polypeptide consisting of amino acid residues 61-219 of SEQ ID NO:2; (g) a polypeptide consisting of amino acid residues 61-268 of SEQ ID NO:2; (h) a polypeptide consisting of amino acid residues 21-219 of SEQ ID NO:2; (i) a polypeptide consisting of amino acid residues 21-268 of SEQ ID NO:2; (j) a polypeptide consisting of amino acid residues 1-219 of SEQ ID NO:2; and (k) a polypeptide consisting of amino acid residues 1-268 of SEQ ID NO:2.

Within another aspect, the present invention provides an isolated nucleic acid molecule encoding a polypeptide selected from the group consisting of: (a) a polypeptide comprising amino acid residues 61-111 of SEQ ID NO:2; (b) a polypeptide comprising amino acid residues 21-111 of SEQ ID NO:2; (c) a polypeptide comprising amino acid residues 1-111 of SEQ ID NO:2; (d) a polypeptide comprising amino acid residues 112-219 of SEQ ID NO:2; (e) a polypeptide comprising amino acid residues 112-268 of SEQ ID NO:2; (f) a polypeptide comprising amino acid residues 61-219 of SEQ ID NO:2; (g) a polypeptide comprising amino acid residues 61-268 of SEQ ID NO:2; (h) a polypeptide comprising amino acid residues 21-219 of SEQ ID NO:2; (i) a polypeptide comprising amino acid residues 21-268 of SEQ ID NO:2; (j) a polypeptide comprising amino acid residues 1-219 of SEQ ID NO:2; and (k) a polypeptide comprising amino acid residues 1-268 of SEQ ID NO:2. In one embodiment, the isolated nucleic acid molecule encoding a polypeptide is as disclosed above, wherein the polypeptide further comprises a moiety selected from the group consisting of: affinity tags, toxins, radionucleotides, enzymes, and fluorophores.

Within another aspect, the present invention provides a nucleic acid molecule encoding a fusion protein comprising a first portion and a second portion joined by a peptide bond, the first portion consisting of a polypeptide selected from the group consisting of: (a) a polypeptide comprising amino acid residues 61-111 of SEQ ID NO:2; (b) a polypeptide comprising amino acid residues 21-111 of SEQ ID NO:2; (c) a polypeptide comprising amino acid residues 1-111 of SEQ ID NO:2; (d) a
polypeptide comprising amino acid residues 112-219 of SEQ ID NO:2; (e) a polypeptide comprising amino acid residues 112-268 of SEQ ID NO:2; (f) a polypeptide comprising amino acid residues 61-219 of SEQ ID NO:2; (g) a polypeptide comprising amino acid residues 61-268 of SEQ ID NO:2; (h) a polypeptide comprising amino acid residues 21-219 of SEQ ID NO:2; (i) a polypeptide comprising amino acid residues 21-268 of SEQ ID NO:2; (j) a polypeptide comprising amino acid residues 1-219 of SEQ ID NO:2; and (k) a polypeptide comprising amino acid residues 1-268 of SEQ ID NO:2; and the second portion comprising another polypeptide. In one embodiment the nucleic acid molecule encoding a fusion protein is as disclosed above, wherein the second portion is a collagen-like domain or a CIQ domain from an adipocyte complement related protein. In another embodiment the isolated nucleic acid molecule is as disclosed above, wherein the nucleic acid encodes a polypeptide selected from the group consisting of: (a) a polypeptide consisting of amino acid residues 61-111 of SEQ ID NO:2; (b) a polypeptide consisting of amino acid residues 21-111 of SEQ ID NO:2; (c) a polypeptide consisting of amino acid residues 1-111 of SEQ ID NO:2; (d) a polypeptide consisting of amino acid residues 112-219 of SEQ ID NO:2; (e) a polypeptide consisting of amino acid residues 112-268 of SEQ ID NO:2; (f) a polypeptide consisting of amino acid residues 61-219 of SEQ ID NO:2; (g) a polypeptide consisting of amino acid residues 61-268 of SEQ ID NO:2; (h) a polypeptide consisting of amino acid residues 21-219 of SEQ ID NO:2; (i) a polypeptide consisting of amino acid residues 21-268 of SEQ ID NO:2; (j) a polypeptide consisting of amino acid residues 1-219 of SEQ ID NO:2; and (k) a polypeptide consisting of amino acid residues 1-268 of SEQ ID NO:2.

Within another aspect, the present invention provides an isolated nucleic acid molecule selected from the group consisting of: a) a nucleic acid molecule consisting of nucleotides 181-333 of SEQ ID NO:1; b) a nucleic acid molecule consisting of nucleotides 61-333 of SEQ ID NO:1; c) a nucleic acid molecule consisting of nucleotides 1-333 of SEQ ID NO:1; d) a nucleic acid molecule consisting of nucleotides 334-657 of SEQ ID NO:1; e) a nucleic acid molecule consisting of nucleotides 334-804 of SEQ ID NO:1; f) a nucleic acid molecule consisting of nucleotides 118-657 of SEQ ID NO:1; g) a nucleic acid molecule consisting of
nucleotides 118-804 of SEQ ID NO:1; h) a nucleic acid molecule consisting of nucleotides 64-657 of SEQ ID NO:1; i) a nucleic acid molecule consisting of nucleotides 64-804 of SEQ ID NO:1; j) a nucleic acid molecule consisting of nucleotides 1-657 of SEQ ID NO:1; k) a nucleic acid molecule consisting of nucleotides 1-804 of SEQ ID NO:1; and l) a nucleic acid molecule consisting of SEQ ID NO:3.

Within another aspect, the present invention provides an expression vector comprising the following operably linked elements: a transcription promoter; a DNA segment encoding a polypeptide with an amino acid sequence consisting of: (a) amino acid residues 61-111 of SEQ ID NO:2; (b) amino acid residues 21-111 of SEQ ID NO:2; (c) amino acid residues 1-111 of SEQ ID NO:2; (d) acid residues 112-219 of SEQ ID NO:2; (e) amino acid residues 112-268 of SEQ ID NO:2; (f) amino acid residues 61-219 of SEQ ID NO:2; (g) amino acid residues 61-268 of SEQ ID NO:2; (h) amino acid residues 21-219 of SEQ ID NO:2; (i) amino acid residues 21-268 of SEQ ID NO:2; (j) amino acid residues 1-219 of SEQ ID NO:2; and (k) amino acid residues 1-268 of SEQ ID NO:2; and a transcription terminator. In one embodiment, the expression vector disclosed above further comprises a secretory signal sequence operably linked to the DNA segment.

Within another aspect, the present invention provides a cultured cell into which has been introduced an expression vector as disclosed above, wherein the cell expresses a polypeptide encoded by the DNA segment.

Within another aspect, the present invention provides a method of producing a polypeptide comprising: culturing a cell as disclosed above; and isolating the polypeptide produced by the cell.

Within another aspect, the present invention provides a method of producing an antibody to a polypeptide comprising: inoculating an animal with a polypeptide selected from the group consisting of: (a) a polypeptide consisting of 9 to 252 amino acids, wherein the polypeptide is a contiguous sequence of amino acids in SEQ ID NO:2 from amino acid residue 1 to amino acid residue 268; (b) a polypeptide consisting of amino acid residues 61-111 of SEQ ID NO:2; (c) a polypeptide consisting of amino acid residues 21-111 of SEQ ID NO:2; (d) a polypeptide consisting of amino
acid residues 1-111 of SEQ ID NO:2; (e) a polypeptide consisting of amino acid residues 112-219 of SEQ ID NO:2; (f) a polypeptide consisting of amino acid residues 112-268 of SEQ ID NO:2; (g) a polypeptide consisting of amino acid residues 61-219 of SEQ ID NO:2; (h) a polypeptide consisting of amino acid residues 61-268 of SEQ ID NO:2; (i) a polypeptide consisting of amino acid residues 21-219 of SEQ ID NO:2; (j) a polypeptide consisting of amino acid residues 21-268 of SEQ ID NO:2; (k) a polypeptide consisting of amino acid residues 1-219 of SEQ ID NO:2; and (l) a polypeptide consisting of amino acid residues 1-268 of SEQ ID NO:2; and wherein the polypeptide elicits an immune response in the animal to produce the antibody; and isolating the antibody from the animal. Within another aspect, the present invention provides an antibody produced by the method as disclosed above, which binds to a polypeptide of SEQ ID NO:2. In one embodiment, the antibody disclosed above is selected from the group consisting of: (a) polyclonal antibody; (b) murine monoclonal antibody; (c) humanized antibody derived from b); (d) an antibody fragment; and (e) human monoclonal antibody. In another embodiment, the antibody fragment is as disclosed above, wherein the antibody fragment is selected from the group consisting of F(ab’), F(ab), Fab’, Fab, Fv, scFv, and minimal recognition unit. Within another aspect, the present invention provides an anti-idiotypic antibody that specifically binds to the antibody as disclosed above. Within another aspect, the present invention provides an antibody that specifically binds to a polypeptide as disclosed above.

The present invention provides nucleic acid molecules that encode a novel adipocyte complement related protein, designated as “zacrp11.” An illustrative nucleotide sequence that encodes zacrp11 is provided by SEQ ID NO:1. The encoded polypeptide has the amino acid sequence of SEQ ID NO:2. Thus, the zacrp11 gene described herein encodes a polypeptide of 252 amino acids, as shown in SEQ ID NO:2.

An illustrative polypeptide is a polypeptide that comprises the amino acid sequence of SEQ ID NO:2.

The present invention further provides antibodies and antibody fragments that specifically bind with such polypeptides. Exemplary antibodies include polyclonal antibodies, murine monoclonal antibodies, humanized antibodies derived
from murine monoclonal antibodies, and human monoclonal antibodies. Illustrative antibody fragments include F(ab')₂, F(ab)₂, Fab', Fab, Fv, scFv, and minimal recognition units. The present invention further includes compositions comprising a carrier and a peptide, polypeptide, or antibody described herein.

The present invention also provides isolated nucleic acid molecules that encode a zacrpl polypeptide, wherein the nucleic acid molecule is selected from the group consisting of: a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:3; a nucleic acid molecule encoding the amino acid sequence of SEQ ID NO:2; and a nucleic acid molecule that remains hybridized following stringent wash conditions to a nucleic acid molecule consisting of a nucleotide sequence selected from the group consisting of: (a) the nucleotide sequence of SEQ ID NO:3, (b) the nucleotide encoding the polypeptide of SEQ ID NO:2, and (c) a nucleotide sequence that is the complement of the nucleotide sequence of (a) or (b).

The present invention further contemplates an isolated nucleic acid molecule that comprise the nucleotide sequence of SEQ ID NO:1.

The present invention also includes vectors and expression vectors comprising such nucleic acid molecules. Such expression vectors may comprise a transcription promoter, and a transcription terminator, wherein the promoter is operably linked with the nucleic acid molecule, and wherein the nucleic acid molecule is operably linked with the transcription terminator. The present invention further includes recombinant host cells comprising these vectors and expression vectors. Illustrative host cells include bacterial, yeast, fungal, insect, mammalian, and plant cells. Recombinant host cells comprising such expression vectors can be used to produce zacrpl polypeptides by culturing such recombinant host cells that comprise the expression vector and that produce the zacrpl protein, and, optionally, isolating the zacrpl protein from the cultured recombinant host cells.

The present invention also contemplates methods for detecting the presence of zacrpl RNA in a biological sample, comprising the steps of (a) contacting a zacrpl nucleic acid probe under hybridizing conditions with either (i) test RNA molecules isolated from the biological sample, or (ii) nucleic acid molecules synthesized from the isolated RNA molecules, wherein the probe has a nucleotide
sequence comprising a portion of the nucleotide sequence of SEQ ID NO:1, or its complement, and (b) detecting the formation of hybrids of the nucleic acid probe and either the test RNA molecules or the synthesized nucleic acid molecules, wherein the presence of the hybrids indicates the presence of zacrp11 RNA in the biological sample. An example of a biological sample is a human biological sample, such as a biopsy or autopsy specimen.

The present invention further provides methods for detecting the presence of zacrp11 polypeptide in a biological sample, comprising the steps of: (a) contacting the biological sample with an antibody or an antibody fragment that specifically binds with a polypeptide having the amino acid sequence of SEQ ID NO:2, wherein the contacting is performed under conditions that allow the binding of the antibody or antibody fragment to the biological sample, and (b) detecting any of the bound antibody or bound antibody fragment. Such an antibody or antibody fragment may further comprise a detectable label selected from the group consisting of radioisotope, fluorescent label, chemiluminescent label, enzyme label, bioluminescent label, and colloidal gold. An exemplary biological sample is a human biological sample.

The present invention also provides kits for performing these detection methods. For example, a kit for detection of zacrp11 gene expression may comprise a container that comprises a nucleic acid molecule, wherein the nucleic acid molecule is selected from the group consisting of (a) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, (b) a nucleic acid molecule comprising the complement of the nucleotide sequence of SEQ ID NO:1, (c) a nucleic acid molecule that is a fragment of (a) consisting of at least eight nucleotides, and (d) a nucleic acid molecule that is a fragment of (b) consisting of at least eight nucleotides. Illustrative nucleic acid molecules include nucleic acid molecules comprising nucleotides 108 to 333, 349 to 756, and 108 to 756 of SEQ ID NO:1, or the complement thereof. Such a kit may also comprise a second container that comprises one or more reagents capable of indicating the presence of the nucleic acid molecule. On the other hand, a kit for detection of zacrp11 protein may comprise a container that comprises an antibody, or an
antibody fragment, that specifically binds with a polypeptide having the amino acid sequence of SEQ ID NO:2.

These and other aspects of the invention will become evident upon reference to the following detailed description. In addition, various references are identified below and are incorporated by reference in their entirety.

Definitions

In the description that follows, a number of terms are used extensively. The following definitions are provided to facilitate understanding of the invention.

As used herein, "nucleic acid" or "nucleic acid molecule" refers to polynucleotides, such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), oligonucleotides, fragments generated by the polymerase chain reaction (PCR), and fragments generated by any of ligation, scission, endonuclease action, and exonuclease action. Nucleic acid molecules can be composed of monomers that are naturally-occurring nucleotides (such as DNA and RNA), or analogs of naturally-occurring nucleotides (e.g., α-enantiomeric forms of naturally-occurring nucleotides), or a combination of both. Modified nucleotides can have alterations in sugar moieties and/or in pyrimidine or purine base moieties. Sugar modifications include, for example, replacement of one or more hydroxyl groups with halogens, alkyl groups, amines, and azido groups, or sugars can be functionalized as ethers or esters.

Moreover, the entire sugar moiety can be replaced with sterically and electronically similar structures, such as aza-sugars and carbocyclic sugar analogs. Examples of modifications in a base moiety include alkylated purines and pyrimidines, acylated purines or pyrimidines, or other well-known heterocyclic substitutes. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Analogos of phosphodiester linkages include phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilidate, phosphoramidate, and the like. The term "nucleic acid molecule" also includes so-called "peptide nucleic acids," which comprise naturally-occurring or modified nucleic acid bases attached to a polyamide backbone. Nucleic acids can be either single stranded or double stranded.
The term “complement of a nucleic acid molecule” refers to a nucleic acid molecule having a complementary nucleotide sequence and reverse orientation as compared to a reference nucleotide sequence. For example, the sequence 5' ATGCACGGG 3' is complementary to 5' CCCGTGCG 3'.

The term “degenerate nucleotide sequence” denotes a sequence of nucleotides that includes one or more degenerate codons as compared to a reference nucleic acid molecule that encodes a polypeptide. Degenerate codons contain different triplets of nucleotides, but encode the same amino acid residue (i.e., GAU and GAC triplets each encode Asp).

The term “structural gene” refers to a nucleic acid molecule that is transcribed into messenger RNA (mRNA), which is then translated into a sequence of amino acids characteristic of a specific polypeptide.

An “isolated nucleic acid molecule” is a nucleic acid molecule that is not integrated in the genomic DNA of an organism. For example, a DNA molecule that encodes a growth factor that has been separated from the genomic DNA of a cell is an isolated DNA molecule. Another example of an isolated nucleic acid molecule is a chemically-synthesized nucleic acid molecule that is not integrated in the genome of an organism. A nucleic acid molecule that has been isolated from a particular species is smaller than the complete DNA molecule of a chromosome from that species.

A “nucleic acid molecule construct” is a nucleic acid molecule, either single- or double-stranded, that has been modified through human intervention to contain segments of nucleic acid combined and juxtaposed in an arrangement not existing in nature.

“Complementary DNA (cDNA)” is a single-stranded DNA molecule that is formed from an mRNA template by the enzyme reverse transcriptase. Typically, a primer complementary to portions of mRNA is employed for the initiation of reverse transcription. Those skilled in the art also use the term “cDNA” to refer to a double-stranded DNA molecule consisting of such a single-stranded DNA molecule and its complementary DNA strand. The term “cDNA” also refers to a clone of a cDNA molecule synthesized from an RNA template.

A “promoter” is a nucleotide sequence that directs the transcription of a structural gene. Typically, a promoter is located in the 5' non-coding region of a gene, proximal to the transcriptional start site of a structural gene. Sequence elements within promoters that function in the initiation of transcription are often characterized by consensus nucleotide sequences. These promoter elements include RNA polymerase binding sites, TATA sequences, CAAT sequences, differentiation-specific elements (DSEs; McGehee et al., Mol. Endocrinol. 7:551 (1993)), cyclic AMP response elements
(CREs), serum response elements (SREs; Treisman, *Seminars in Cancer Biol.* 1:47 (1990)), glucocorticoid response elements (GREs), and binding sites for other transcription factors, such as CRE/ATF (O'Reilly et al., *J. Biol. Chem.* 267:19938 (1992)), AP2 (Ye et al., *J. Biol. Chem.* 269:25728 (1994)), SP1, cAMP response element binding protein (CREB; Loeken, *Gene Expr.* 3:253 (1993)) and octamer factors (see, in general, Watson et al., eds., *Molecular Biology of the Gene*, 4th ed. (The Benjamin/Cummings Publishing Company, Inc. 1987), and Lemaigre and Rousseau, *Biochem. J.* 303:1 (1994)). If a promoter is an inducible promoter, then the rate of transcription increases in response to an inducing agent. In contrast, the rate of transcription is not regulated by an inducing agent if the promoter is a constitutive promoter. Repressible promoters are also known.

A “core promoter” contains essential nucleotide sequences for promoter function, including the TATA box and start of transcription. By this definition, a core promoter may or may not have detectable activity in the absence of specific sequences that may enhance the activity or confer tissue specific activity.

An “enhancer” is a type of regulatory element that can increase the efficiency of transcription, regardless of the distance or orientation of the enhancer relative to the start site of transcription.

“Heterologous DNA” refers to a DNA molecule, or a population of DNA molecules, that does not exist naturally within a given host cell. DNA molecules heterologous to a particular host cell may contain DNA derived from the host cell species (*i.e.*, endogenous DNA) so long as that host DNA is combined with non-host DNA (*i.e.*, exogenous DNA). For example, a DNA molecule containing a non-host DNA segment encoding a polypeptide operably linked to a host DNA segment comprising a transcription promoter is considered to be a heterologous DNA molecule. Conversely, a heterologous DNA molecule can comprise an endogenous gene operably linked with an exogenous promoter. As another illustration, a DNA molecule comprising a gene derived from a wild-type cell is considered to be heterologous DNA if that DNA molecule is introduced into a mutant cell that lacks the wild-type gene.

A “polypeptide” is a polymer of amino acid residues joined by peptide bonds, whether produced naturally or synthetically. Polypeptides of less than about 10 amino acid residues are commonly referred to as “peptides.”

A “protein” is a macromolecule comprising one or more polypeptide chains. A protein may also comprise non-peptidic components, such as carbohydrate groups. Carbohydrates and other non-peptidic substituents may be added to a protein by the cell in which the protein is produced, and will vary with the type of cell. Proteins are defined herein in terms of their amino acid backbone structures;
substituents such as carbohydrate groups are generally not specified, but may be present nonetheless.

A peptide or polypeptide encoded by a non-host DNA molecule is a "heterologous" peptide or polypeptide.

A "cloning vector" is a nucleic acid molecule, such as a plasmid, cosmid, or bacteriophage, that has the capability of replicating autonomously in a host cell. Cloning vectors typically contain one or a small number of restriction endonuclease recognition sites that allow insertion of a nucleic acid molecule in a determinable fashion without loss of an essential biological function of the vector, as well as nucleotide sequences encoding a marker gene that is suitable for use in the identification and selection of cells transformed with the cloning vector. Marker genes typically include genes that provide tetracycline resistance or ampicillin resistance.

An "expression vector" is a nucleic acid molecule encoding a gene that is expressed in a host cell. Typically, an expression vector comprises a transcription promoter, a gene, and a transcription terminator. Gene expression is usually placed under the control of a promoter, and such a gene is said to be "operably linked to" the promoter. Similarly, a regulatory element and a core promoter are operably linked if the regulatory element modulates the activity of the core promoter.

A "recombinant host" is a cell that contains a heterologous nucleic acid molecule, such as a cloning vector or expression vector. In the present context, an example of a recombinant host is a cell that produces zacrp11 from an expression vector. In contrast, zacrp11 can be produced by a cell that is a "natural source" of zacrp11, and that lacks an expression vector.

A "fusion protein" is a hybrid protein expressed by a nucleic acid molecule comprising nucleotide sequences of at least two genes. For example, a fusion protein can comprise at least part of a zacrp11 polypeptide fused with a polypeptide that binds an affinity matrix. Such a fusion protein provides a means to isolate large quantities of zacrp11 using affinity chromatography.

The term "receptor" denotes a cell-associated protein that binds to a bioactive molecule termed a "ligand." This interaction mediates the effect of the ligand on the cell. Receptors can be membrane bound, cytosolic or nuclear; monomeric (e.g., thyroid stimulating hormone receptor, beta-adrenergic receptor) or multimeric (e.g., PDGF receptor, growth hormone receptor, IL-3 receptor, GM-CSF receptor, G-CSF receptor, erythropoietin receptor and IL-6 receptor). Membrane-bound receptors are characterized by a multi-domain structure comprising an extracellular ligand-binding domain and an intracellular effector domain that is typically involved in signal transduction. In certain membrane-bound receptors, the extracellular ligand-binding
domain and the intracellular effector domain are located in separate polypeptides that comprise the complete functional receptor.

In general, the binding of ligand to receptor results in a conformational change in the receptor that causes an interaction between the effector domain and other molecule(s) in the cell, which in turn leads to an alteration in the metabolism of the cell. Metabolic events that are often linked to receptor-ligand interactions include gene transcription, phosphorylation, dephosphorylation, increases in cyclic AMP production, mobilization of cellular calcium, mobilization of membrane lipids, cell adhesion, hydrolysis of inositol lipids and hydrolysis of phospholipids.

The term “secretory signal sequence” denotes a nucleotide sequence that encodes a peptide (a “secretory peptide”) that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in which it is synthesized. The larger polypeptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

An “isolated polypeptide” is a polypeptide that is essentially free from contaminating cellular components, such as carbohydrate, lipid, or other proteinaceous impurities associated with the polypeptide in nature. Typically, a preparation of isolated polypeptide contains the polypeptide in a highly purified form, i.e., at least about 80% pure, at least about 90% pure, at least about 95% pure, greater than 95% pure, or greater than 99% pure. One way to show that a particular protein preparation contains an isolated polypeptide is by the appearance of a single band following sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of the protein preparation and Coomassie Brilliant Blue staining of the gel. However, the term “isolated” does not exclude the presence of the same polypeptide in alternative physical forms, such as dimers or alternatively glycosylated or derivatized forms.

The terms “amino-terminal” and “carboxyl-terminal” are used herein to denote positions within polypeptides. Where the context allows, these terms are used with reference to a particular sequence or portion of a polypeptide to denote proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a polypeptide is located proximal to the carboxyl terminus of the reference sequence, but is not necessarily at the carboxyl terminus of the complete polypeptide.

The term “expression” refers to the biosynthesis of a gene product. For example, in the case of a structural gene, expression involves transcription of the structural gene into mRNA and the translation of mRNA into one or more polypeptides.

The term “splice variant” is used herein to denote alternative forms of RNA transcribed from a gene. Splice variation arises naturally through use of
alternative splicing sites within a transcribed RNA molecule, or less commonly between separately transcribed RNA molecules, and may result in several mRNAs transcribed from the same gene. Splice variants may encode polypeptides having altered amino acid sequence. The term splice variant is also used herein to denote a polypeptide encoded by a splice variant of an mRNA transcribed from a gene.

As used herein, the term “immunomodulator” includes cytokines, stem cell growth factors, lymphotoxins, co-stimulatory molecules, hematopoietic factors, and synthetic analogs of these molecules.

The term “complement/anti-complement pair” denotes non-identical moieties that form a non-covalently associated, stable pair under appropriate conditions. For instance, biotin and avidin (or streptavidin) are prototypical members of a complement/anti-complement pair. Other exemplary complement/anti-complement pairs include receptor/ligand pairs, antibody/antigen (or hapten or epitope) pairs, sense/antisense polynucleotide pairs, and the like. Where subsequent dissociation of the complement/anti-complement pair is desirable, the complement/anti-complement pair preferably has a binding affinity of less than $10^9 \text{ M}^{-1}$.

An “anti-idiotypic antibody” is an antibody that binds with the variable region domain of an immunoglobulin. In the present context, an anti-idiotypic antibody binds with the variable region of an anti-zacrp11 antibody, and thus, an anti-idiotypic antibody mimics an epitope of zacrp11.

An “antibody fragment” is a portion of an antibody such as F(ab')$_2$, F(ab)$_2$, Fab', Fab, and the like. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the intact antibody. For example, an anti-zacrp11 monoclonal antibody fragment binds with an epitope of zacrp11.

The term “antibody fragment” also includes a synthetic or a genetically engineered polypeptide that binds to a specific antigen, such as polypeptides consisting of the light chain variable region, “Fv” fragments consisting of the variable regions of the heavy and light chains, recombinant single chain polypeptide molecules in which light and heavy variable regions are connected by a peptide linker (“scFv proteins”), and minimal recognition units consisting of the amino acid residues that mimic the hypervariable region.

A “chimeric antibody” is a recombinant protein that contains the variable domains and complementary determining regions derived from a rodent antibody, while the remainder of the antibody molecule is derived from a human antibody.

“Humanized antibodies” are recombinant proteins in which murine complementarity determining regions of a monoclonal antibody have been transferred
from heavy and light variable chains of the murine immunoglobulin into a human variable domain.

As used herein, a "therapeutic agent" is a molecule or atom which is conjugated to an antibody moiety to produce a conjugate which is useful for therapy. Examples of therapeutic agents include drugs, toxins, immunomodulators, chelators, boron compounds, photoactive agents or dyes, and radioisotopes.

A "detectable label" is a molecule or atom which can be conjugated to an antibody moiety to produce a molecule useful for diagnosis. Examples of detectable labels include chelators, photoactive agents, radioisotopes, fluorescent agents, paramagnetic ions, or other marker moieties.

The term "affinity tag" is used herein to denote a polypeptide segment that can be attached to a second polypeptide to provide for purification or detection of the second polypeptide or provide sites for attachment of the second polypeptide to a substrate. In principal, any peptide or protein for which an antibody or other specific binding agent is available can be used as an affinity tag. Affinity tags include a polyhistidine tract, protein A (Nilsson et al., EMBO J. 4:1075 (1985); Nilsson et al., Methods Enzymol. 198:3 (1991)), glutathione S transferase (Smith and Johnson, Gene 67:31 (1988)), Glu-Glu affinity tag (Grussenmeyer et al., Proc. Natl. Acad. Sci. USA 82:7952 (1985)), substance P, FLAG peptide (Hopp et al., Biotechnology 6:1204 (1988)), streptavidin binding peptide, or other antigenic epitope or binding domain. See, in general, Ford et al., Protein Expression and Purification 2:95 (1991). Nucleic acid molecules encoding affinity tags are available from commercial suppliers (e.g., Pharmacia Biotech, Piscataway, NJ).

A "naked antibody" is an entire antibody, as opposed to an antibody fragment, which is not conjugated with a therapeutic agent. Naked antibodies include both polyclonal and monoclonal antibodies, as well as certain recombinant antibodies, such as chimeric and humanized antibodies.

As used herein, the term "antibody component" includes both an entire antibody and an antibody fragment.

An "immunoconjugate" is a conjugate of an antibody component with a therapeutic agent or a detectable label.

As used herein, the term "antibody fusion protein" refers to a recombinant molecule that comprises an antibody component and a therapeutic agent. Examples of therapeutic agents suitable for such fusion proteins include immunomodulators ("antibody-immunomodulator fusion protein") and toxins ("antibody-toxin fusion protein").
A "target polypeptide" or a "target peptide" is an amino acid sequence that comprises at least one epitope, and that is expressed on a target cell, such as a tumor cell, or a cell that carries an infectious agent antigen. T cells recognize peptide epitopes presented by a major histocompatibility complex molecule to a target polypeptide or target peptide and typically lyse the target cell or recruit other immune cells to the site of the target cell, thereby killing the target cell.

An "antigenic peptide" is a peptide which will bind a major histocompatibility complex molecule to form an MHC-peptide complex which is recognized by a T cell, thereby inducing a cytotoxic lymphocyte response upon presentation to the T cell. Thus, antigenic peptides are capable of binding to an appropriate major histocompatibility complex molecule and inducing a cytotoxic T cells response, such as cell lysis or specific cytokine release against the target cell which binds or expresses the antigen. The antigenic peptide can be bound in the context of a class I or class II major histocompatibility complex molecule, on an antigen presenting cell or on a target cell.

In eukaryotes, RNA polymerase II catalyzes the transcription of a structural gene to produce mRNA. A nucleic acid molecule can be designed to contain an RNA polymerase II template in which the RNA transcript has a sequence that is complementary to that of a specific mRNA. The RNA transcript is termed an "anti-sense RNA" and a nucleic acid molecule that encodes the anti-sense RNA is termed an "anti-sense gene." Anti-sense RNA molecules are capable of binding to mRNA molecules, resulting in an inhibition of mRNA translation.

An "anti-sense oligonucleotide specific for zacrplI" or an "zacrp11 anti-sense oligonucleotide" is an oligonucleotide having a sequence (a) capable of forming a stable triplex with a portion of the zacrplI gene, or (b) capable of forming a stable duplex with a portion of an mRNA transcript of the zacrplI gene.

A "ribozyme" is a nucleic acid molecule that contains a catalytic center. The term includes RNA enzymes, self-splicing RNAs, self-cleaving RNAs, and nucleic acid molecules that perform these catalytic functions. A nucleic acid molecule that encodes a ribozyme is termed a "ribozyme gene."

An "external guide sequence" is a nucleic acid molecule that directs the endogenous ribozyme, RNase P, to a particular species of intracellular mRNA, resulting in the cleavage of the mRNA by RNase P. A nucleic acid molecule that encodes an external guide sequence is termed an "external guide sequence gene."

The term "variant zacrplI gene" refers to nucleic acid molecules that encode a polypeptide having an amino acid sequence that is a modification of SEQ ID NO:2. Such variants include naturally-occurring polymorphisms of zacrplI genes, as
well as synthetic genes that contain conservative amino acid substitutions of the amino acid sequence of SEQ ID NO:2. Additional variant forms of zacrp11 genes are nucleic acid molecules that contain insertions or deletions of the nucleotide sequences described herein. A variant zacrp11 gene can be identified by determining whether the gene hybridizes with a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, or its complement, under stringent conditions.

Alternatively, variant zacrp11 genes can be identified by sequence comparison. Two amino acid sequences have “100% amino acid sequence identity” if the amino acid residues of the two amino acid sequences are the same when aligned for maximal correspondence. Similarly, two nucleotide sequences have “100% nucleotide sequence identity” if the nucleotide residues of the two nucleotide sequences are the same when aligned for maximal correspondence. Sequence comparisons can be performed using standard software programs such as those included in the LASERGENE bioinformatics computing suite, which is produced by DNASTAR (Madison, Wisconsin). Other methods for comparing two nucleotide or amino acid sequences by determining optimal alignment are well-known to those of skill in the art (see, for example, Peruski and Peruski, *The Internet and the New Biology: Tools for Genomic and Molecular Research* (ASM Press, Inc. 1997), Wu et al. (eds.), “Information Superhighway and Computer Databases of Nucleic Acids and Proteins,” in *Methods in Gene Biotechnology*, pages 123-151 (CRC Press, Inc. 1997), and Bishop (ed.), *Guide to Human Genome Computing*, 2nd Edition (Academic Press, Inc. 1998)). Particular methods for determining sequence identity are described below.

The term “allelic variant” is used herein to denote any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in phenotypic polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequence. The term allelic variant is also used herein to denote a protein encoded by an allelic variant of a gene.

The term “ortholog” denotes a polypeptide or protein obtained from one species that is the functional counterpart of a polypeptide or protein from a different species. Sequence differences among orthologs are the result of speciation.

“Paralogs” are distinct but structurally related proteins made by an organism. Paralogs are believed to arise through gene duplication. For example, α-globin, β-globin, and myoglobin are paralogs of each other.

The present invention includes functional fragments of zacrp11 genes. Within the context of this invention, a “functional fragment” of a zacrp11 gene refers to a nucleic acid molecule that encodes a portion of a zacrp11 polypeptide which
specifically binds with an anti-zacrp11 antibody. For example, a functional fragment of a zacrp11 gene described herein comprises a portion of the nucleotide sequence of SEQ ID NO:1, and encodes a polypeptide that specifically binds with an anti-zacrp11 antibody.

Due to the imprecision of standard analytical methods, molecular weights and lengths of polymers are understood to be approximate values. When such a value is expressed as “about” X or “approximately” X, the stated value of X will be understood to be accurate to ±10%.

The present invention is based in part upon the discovery of a novel DNA sequence that encodes a polypeptide having homology to the adipocyte complement related protein family. The polypeptide has been designated zacrp11. The nucleotide sequence of zacrp11 is described in SEQ ID NO:1, and its deduced amino acid sequence is described in SEQ ID NO:2. The zacrp11 polypeptide includes a signal sequence, ranging from amino acid 1 (Met) to amino acid residue 20 (Ala) of SEQ ID NO:2, nucleotides 1-60 of SEQ ID NO:1. The mature polypeptide ranges from amino acid 21 (His) to amino acid 268 (Thr) of SEQ ID NO:2, nucleotides 61-804 of SEQ ID NO:1. Within the mature polypeptide is found an N-terminal region of no known homology, ranging between amino acid residue 21 (His) and 60 (Gln) of SEQ ID NO:2, nucleotides 61-180 of SEQ ID NO:1. In addition, a collagen-like domain is found between amino acid 61 (Gly) and 111 (Asn) of SEQ ID NO:2, nucleotides 181-333 of SEQ ID NO:1. In the collagen-like domain there are 16 collagen repeats, eight perfect Gly-Xaa-Pro repeats and eight imperfect Gly-Xaa-Xaa repeats, and one Ser-Leu-Pro triplet. The zacrp11 polypeptide also includes a carboxy-terminal Clq domain, ranging from about amino acid 112 (Ala) to 219 (Phe) of SEQ ID NO:2, nucleotides 334-657 of SEQ ID NO:1. A modified aromatic motif [FA]-X(5)-[NDV]-X(4)-[FYWLD]-X(6)-[FS]-X(5)-G-X-Y-X(4) (SEQ ID NO:4) is also found within this domain between residues 112 (Ala) and 142 (Tyr) of SEQ ID NO:2, nucleotides 334-425 of SEQ ID NO:1. X represents any amino acid residue and the number in parentheses () indicates the amino acid number of residues. The amino acid residues contained within the square parentheses [ ] restrict the choice of amino acid residues at that particular position. There is a fair amount of conserved structure within the Clq domain to enable proper folding.

Zacrp11 shares 34% identity at the amino acid level with ACRP 30 (Scherer et al., J. Biol. Chem. 270:26746-49, 1995), 31% identity at the amino acid level with C1QC (Sellar et al., Biochem. J. 274:481-90, 1991; and Reid, Biochem. J. 179:367-71, 1979; Genbank Accession No. PO2747), and 91% identity at the amino
acrid level within the C1q domain of CRF (C1q—related factor, Genbank Accession No: AF095154).

Another aspect of the present invention includes zacrpl polypeptide fragments. Preferred fragments include those containing the collagen-like domain of zacrpl polypeptides, ranging from amino acid 1 (Met), 21 (His), or 61 (Gly) to amino acid 111 (Asn) of SEQ ID NO:2, a portion of the zacrpl polypeptide containing the collagen-like domain or a portion of the collagen-like domain capable of dimerization or oligomerization. As used herein the term “collagen” or “collagen-like domain” refers to a series of repeating triplet amino acid sequences, “repeats” or “collagen repeats” represented by the motifs Gly-Xaa-Pro or Gly-Xaa-Xaa, where Xaa is any amino acid residue. The number of collagen repeats within a collagen-like domain varies within the adipocyte complement related protein family. Fragments or proteins containing such collagen-like domains may form homomeric constructs (dimers or oligomers of the same fragment or protein). Moreover, such fragments or proteins containing such collagen-like domains may form heteromeric constructs (dimers, trimers or oligomers of different fragments or proteins).

These fragments are particularly useful in the study of collagen dimerization or oligomerization or in formation of fusion proteins as described more fully below. Polynucleotides encoding such fragments are also encompassed by the present invention, including the group consisting of (a) polynucleotide molecule comprising a sequence of nucleotides as shown in SEQ ID NO:1 from nucleotide 1, 61, or 118 to nucleotide 333; (b) polynucleotide molecules that encode a zacrpl polypeptide fragment that is at least 80%, 90%, or 95% identical to the amino acid sequence of SEQ ID NO:2 from amino acid residue 61 (Gly) to amino acid residue 111 (Asn); (c) molecules complementary to (a) or (b); and (d) degenerate nucleotide sequences encoding a zacrpl polypeptide collagen-like domain fragment.

Other preferred fragments include the globular C1q domain of zacrpl polypeptides, ranging from amino acid 112 (Ala) to 219 (Phe) of SEQ ID NO:2 or amino acid 112 (Ala) to 268 (Thr) of SEQ ID NO:2, a portion of the zacrpl polypeptide containing the C1q domain or an active portion of the C1q domain. Other C1q domain containing proteins include C1q A, B and C (Sellar et al., ibid., Reid, ibid., and Reid et al., 1982, ibid), chipmunk hibernation-associated plasma proteins HP-20, HP-25 and HP-27 (Takamatsu et al., ibid and Kondo & Kondo, ibid), human precerebellin (Urade et al., ibid), human endothelial cell multimerin (Hayward et al., ibid), vertebrate collagens type VIII and X (Muragaki et al., ibid), adipocyte complement related proteins Acrp30 (Scherer et al., ibid), apM1 (Maeda et al., ibid), GBP28 (Nakano et al., ibid), zsig39 (Sheppard and Humes, WIPO Published Patent No:
WO99/10492), zsig37 (Sheppard, WIPO Published Patent No: WO99/04000), ZCRP30R1 (Smith et al., WIPO Published Patent No. WO99/56619), ACRP30RIL (Hensley et al., WIPO Published Patent No: WO99/59618), ACRP30R2 (Hensley et al., WIPO Published Patent No: WO99/64629), PRO 353 and PRO 344 (Wood et al., WIPO Published Patent No. WO99/28462), zacrpl2 (Piddingtton et al.,WO 00/63376), zacrpl3 (Piddingtton et al., WO 00/63377), zacrpl4 (Piddingtton et al., WO 01/02565), zacrpl5 (Piddingtton et al., WO 00/73444), zacrpl6 (Piddingtton et al., WO 00/73466), and zacrpl12 (Piddingtton et al., WO 00/****).


These fragments are particularly useful in the study or modulation of energy balance or neurotransmission, particularly diet- or stress-related neurotransmission, collagen inhibition, and complement inhibition. Anti-microbial activity may also be present in such fragments. The homology of adipocyte complement related protein C1q domains to TNF proteins (Shapiro and Scherer, ibid) suggests such fragments would be useful in obesity-related insulin resistance, immune regulation, inflammatory response, platelet adhesion modulation, apoptosis and osteoclast maturation. Polynucleotides encoding such fragments are also encompassed by the present invention, including the group consisting of (a) polynucleotide molecules comprising a sequence of nucleotides as shown in SEQ ID NO:1 from nucleotide 334 to nucleotide 657, or nucleotide 334 to 804; (b) polynucleotide molecules that encode a zacrpl1 polypeptide fragment that is at least 80%, 90%, or 95% identical to the amino acid sequence of SEQ ID NO:2 from amino acid residue 112 (Ala) to amino acid residue 219 (Phe) or amino acid residues 112 (Ala) to amino acid residue 268 (Thr); (c) molecules complementary to (a) or (b); and (d) degenerate nucleotide sequences encoding a zacrpl1 polypeptide C1q domain fragment.

Other zacrpl1 polypeptide fragments of the present invention include both the collagen-like domain and the C1q domain ranging from amino acid residue 21 (His), or 61 (Gly) to 219 (Phe) or 268 (Thr) of SEQ ID NO:2. Polynucleotides encoding such fragments are also encompassed by the present invention, including the group consisting of (a) polynucleotide molecules comprising a sequence of nucleotides as shown in SEQ ID NO:1 from nucleotide 61 or 181 to nucleotide 657 or 804; (b) polynucleotide molecules that encode a zacrpl1 polypeptide fragment that is at least 80%, 90%, or 95% identical to the amino acid sequence of SEQ ID NO:2 from amino
acid residue 21 (His) or 61 (Gly) to amino acid residue 219 (Phe) or 268 (Thr); (c) molecules complementary to (a) or (b); and (d) degenerate nucleotide sequences encoding a zacrp11 polypeptide collagen-like domain-C1q domain fragment.

Production of a human zacrp11 Gene

Nucleic acid molecules encoding a human zacrp11 gene can be obtained by screening a human cDNA or genomic library using polynucleotide probes based upon SEQ ID NO:1. These techniques are standard and well-established. As an illustration, a nucleic acid molecule that encodes a human zacrp11 gene can be isolated from a human cDNA library. In this case, the first step would be to prepare the cDNA library using methods well-known to those of skill in the art. In general, RNA isolation techniques must provide a method for breaking cells, a means of inhibiting RNase-directed degradation of RNA, and a method of separating RNA from DNA, protein, and polysaccharide contaminants. For example, total RNA can be isolated by freezing tissue in liquid nitrogen, grinding the frozen tissue with a mortar and pestle to lyse the cells, extracting the ground tissue with a solution of phenol/chloroform to remove proteins, and separating RNA from the remaining impurities by selective precipitation with lithium chloride (see, for example, Ausubel et al. (eds.), Short Protocols in Molecular Biology, 3rd Edition, pages 4-1 to 4-6 (John Wiley & Sons 1995) ["Ausubel (1995)"]; Wu et al., Methods in Gene Biotechnology, pages 33-41 (CRC Press, Inc. 1997) ["Wu (1997)"]). Alternatively, total RNA can be isolated by extracting ground tissue with guanidinium isothiocyanate, extracting with organic solvents, and separating RNA from contaminants using differential centrifugation (see, for example, Chirgwin et al., Biochemistry 18:52 (1979); Ausubel (1995) at pages 4-1 to 4-6; Wu (1997) at pages 33-41).

In order to construct a cDNA library, poly(A)+ RNA must be isolated from a total RNA preparation. Poly(A)+ RNA can be isolated from total RNA using the standard technique of oligo(dT)-cellulose chromatography (see, for example, Aviv and Leder, Proc. Nat’l Acad. Sci. USA 69:1408 (1972); Ausubel (1995) at pages 4-11 to 4-12).

Double-stranded cDNA molecules are synthesized from poly(A)+ RNA using techniques well-known to those in the art. (see, for example, Wu (1997) at pages 41-46). Moreover, commercially available kits can be used to synthesize double-stranded cDNA molecules. For example, such kits are available from Life Technologies, Inc. (Gaithersburg, MD), CLONTECH Laboratories, Inc. (Palo Alto, CA), Promega Corporation (Madison, WI) and STRATAGENE (La Jolla, CA).

Various cloning vectors are appropriate for the construction of a cDNA library. For example, a cDNA library can be prepared in a vector derived from
bacteriophage, such as a λgt10 vector. See, for example, Huynh et al., “Constructing and Screening cDNA Libraries in λgt10 and λgt11,” in DNA Cloning: A Practical Approach Vol. I, Glover (ed.), page 49 (IRL Press, 1985); Wu (1997) at pages 47-52.

Alternatively, double-stranded cDNA molecules can be inserted into a plasmid vector, such as a PBLUESCRIPT vector (STRATAGENE; La Jolla, CA), a LAMDAGEM-4 (Promega Corp.) or other commercially available vectors. Suitable cloning vectors also can be obtained from the American Type Culture Collection (Manassas, VA).

To amplify the cloned cDNA molecules, the cDNA library is inserted into a prokaryotic host, using standard techniques. For example, a cDNA library can be introduced into competent E. coli DH5 cells, which can be obtained, for example, from Life Technologies, Inc. (Gaithersburg, MD).

A human genomic library can be prepared by means well-known in the art (see, for example, Ausubel (1995) at pages 5-1 to 5-6; Wu (1997) at pages 307-327). Genomic DNA can be isolated by lysing tissue with the detergent Sarkosyl, digesting the lysate with proteinase K, clearing insoluble debris from the lysate by centrifugation, precipitating nucleic acid from the lysate using isopropanol, and purifying resuspended DNA on a cesium chloride density gradient.

DNA fragments that are suitable for the production of a genomic library can be obtained by the random shearing of genomic DNA or by the partial digestion of genomic DNA with restriction endonucleases. Genomic DNA fragments can be inserted into a vector, such as a bacteriophage or cosmid vector, in accordance with conventional techniques, such as the use of restriction enzyme digestion to provide appropriate termini, the use of alkaline phosphatase treatment to avoid undesirable joining of DNA molecules, and ligation with appropriate ligases. Techniques for such manipulation are well-known in the art (see, for example, Ausubel (1995) at pages 5-1 to 5-6; Wu (1997) at pages 307-327).

Nucleic acid molecules that encode a human Zacp11 gene can also be obtained using the polymerase chain reaction (PCR) with oligonucleotide primers having nucleotide sequences that are based upon the nucleotide sequences of the Zacp11 gene, as described herein. General methods for screening libraries with PCR are provided by, for example, Yu et al., “Use of the Polymerase Chain Reaction to Screen Phage Libraries,” in Methods in Molecular Biology, Vol. 15: PCR Protocols: Current Methods and Applications, White (ed.), pages 211-215 (Humana Press, Inc. 1993). Moreover, techniques for using PCR to isolate related genes are described by, for example, Preston, “Use of Degenerate Oligonucleotide Primers and the Polymerase Chain Reaction to Clone Gene Family Members,” in Methods in Molecular Biology,

Alternatively, human genomic libraries can be obtained from commercial sources such as Research Genetics (Huntsville, AL) and the American Type Culture Collection (Manassas, VA).

A library containing cDNA or genomic clones can be screened with one or more polynucleotide probes based upon SEQ ID NO:1, using standard methods (see, for example, Ausubel (1995) at pages 6-1 to 6-11).

Anti-zacrp11 antibodies, produced as described below, can also be used to isolate DNA sequences that encode human Zacrp11 genes from cDNA libraries. For example, the antibodies can be used to screen λgt11 expression libraries, or the antibodies can be used for immunoscreening following hybrid selection and translation (see, for example, Ausubel (1995) at pages 6-12 to 6-16; Margolis et al., “Screening λ expression libraries with antibody and protein probes,” in DNA Cloning 2: Expression Systems, 2nd Edition, Glover et al. (eds.), pages 1-14 (Oxford University Press 1995)).

As an alternative, a Zacrp11 gene can be obtained by synthesizing nucleic acid molecules using mutually priming long oligonucleotides and the nucleotide sequences described herein (see, for example, Ausubel (1995) at pages 8-8 to 8-9). Established techniques using the polymerase chain reaction provide the ability to synthesize DNA molecules at least two kilobases in length (Adang et al., Plant Molec. Biol. 21:1131 (1993), Bambot et al., PCR Methods and Applications 2:266 (1993), Dillon et al., “Use of the Polymerase Chain Reaction for the Rapid Construction of Synthetic Genes,” in Methods in Molecular Biology, Vol. 15: PCR Protocols: Current Methods and Applications, White (ed.), pages 263-268, (Humana Press, Inc. 1993), and Holowachuk et al., PCR Methods Appl. 4:299 (1995)).

The nucleic acid molecules of the present invention can also be synthesized with “gene machines” using protocols such as the phosphoramidite method. If chemically-synthesized double stranded DNA is required for an application such as the synthesis of a gene or a gene fragment, then each complementary strand is made separately. The production of short genes (60 to 80 base pairs) is technically straightforward and can be accomplished by synthesizing the complementary strands and then annealing them. For the production of longer genes (>300 base pairs), however, special strategies may be required, because the coupling efficiency of each cycle during chemical DNA synthesis is seldom 100%. To overcome this problem, synthetic genes (double-stranded) are assembled in modular form from single-stranded fragments that are from 20 to 100 nucleotides in length.
One method for building a synthetic gene requires the initial production of a set of overlapping, complementary oligonucleotides, each of which is between 20 to 60 nucleotides long. The sequences of the strands are planned so that, after annealing, the two end segments of the gene are aligned to give blunt ends. Each internal section of the gene has complementary 3’ and 5’ terminal extensions that are designed to base pair precisely with an adjacent section. Thus, after the gene is assembled, the only remaining requirement to complete the process is to seal the nicks along the backbones of the two strands with T4 DNA ligase. In addition to the protein coding sequence, synthetic genes can be designed with terminal sequences that facilitate insertion into a restriction endonuclease sites of a cloning vector and other sequences should also be added that contain signals for the proper initiation and termination of transcription and translation.

An alternative way to prepare a full-size gene is to synthesize a specified set of overlapping oligonucleotides (40 to 100 nucleotides). After the 3’ and 5’ extensions (6 to 10 nucleotides) are annealed, large gaps still remain, but the base-paired regions are both long enough and stable enough to hold the structure together. The duplex is completed and the gaps filled by enzymatic DNA synthesis with *E. coli* DNA polymerase I. This enzyme uses the 3’-hydroxyl groups as replication initiation points and the single-stranded regions as templates. After the enzymatic synthesis is completed, the nicks are sealed with T4 DNA ligase. For larger genes, the complete gene sequence is usually assembled from double-stranded fragments that are each put together by joining four to six overlapping oligonucleotides (20 to 60 base pairs each). If there is a sufficient amount of the double-stranded fragments after each synthesis and annealing step, they are simply joined to one another. Otherwise, each fragment is cloned into a vector to amplify the amount of DNA available. In both cases, the double-stranded constructs are sequentially linked to one another to form the entire gene sequence. Each double-stranded fragment and the complete sequence should be characterized by DNA sequence analysis to verify that the chemically synthesized gene has the correct nucleotide sequence. For reviews on polynucleotide synthesis, see, for example, Glick and Pasternak, *Molecular Biotechnology, Principles and Applications of Recombinant DNA* (ASM Press 1994), Itakura et al., *Annu. Rev. Biochem.* 53:323 (1984), and Clunie et al., *Proc. Nat’l Acad. Sci. USA* 87:633 (1990).

The sequence of a *zacrpII* cDNA or *zacrpII* genomic fragment can be determined using standard methods. *zacrpII* polynucleotide sequences disclosed herein can also be used as probes or primers to clone 5’ non-coding regions of a *zacrpII* gene. Promoter elements from a *zacrpII* gene can be used to direct the expression of heterologous genes in, for example, transgenic animals or patients undergoing gene
therapy. The identification of genomic fragments containing a zacrp11 promoter or regulatory element can be achieved using well-established techniques, such as deletion analysis (see, generally, Ausubel (1995)).

Cloning of 5' flanking sequences also facilitates production of zacrp11 proteins by “gene activation,” as disclosed in U.S. Patent No. 5,641,670. Briefly, expression of an endogenous zacrp11 gene in a cell is altered by introducing into the zacrp11 locus a DNA construct comprising at least a targeting sequence, a regulatory sequence, an exon, and an unpaired splice donor site. The targeting sequence is a zacrp11 5' non-coding sequence that permits homologous recombination of the construct with the endogenous zacrp11 locus, whereby the sequences within the construct become operably linked with the endogenous zacrp11 coding sequence. In this way, an endogenous zacrp11 promoter can be replaced or supplemented with other regulatory sequences to provide enhanced, tissue-specific, or otherwise regulated expression.

Production of zacrp11 Gene Variants

The present invention provides a variety of nucleic acid molecules, including DNA and RNA molecules, that encode the zacrp11 polypeptides disclosed herein. Those skilled in the art will readily recognize that, in view of the degeneracy of the genetic code, considerable sequence variation is possible among these polynucleotide molecules. SEQ ID NO:3 is a degenerate nucleotide sequence that encompasses all nucleic acid molecules that encode the zacrp11 polypeptide of SEQ ID NO:2. Those skilled in the art will recognize that the degenerate sequence of SEQ ID NO:3 also provides all RNA sequences encoding SEQ ID NO:2, by substituting U for T. Thus, the present invention contemplates zacrp11 polypeptide-encoding nucleic acid molecules comprising nucleotides 1 to 804 of SEQ ID NO:1, and their RNA equivalents.

Table 1 sets forth the one-letter codes used within SEQ ID NO:3 to denote degenerate nucleotide positions. “Resolutions” are the nucleotides denoted by a code letter. “Complement” indicates the code for the complementary nucleotide(s). For example, the code Y denotes either C or T, and its complement R denotes A or G, A being complementary to T, and G being complementary to C.
The degenerate codons used in SEQ ID NO:3, encompassing all possible codons for a given amino acid, are set forth in Table 2.
<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>One Letter Code</th>
<th>Codons</th>
<th>Degenerate Codon</th>
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<td>K</td>
<td>AAA AAG</td>
<td>AAR</td>
</tr>
<tr>
<td>Met</td>
<td>M</td>
<td>ATG</td>
<td>ATG</td>
</tr>
<tr>
<td>Ile</td>
<td>I</td>
<td>ATA ATC ATT</td>
<td>ATH</td>
</tr>
<tr>
<td>Leu</td>
<td>L</td>
<td>CTA CTC CTG CTT TTA TTA TTG</td>
<td>YTN</td>
</tr>
<tr>
<td>Val</td>
<td>V</td>
<td>GTA GTC GTG GTT</td>
<td>GTN</td>
</tr>
<tr>
<td>Phe</td>
<td>F</td>
<td>TTC TTT</td>
<td>TTY</td>
</tr>
<tr>
<td>Tyr</td>
<td>Y</td>
<td>TAC TAT</td>
<td>TAY</td>
</tr>
<tr>
<td>Trp</td>
<td>W</td>
<td>TGG</td>
<td>TGG</td>
</tr>
<tr>
<td>Ter</td>
<td>.</td>
<td>TAA TAG TGA</td>
<td>TRR</td>
</tr>
<tr>
<td>Asn</td>
<td>Asp</td>
<td>B</td>
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</tr>
<tr>
<td>Glu</td>
<td>Gln</td>
<td>Z</td>
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<tr>
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One of ordinary skill in the art will appreciate that some ambiguity is introduced in determining a degenerate codon, representative of all possible codons encoding an amino acid. For example, the degenerate codon for serine (WSN) can, in some circumstances, encode arginine (AGR), and the degenerate codon for arginine (MGN) can, in some circumstances, encode serine (AGY). A similar relationship exists between codons encoding phenylalanine and leucine. Thus, some polynucleotides encompassed by the degenerate sequence may encode variant amino acid sequences, but one of ordinary skill in the art can easily identify such variant sequences by reference to the amino acid sequence of SEQ ID NO:2. Variant sequences can be readily tested for functionality as described herein.

Different species can exhibit “preferential codon usage.” In general, see, Grantham et al., Nuc. Acids Res. 8:1893 (1980), Haas et al. Curr. Biol. 6:315 (1996), Wain-Hobson et al., Gene 13:355 (1981), Grosjean and Fiers, Gene 18:199 (1982), Holm, Nuc. Acids Res. 14:3075 (1986), Ikemura, J. Mol. Biol. 158:573 (1982), Sharp and Matassi, Curr. Opin. Genet. Dev. 4:851 (1994), Kane, Curr. Opin. Biotechnol. 6:494 (1995), and Makrides, Microbiol. Rev. 60:512 (1996). As used herein, the term “preferential codon usage” or “preferential codons” is a term of art referring to protein translation codons that are most frequently used in cells of a certain species, thus favoring one or a few representatives of the possible codons encoding each amino acid (see Table 2). For example, the amino acid threonine (Thr) may be encoded by ACA, ACC, ACG, or ACT, but in mammalian cells ACC is the most commonly used codon; in other species, for example, insect cells, yeast, viruses or bacteria, different Thr codons may be preferential. Preferential codons for a particular species can be introduced into the polynucleotides of the present invention by a variety of methods known in the art. Introduction of preferential codon sequences into recombinant DNA can, for example, enhance production of the protein by making protein translation more efficient within a particular cell type or species. Therefore, the degenerate codon sequence disclosed in SEQ ID NO:3 serves as a template for optimizing expression of polynucleotides in various cell types and species commonly used in the art and disclosed herein. Sequences containing preferential codons can be tested and optimized for expression in various species, and tested for functionality as disclosed herein.

The present invention further provides variant polypeptides and nucleic acid molecules that represent counterparts from other species (orthologs). These species include, but are not limited to mammalian, avian, amphibian, reptile, fish, insect and other vertebrate and invertebrate species. Of particular interest are zacrpl1 polypeptides from other mammalian species, including porcine, ovine, bovine, canine, feline, equine, and other primate polypeptides. Such orthologs of zacrpl1 can be
cloned using information and compositions provided by the present invention in combination with conventional cloning techniques. For example, a cDNA can be cloned using mRNA obtained from a tissue or cell type that expresses zacrp11 as disclosed herein. Suitable sources of mRNA can be identified by probing northern blots with probes designed from the sequences disclosed herein. A library is then prepared from mRNA of a positive tissue or cell line.

A zacrp11-encoding cDNA can then be isolated by a variety of methods, such as by probing with a complete or partial cDNA or with one or more sets of degenerate probes based on the disclosed sequences. A cDNA can also be cloned using the polymerase chain reaction with primers designed from the representative zacrp11 sequences disclosed herein. Within an additional method, the cDNA library can be used to transform or transfect host cells, and expression of the cDNA of interest can be detected with an antibody to zacrp11 polypeptide. Similar techniques can also be applied to the isolation of genomic clones.

Those skilled in the art will recognize that the sequence disclosed in SEQ ID NO:1 represents a single allele of human zacrp11, and that allelic variation and alternative splicing are expected to occur. Allelic variants of this sequence can be cloned by probing cDNA or genomic libraries from different individuals according to standard procedures. Allelic variants of the nucleotide sequence shown in SEQ ID NO:1, including those containing silent mutations and those in which mutations result in amino acid sequence changes, are within the scope of the present invention, as are proteins which are allelic variants of SEQ ID NO:2. cDNA molecules generated from alternatively spliced mRNAs, which retain the properties of the zacrp11 polypeptide are included within the scope of the present invention, as are polypeptides encoded by such cDNAs and mRNAs. Allelic variants and splice variants of these sequences can be cloned by probing cDNA or genomic libraries from different individuals or tissues according to standard procedures known in the art.

Within certain embodiments of the invention, the isolated nucleic acid molecules can hybridize under stringent conditions to nucleic acid molecules comprising nucleotide sequences disclosed herein. For example, such nucleic acid molecules can hybridize under stringent conditions to nucleic acid molecules comprising the nucleotide sequence of SEQ ID NO:1, to nucleic acid molecules consisting of the nucleotide sequence of SEQ ID NO:1, or to nucleic acid molecules consisting of a nucleotide sequence complementary to SEQ ID NO:1. In general, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the
temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe.

A pair of nucleic acid molecules, such as DNA-DNA, RNA-RNA and DNA-RNA, can hybridize if the nucleotide sequences have some degree of complementarity. Hybrids can tolerate mismatched base pairs in the double helix, but the stability of the hybrid is influenced by the degree of mismatch. The Tₘ of the mismatched hybrid decreases by 1°C for every 1-1.5% base pair mismatch. Varying the stringency of the hybridization conditions allows control over the degree of mismatch that will be present in the hybrid. The degree of stringency increases as the hybridization temperature increases and the ionic strength of the hybridization buffer decreases. Stringent hybridization conditions encompass temperatures of about 5-25°C below the Tₘ of the hybrid and a hybridization buffer having up to 1 M Na⁺. Higher degrees of stringency at lower temperatures can be achieved with the addition of formamide which reduces the Tₘ of the hybrid about 1°C for each 1% formamide in the buffer solution. Generally, such stringent conditions include temperatures of 20-70°C and a hybridization buffer containing up to 6x SSC and 0-50% formamide. A higher degree of stringency can be achieved at temperatures of from 40-70°C with a hybridization buffer having up to 4x SSC and from 0-50% formamide. Highly stringent conditions typically encompass temperatures of 42-70°C with a hybridization buffer having up to 1x SSC and 0-50% formamide. Different degrees of stringency can be used during hybridization and washing to achieve maximum specific binding to the target sequence. Typically, the washes following hybridization are performed at increasing degrees of stringency to remove non-hybridized polynucleotide probes from hybridized complexes.

The above conditions are meant to serve as a guide and it is well within the abilities of one skilled in the art to adapt these conditions for use with a particular polypeptide hybrid. The Tₘ for a specific target sequence is the temperature (under defined conditions) at which 50% of the target sequence will hybridize to a perfectly matched probe sequence. Those conditions which influence the Tₘ include, the size and base pair content of the polynucleotide probe, the ionic strength of the hybridization solution, and the presence of destabilizing agents in the hybridization solution. Numerous equations for calculating Tₘ are known in the art, and are specific for DNA, RNA and DNA-RNA hybrids and polynucleotide probe sequences of varying length (see, for example, Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition (Cold Spring Harbor Press 1989); Ausubel et al., (eds.), Current Protocols in Molecular Biology (John Wiley and Sons, Inc. 1987); Berger and Kimmel (eds.), Guide to Molecular Cloning Techniques, (Academic Press, Inc. 1987); and Wetmur,
Crit. Rev. Biochem. Mol. Biol. 26:227 (1990)). Sequence analysis software, as well as sites on the Internet, are available tools for analyzing a given sequence and calculating T_m based on user defined criteria. Such programs can also analyze a given sequence under defined conditions and identify suitable probe sequences. Typically, hybridization of longer polynucleotide sequences, >50 base pairs, is performed at temperatures of about 20-25°C below the calculated T_m. For smaller probes, <50 base pairs, hybridization is typically carried out at the T_m or 5-10°C below. This allows for the maximum rate of hybridization for DNA-DNA and DNA-RNA hybrids.

The length of the polynucleotide sequence influences the rate and stability of hybrid formation. Smaller probe sequences, <50 base pairs, reach equilibrium with complementary sequences rapidly, but may form less stable hybrids. Incubation times of anywhere from minutes to hours can be used to achieve hybrid formation. Longer probe sequences come to equilibrium more slowly, but form more stable complexes even at lower temperatures. Incubations are allowed to proceed overnight or longer. Generally, incubations are carried out for a period equal to three times the calculated Cot time. Cot time, the time it takes for the polynucleotide sequences to reassociate, can be calculated for a particular sequence by methods known in the art.

The base pair composition of polynucleotide sequence will effect the thermal stability of the hybrid complex, thereby influencing the choice of hybridization temperature and the ionic strength of the hybridization buffer. A-T pairs are less stable than G-C pairs in aqueous solutions containing sodium chloride. Therefore, the higher the G-C content, the more stable the hybrid. Even distribution of G and C residues within the sequence also contribute positively to hybrid stability. In addition, the base pair composition can be manipulated to alter the T_m of a given sequence. For example, 5-methyldeoxycytidine can be substituted for deoxycytidine and 5-bromodeoxuridine can be substituted for thymidine to increase the T_m, whereas 7-deaz-2'-deoxyguanosine can be substituted for guanosine to reduce dependence on T_m.

The ionic concentration of the hybridization buffer also affects the stability of the hybrid. Hybridization buffers generally contain blocking agents such as Denhardt’s solution (Sigma Chemical Co., St. Louis, Mo.), denatured salmon sperm DNA, tRNA, milk powders (BLOTTO), heparin or SDS, and a Na^+ source, such as SSC (1x SSC: 0.15 M sodium chloride, 15 mM sodium citrate) or SSPE (1x SSPE: 1.8 M NaCl, 10 mM NaH_2PO_4, 1 mM EDTA, pH 7.7). By decreasing the ionic concentration of the buffer, the stability of the hybrid is increased. Typically, hybridization buffers contain from between 10 mM - 1 M Na^+ The addition of destabilizing or denaturing agents such as formamide, tetralkylammonium salts, guanidinium cations or
thiocyanate cations to the hybridization solution will alter the \( T_m \) of a hybrid. Typically, formamide is used at a concentration of up to 50% to allow incubations to be carried out at more convenient and lower temperatures. Formamide also acts to reduce non-specific background when using RNA probes.

As an illustration, a nucleic acid molecule encoding a variant zacrpl1 polypeptide can be hybridized with a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 (or its complement) at 42°C overnight in a solution comprising 50% formamide, 5xSSC (1xSSC: 0.15 M sodium chloride and 15 mM sodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt’s solution (100x Denhardt’s solution: 2% (w/v) Ficoll 400, 2% (w/v) polyvinylpyrrolidone, and 2% (w/v) bovine serum albumin, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA. One of skill in the art can devise variations of these hybridization conditions. For example, the hybridization mixture can be incubated at a higher temperature, such as about 65°C, in a solution that does not contain formamide. Moreover, premixed hybridization solutions are available (e.g., EXPRESSHYB Hybridization Solution from CLONTECH Laboratories, Inc.), and hybridization can be performed according to the manufacturer’s instructions.

Following hybridization, the nucleic acid molecules can be washed to remove non-hybridized nucleic acid molecules under stringent conditions, or under highly stringent conditions. Typical stringent washing conditions include washing in a solution of 0.5x - 2x SSC with 0.1% sodium dodecyl sulfate (SDS) at 55 - 65°C. That is, nucleic acid molecules encoding a variant zacrpl1 polypeptide remained hybridized following stringent washing conditions with a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 (or its complement), in which the wash stringency is equivalent to 0.5x - 2x SSC with 0.1% SDS at 55 - 65°C, including 0.5x SSC with 0.1% SDS at 55°C, or 2x SSC with 0.1% SDS at 65°C. One of skill in the art can readily devise equivalent conditions, for example, by substituting the SSPE for SSC in the wash solution.

Typical highly stringent washing conditions include washing in a solution of 0.1x - 0.2x SSC with 0.1% sodium dodecyl sulfate (SDS) at 50 - 65°C. In other words, nucleic acid molecules encoding a variant zacrpl1 polypeptide remained hybridized following stringent washing conditions with a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 (or its complement), in which the wash stringency is equivalent to 0.1x - 0.2x SSC with 0.1% SDS at 50 - 65°C, including 0.1x SSC with 0.1% SDS at 50°C, or 0.2x SSC with 0.1% SDS at 65°C.

The present invention also provides isolated zacrpl1 polypeptides that have a substantially similar sequence identity to the polypeptide of SEQ ID NO:2, or
orthologs. The term “substantially similar sequence identity” is used herein to denote polypeptides having 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence shown in SEQ ID NO:2.

The present invention also contemplates zacr11 variant nucleic acid molecules that can be identified using two criteria: a determination of the similarity between the encoded polypeptide with the amino acid sequence of SEQ ID NO:2, and a hybridization assay, as described above. Such zacr11 variants include nucleic acid molecules (1) that remain hybridized following stringent washing conditions with a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 (or its complement), in which the wash stringency is equivalent to 0.5x - 2x SSC with 0.1% SDS at 55 - 65°C, and (2) that encode a polypeptide comprising the amino acid sequence amino acid residue 61 to amino acid residue 111 of SEQ ID NO:2.

Alternatively, zacr11 variants can be characterized as nucleic acid molecules (1) that remain hybridized following highly stringent washing conditions with a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 (or its complement), in which the wash stringency is equivalent to 0.1x - 0.2x SSC with 0.1% SDS at 50 - 65°C, and (2) that encode a polypeptide comprising the amino acid sequence amino acid residue 61 to amino acid residue 219 of SEQ ID NO:2.

The present invention also includes particular zacr11 variants are characterized using hybridization analysis with a reference nucleic acid molecule that is a fragment of a nucleic acid molecule consisting of the nucleotide sequence of SEQ ID NO:1, or its complement. For example, such reference nucleic acid molecules include nucleic acid molecules consisting of the following nucleotide sequences, or complements thereof, SEQ ID NO:1, nucleotides 181 to 657 of SEQ ID NO:1.

Percent sequence identity is determined by conventional methods. See, for example, Altschul et al., Bull. Math. Bio. 48:603 (1986), and Henikoff and Henikoff, Proc. Nat’l Acad. Sci. USA 89:10915 (1992). Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the “BLOSUM62” scoring matrix of Henikoff and Henikoff (ibid.) as shown in Table 3 (amino acids are indicated by the standard one-letter codes). The percent identity is then calculated as: ([Total number of identical matches]/ [length of the longer sequence plus the number of gaps introduced into the longer sequence in order to align the two sequences]) 100.
Table 3

| A | R | N | D | C | Q | E | G | H | I | L | K | M | F | P | S | T | W | Y | V |
|   | 4 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 5 | -1| 5 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|   | -2| 0 | 6 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| D | -2| 1 | 6 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| C | 0 | -3| -3| -3| 9 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Q | -1| 1 | 0 | 0 | -3| 5 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| E | -1| 0 | 0 | 2 | -4| 2 | 5 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 10| G | 0 | -2| 0 | -1| 3 | -2| -2| 6 |   |   |   |   |   |   |   |   |   |   |   |   |   |
| H | -2| 0 | 1 | -1| -3| 0 | 0 | -2| 8 |   |   |   |   |   |   |   |   |   |   |   |   |   |
| I | -1| -3| -3| -3| -1| -3| -3| -4| -3| 4 |   |   |   |   |   |   |   |   |   |   |   |   |
| L | -1| -2| -3| -4| -1| -2| -3| -4| -3| 2 | 4 |   |   |   |   |   |   |   |   |   |   |   |
| K | -1| 2 | 0 | -1| -3| 1 | 1 | -2| -1| -3| -2| 5 |   |   |   |   |   |   |   |   |   |   |
| 15| M | -1| -1| -2| -3| -1| 0 | -2| -3| -2| 1 | 2 | -1| 5 |   |   |   |   |   |   |   |   |   |
| F | -2| -3| -3| -2| -3| -3| -3| -1| 0 | 0 | -3| 0 | 6 |   |   |   |   |   |   |   |   |   |   |
| P | -1| -2| -2| -1| -3| -1| -1| -2| -2| -3| -3| -1| -2| -4| 7 |   |   |   |   |   |   |   |   |   |   |
| S | 1 | -1| 1 | 0 | 0 | 0 | 0 | -1| -2| -2| 0 | -1| -2| -1| 4 |   |   |   |   |   |   |   |   |   |   |
| T | 0 | -1| 0 | -1| -1| -1| -1| -2| -2| -1| -1| -1| -2| -1| 1 | 5 |   |   |   |   |   |   |   |   |   |   |
| 20| W | -3| -3| -4| -4| -2| -2| -3| -3| -2| -3| -1| 1 | -4| -3| -2| 1 |   |   |   |   |   |   |   |   |   |
| Y | -2| -2| -2| -3| -2| -3| -2| -3| -2| -3| -3| -2| -2| 2 | 7 |   |   |   |   |   |   |   |   |   |   |
| V | 0 | -3| -3| -3| -1| -2| -2| -3| -3| 3 | 1 | -2| 1 | -1| -2| -2| 0 | -3| -1| 4 |   |   |   |   |
Those skilled in the art appreciate that there are many established algorithms available to align two amino acid sequences. The "FASTA" similarity search algorithm of Pearson and Lipman is a suitable protein alignment method for examining the level of identity shared by an amino acid sequence disclosed herein and the amino acid sequence of a putative zacrp11 variant. The FASTA algorithm is described by Pearson and Lipman, *Proc. Nat'l Acad. Sci. USA* 85:2444 (1988), and by Pearson, *Meth. Enzymol. 183*:63 (1990). Briefly, FASTA first characterizes sequence similarity by identifying regions shared by the query sequence (e.g., SEQ ID NO:2) and a test sequence that have either the highest density of identities (if the ktup variable is 1) or pairs of identities (if ktup=2), without considering conservative amino acid substitutions, insertions, or deletions. The ten regions with the highest density of identities are then rescored by comparing the similarity of all paired amino acids using an amino acid substitution matrix, and the ends of the regions are "trimmed" to include only those residues that contribute to the highest score. If there are several regions with scores greater than the "cutoff" value (calculated by a predetermined formula based upon the length of the sequence and the ktup value), then the trimmed initial regions are examined to determine whether the regions can be joined to form an approximate alignment with gaps. Finally, the highest scoring regions of the two amino acid sequences are aligned using a modification of the Needleman-Wunsch-Sellers algorithm (Needleman and Wunsch, *J. Mol. Biol. 48*:444 (1970); Sellers, *SIAM J. Appl. Math. 26*:787 (1974)), which allows for amino acid insertions and deletions. Illustrative parameters for FASTA analysis are: ktup=1, gap opening penalty=10, gap extension penalty=1, and substitution matrix=BLOSUM62. These parameters can be introduced into a FASTA program by modifying the scoring matrix file ("SMATRIX"), as explained in Appendix 2 of Pearson, *Meth. Enzymol. 183*:63 (1990).

FASTA can also be used to determine the sequence identity of nucleic acid molecules using a ratio as disclosed above. For nucleotide sequence comparisons, the ktup value can range between one to six, preferably from three to six, most preferably three, with other parameters set as described above.

The present invention includes nucleic acid molecules that encode a polypeptide having a conservative amino acid change, compared with the amino acid sequence of SEQ ID NO:2. That is, variants can be obtained that contain one or more amino acid substitutions of SEQ ID NO:2, in which an alkyl amino acid is substituted for an alkyl amino acid in a zacrp11 amino acid sequence, an aromatic amino acid is substituted for an aromatic amino acid in a zacrp11 amino acid sequence, a sulfur-containing amino acid is substituted for a sulfur-containing amino acid in a zacrp11 amino acid sequence, a hydroxy-containing amino acid is substituted for a hydroxy-
containing amino acid in a zacrp11 amino acid sequence, an acidic amino acid is
substituted for an acidic amino acid in a zacrp11 amino acid sequence, a basic amino
acid is substituted for a basic amino acid in a zacrp11 amino acid sequence, or a dibasic
monocarboxylic amino acid is substituted for a dibasic monocarboxylic amino acid in a
zacrp11 amino acid sequence.

Among the common amino acids, for example, a "conservative amino
acid substitution" is illustrated by a substitution among amino acids within each of the
following groups: (1) glycine, alanine, valine, leucine, and isoleucine, (2)
phenylalanine, tyrosine, and tryptophan, (3) serine and threonine, (4) aspartate and
glutamate, (5) glutamine and asparagine, and (6) lysine, arginine and histidine.

The BLOSUM62 table is an amino acid substitution matrix derived from
about 2,000 local multiple alignments of protein sequence segments, representing
highly conserved regions of more than 500 groups of related proteins (Henikoff and
substitution frequencies can be used to define conservative amino acid substitutions that
may be introduced into the amino acid sequences of the present invention. Although it
is possible to design amino acid substitutions based solely upon chemical properties (as
discussed above), the language "conservative amino acid substitution" preferably refers
to a substitution represented by a BLOSUM62 value of greater than -1. For example,
an amino acid substitution is conservative if the substitution is characterized by a
BLOSUM62 value of 0, 1, 2, or 3. According to this system, preferred conservative
amino acid substitutions are characterized by a BLOSUM62 value of at least 1 (e.g., 1,
2 or 3), while more preferred conservative amino acid substitutions are characterized by
a BLOSUM62 value of at least 2 (e.g., 2 or 3).

Particular variants of zacrp11 are characterized by having greater than
96%, at least 97%, at least 98%, or at least 99% sequence identity to the corresponding
amino acid sequence (e.g., SEQ ID NO:2), wherein the variation in amino acid
sequence is due to one or more conservative amino acid substitutions.

Conservative amino acid changes in a zacrp11 gene can be introduced
by substituting nucleotides for the nucleotides recited in SEQ ID NO:1. Such
"conservative amino acid" variants can be obtained, for example, by oligonucleotide-
directed mutagenesis, linker-scanning mutagenesis, mutagenesis using the polymerase
chain reaction, and the like (see Ausubel (1995) at pages 8-10 to 8-22; and McPherson
(ed.), Directed Mutagenesis: A Practical Approach (IRL Press 1991)).

The proteins of the present invention can also comprise non-naturally
occurring amino acid residues. Non-naturally occurring amino acids include, without
limitation, trans-3-methylproline, 2,4-methanoproline, cis-4-hydroxyproline, trans-4-
hydroxyproline, N-methylglycine, allo-threonine, methylthreonine, hydroxyethylcysteine, hydroxyethylhomocysteine, nitroglutamine, homoglutamine, piperolic acid, thiazolidine carboxylic acid, dehydroproline, 3- and 4-methylproline, 3,3-dimethylproline, tert-leucine, norvaline, 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, and 4-fluorophenylalanine. Several methods are known in the art for incorporating non-naturally occurring amino acid residues into proteins. For example, an in vitro system can be employed wherein nonsense mutations are suppressed using chemically aminoacylated suppressor tRNAs. Methods for synthesizing amino acids and aminoacylating tRNA are known in the art. Transcription and translation of plasmids containing nonsense mutations is typically carried out in a cell-free system comprising an E. coli S30 extract and commercially available enzymes and other reagents. Proteins are purified by chromatography. See, for example, Robertson et al., J. Am. Chem. Soc. 113:2722 (1991), Ellman et al., Methods Enzymol. 202:301 (1991), Chung et al., Science 259:806 (1993), and Chung et al., Proc. Nat'l Acad. Sci. USA 90:10145 (1993).

In a second method, translation is carried out in Xenopus oocytes by microinjection of mutated mRNA and chemically aminoacylated suppressor tRNAs (Turcatti et al., J. Biol. Chem. 271:19991 (1996)). Within a third method, E. coli cells are cultured in the absence of a natural amino acid that is to be replaced (e.g., phenylalanine) and in the presence of the desired non-naturally occurring amino acid(s) (e.g., 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, or 4-fluorophenylalanine). The non-naturally occurring amino acid is incorporated into the protein in place of its natural counterpart. See, Koide et al., Biochem. 33:7470 (1994). Naturally occurring amino acid residues can be converted to non-naturally occurring species by in vitro chemical modification. Chemical modification can be combined with site-directed mutagenesis to further expand the range of substitutions (Wynn and Richards, Protein Sci. 2:395 (1993)).

A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, non-naturally occurring amino acids, and unnatural amino acids may be substituted for zacp11 amino acid residues.

Essential amino acids in the polypeptides of the present invention can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, Science 244:1081 (1989), Bass et al., Proc. Nat'l Acad. Sci. USA 88:4498 (1991), Coombs and Corey, "Site-Directed Mutagenesis and Protein Engineering," in Proteins: Analysis and Design, Angeletti (ed.), pages 259-311 (Academic Press, Inc. 1998)). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the
resultant mutant molecules are tested for biological activity as disclosed below to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton et al., J. Biol. Chem. 271:4699 (1996).

The location of zacrp11 activity domains can also be determined by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids. See, for example, de Vos et al., Science 255:306 (1992), Smith et al., J. Mol. Biol. 224:899 (1992), and Wlodaver et al., FEBS Lett. 309:59 (1992). Moreover, zacrp11 labeled with biotin or FITC can be used for expression cloning of zacrp11 substrates and inhibitors.

Multiple amino acid substitutions can be made and tested using known methods of mutagenesis and screening, such as those disclosed by Reidhaar-Olson and Sauer (Science 241:53 (1988)) or Bowie and Sauer (Proc. Nat’l Acad. Sci. USA 86:2152 (1989)). Briefly, these authors disclose methods for simultaneously randomizing two or more positions in a polypeptide, selecting for functional polypeptide, and then sequencing the mutagenized polypeptides to determine the spectrum of allowable substitutions at each position. Other methods that can be used include phage display (e.g., Lowman et al., Biochem. 30:10832 (1991), Ladner et al., U.S. Patent No. 5,223,409, Huse, international publication No. WO 92/06204, and region-directed mutagenesis (Derbyshire et al., Gene 46:145 (1986), and Ner et al., DNA 7:127, (1988)).

Variants of the disclosed zacrp11 nucleotide and polypeptide sequences can also be generated through DNA shuffling as disclosed by Stemmer, Nature 370:389 (1994), Stemmer, Proc. Nat’l Acad. Sci. USA 91:10747 (1994), and international publication No. WO 97/20078. Briefly, variant DNAs are generated by in vitro homologous recombination by random fragmentation of a parent DNA followed by reassembly using PCR, resulting in randomly introduced point mutations. This technique can be modified by using a family of parent DNAs, such as allelic variants or DNAs from different species, to introduce additional variability into the process. Selection or screening for the desired activity, followed by additional iterations of mutagenesis and assay provides for rapid “evolution” of sequences by selecting for desirable mutations while simultaneously selecting against detrimental changes.

Mutagenesis methods as disclosed herein can be combined with high-throughput, automated screening methods to detect activity of cloned, mutagenized polypeptides in host cells. Mutagenized DNA molecules that encode biologically active polypeptides, or polypeptides that bind with anti-zacrp11 antibodies, can be recovered from the host cells and rapidly sequenced using modern equipment. These methods
allow the rapid determination of the importance of individual amino acid residues in a polypeptide of interest, and can be applied to polypeptides of unknown structure.

The present invention also includes “functional fragments” of zacrpl polypeptides and nucleic acid molecules encoding such functional fragments. Routine deletion analyses of nucleic acid molecules can be performed to obtain functional fragments of a nucleic acid molecule that encodes a zacrpl polypeptide. As an illustration, DNA molecules having the nucleotide sequence of SEQ ID NO:1 can be digested with Bal31 nuclease to obtain a series of nested deletions. One alternative to exonuclease digestion is to use oligonucleotide-directed mutagenesis to introduce deletions or stop codons to specify production of a desired fragment. Alternatively, particular fragments of a zacrpl gene can be synthesized using the polymerase chain reaction.


The present invention also contemplates functional fragments of a zacrpl gene that has amino acid changes, compared with the amino acid sequence of SEQ ID NO:2. A variant zacrpl gene can be identified on the basis of structure by determining the level of identity with nucleotide and amino acid sequences of SEQ ID NOs:1 and 2, as discussed above. An alternative approach to identifying a variant gene on the basis of structure is to determine whether a nucleic acid molecule encoding a potential variant zacrpl gene can hybridize to a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, as discussed above.

The present invention also provides polypeptide fragments or peptides comprising an epitope-bearing portion of a zacrpl polypeptide described herein. Such fragments or peptides may comprise an “immunogenic epitope,” which is a part of a protein that elicits an antibody response when the entire protein is used as an
immunogen. Immunogenic epitope-bearing peptides can be identified using standard methods (see, for example, Geysen et al., Proc. Nat’l Acad. Sci. USA 81:3998 (1983)).

In contrast, polypeptide fragments or peptides may comprise an “antigenic epitope,” which is a region of a protein molecule to which an antibody can specifically bind. Certain epitopes consist of a linear or contiguous stretch of amino acids, and the antigenicity of such an epitope is not disrupted by denaturing agents. It is known in the art that relatively short synthetic peptides that can mimic epitopes of a protein can be used to stimulate the production of antibodies against the protein (see, for example, Sutcliffe et al., Science 219:660 (1983)). Accordingly, antigenic epitope-bearing peptides and polypeptides of the present invention are useful to raise antibodies that bind with the polypeptides described herein.

Antigenic epitope-bearing peptides and polypeptides preferably contain at least four to ten amino acids, at least ten to fifteen amino acids, or about 15 to about 30 amino acids of SEQ ID NO:2. Such epitope-bearing peptides and polypeptides can be produced by fragmenting a zacrp11 polypeptide, or by chemical peptide synthesis, as described herein. Moreover, epitopes can be selected by phage display of random peptide libraries (see, for example, Lane and Stephen, Curr. Opin. Immunol. 5:268 (1993), and Cortese et al., Curr. Opin. Biotechnol. 7:616 (1996)). Standard methods for identifying epitopes and producing antibodies from small peptides that comprise an epitope are described, for example, by Mole, “Epitope Mapping,” in Methods in Molecular Biology, Vol. 10, Manson (ed.), pages 105-116 (The Humana Press, Inc. 1992), Price, “Production and Characterization of Synthetic Peptide-Derived Antibodies,” in Monoclonal Antibodies: Production, Engineering, and Clinical Application, Ritter and Ladyman (eds.), pages 60-84 (Cambridge University Press 1995), and Coligan et al. (eds.), Current Protocols in Immunology, pages 9.3.1 - 9.3.5 and pages 9.4.1 - 9.4.11 (John Wiley & Sons 1997).

For any zacrp11 polypeptide, including variants and fusion proteins, one of ordinary skill in the art can readily generate a fully degenerate polynucleotide sequence encoding that variant using the information set forth in Tables 1 and 2 above. Moreover, those of skill in the art can use standard software to devise zacrp11 variants based upon the nucleotide and amino acid sequences described herein. Accordingly, the present invention includes a computer-readable medium encoded with a data structure that provides at least one of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3. Suitable forms of computer-readable media include magnetic media and optically-readable media. Examples of magnetic media include a hard or fixed drive, a random access memory (RAM) chip, a floppy disk, digital linear tape (DLT), a disk cache, and a ZIP disk. Optically readable media are exemplified by compact discs (e.g., CD-read
only memory (ROM), CD-rewritable (RW), and CD-recordable), and digital versatile/video discs (DVD) (e.g., DVD-ROM, DVD-RAM, and DVD+RW).

Production of zacrpl11 Fusion Proteins

Fusion proteins of zacrpl11 can be used to express zacrpl11 in a recombinant host, and to isolate expressed zacrpl11. As described below, particular zacrpl11 fusion proteins also have uses in diagnosis and therapy.

One type of fusion protein comprises a peptide that guides a zacrpl11 polypeptide from a recombinant host cell. To direct a zacrpl11 polypeptide into the secretory pathway of a eukaryotic host cell, a secretory signal sequence (also known as a signal peptide, a leader sequence, prepro sequence or pro sequence) is provided in the zacrpl11 expression vector. While the secretory signal sequence may be derived from zacrpl11, a suitable signal sequence may also be derived from another secreted protein or synthesized de novo. The secretory signal sequence is operably linked to a zacrpl11-encoding sequence such that the two sequences are joined in the correct reading frame and positioned to direct the newly synthesized polypeptide into the secretory pathway of the host cell. Secretory signal sequences are commonly positioned 5' to the nucleotide sequence encoding the polypeptide of interest, although certain secretory signal sequences may be positioned elsewhere in the nucleotide sequence of interest (see, e.g., Welch et al., U.S. Patent No. 5,037,743; Holland et al., U.S. Patent No. 5,143,830).

While the secretory signal sequence of zacrpl11 or another protein produced by mammalian cells (e.g., tissue-type plasminogen activator signal sequence, as described, for example, in U.S. Patent No. 5,641,655) is useful for expression of zacrpl11 in recombinant mammalian hosts, a yeast signal sequence is preferred for expression in yeast cells. Examples of suitable yeast signal sequences are those derived from yeast mating pheromone α-factor (encoded by the MFA1 gene), invertase (encoded by the SUC2 gene), or acid phosphatase (encoded by the PHO5 gene). See, for example, Romanos et al., “Expression of Cloned Genes in Yeast,” in DNA Cloning 2: A Practical Approach, 2nd Edition, Glover and Hames (eds.), pages 123-167 (Oxford University Press 1995).

In bacterial cells, it is often desirable to express a heterologous protein as a fusion protein to decrease toxicity, increase stability, and to enhance recovery of the expressed protein. For example, zacrpl11 can be expressed as a fusion protein comprising a glutathione S-transferase polypeptide. Glutathione S-transferase fusion proteins are typically soluble, and easily purifiable from E. coli lysates on immobilized glutathione columns. In similar approaches, a zacrpl11 fusion protein comprising a maltose binding protein polypeptide can be isolated with an amylose resin column,
while a fusion protein comprising the C-terminal end of a truncated Protein A gene can be purified using IgG-Sepharose. Established techniques for expressing a heterologous polypeptide as a fusion protein in a bacterial cell are described, for example, by Williams et al., "Expression of Foreign Proteins in E. coli Using Plasmid Vectors and Purification of Specific Polyclonal Antibodies," in DNA Cloning 2: A Practical Approach, 2nd Edition, Glover and Hames (Eds.), pages 15-58 (Oxford University Press 1995). In addition, commercially available expression systems are available. For example, the PINPOINT Xa protein purification system (Promega Corporation; Madison, WI) provides a method for isolating a fusion protein comprising a polypeptide that becomes biotinylated during expression with a resin that comprises avidin.

Peptide tags that are useful for isolating heterologous polypeptides expressed by either prokaryotic or eukaryotic cells include polyhistidine tags (which have an affinity for nickel-chelating resin), c-myc tags, calmodulin binding protein (isolated with calmodulin affinity chromatography), substance P, the RYIRS tag (which binds with anti-RYIRS antibodies), the Glu-Glu tag, and the FLAG tag (which binds with anti-FLAG antibodies). See, for example, Luo et al., Arch. Biochem. Biophys. 329:215 (1996), Morganti et al., Biotecnol. Appl. Biochem. 23:67 (1996), and Zheng et al., Gene 186:55 (1997). Nucleic acid molecules encoding such peptide tags are available, for example, from Sigma-Aldrich Corporation (St. Louis, MO).

Another form of fusion protein comprises a zacrpl1 polypeptide and an immunoglobulin heavy chain constant region, typically an Fc fragment, which contains two constant region domains and a hinge region but lacks the variable region. As an illustration, Chang et al., U.S. Patent No. 5,723,125, describe a fusion protein comprising a human interferon and a human immunoglobulin Fc fragment, in which the C-terminal of the interferon is linked to the N-terminal of the Fc fragment by a peptide linker moiety. An example of a peptide linker is a peptide comprising primarily a T cell inert sequence, which is immunologically inert. In such a fusion protein, an illustrative Fc moiety is a human γ4 chain, which is stable in solution and has little or no complement activating activity. Accordingly, the present invention contemplates a zacrpl1 fusion protein that comprises a zacrpl1 moiety and a human Fc fragment, wherein the C-terminus of the zacrpl1 moiety is attached to the N-terminus of the Fc fragment via a peptide linker. The zacrpl1 moiety can be a zacrpl1 molecule or a fragment thereof.

In another variation, a zacrpl1 fusion protein comprises an IgG sequence, a zacrpl1 moiety covalently joined to the amino terminal end of the IgG sequence, and a signal peptide that is covalently joined to the amino terminal of the zacrpl1 moiety, wherein the IgG sequence consists of the following elements in the
following order: a hinge region, a CH₂ domain, and a CH₃ domain. Accordingly, the
IgG sequence lacks a CH₁ domain. The zacrpl1 moiety displays a zacrpl1 activity, as
described herein, such as the ability to bind with a zacrpl1 antibody. This general
approach to producing fusion proteins that comprise both antibody and nonantibody
portions has been described by LaRochelle et al., EP 742830 (WO 95/21258).

Fusion proteins comprising a zacrpl1 moiety and an Fc moiety can be
used, for example, as an in vitro assay tool. For example, the presence of a zacrpl1
inhibitor in a biological sample can be detected using a zacrpl1-antibody fusion
protein, in which the zacrpl1 moiety is used to target the substrate or inhibitor, and a
macromolecule, such as Protein A or anti-Fc antibody; is used to detect the bound
fusion protein-receptor complex. Furthermore, such fusion proteins can be used to
identify molecules that interfere with the binding of zacrpl1 and a substrate.

Fusion proteins can be prepared by methods known to those skilled in
the art by preparing each component of the fusion protein and chemically conjugating
the components. Alternatively, a polynucleotide encoding both components of the
fusion protein in the proper reading frame can be generated using known techniques
and expressed by the methods described herein. General methods for enzymatic and
chemical cleavage of fusion proteins are described, for example, by Ausubel (1995) at
pages 16-19 to 16-25.

zacrp11 Analogs and zacrpl1 Inhibitors

One general class of zacrpl1 analogs are variants having an amino acid
sequence that is a mutation of the amino acid sequence disclosed herein. Another
general class of zacrpl1 analogs is provided by anti-idiotypic antibodies, and fragments
thereof, as described below. Moreover, recombinant antibodies comprising anti-
idiotypic variable domains can be used as analogs (see, for example, Monfardini et al.,
idiotypic zacrpl1 antibodies mimic zacrpl1, these domains can provide zacrpl1
activity. Methods of producing anti-idiotypic catalytic antibodies are known to those of
skill in the art (see, for example, Joron et al., Ann. N Y Acad. Sci. 672:216 (1992),
Acad. Sci. 864:118 (1998)).

Another approach to identifying zacrpl1 analogs is provided by the use
of combinatorial libraries. Methods for constructing and screening phage display and
other combinatorial libraries are provided, for example, by Kay et al., Phage Display of
Solution in vitro assays can be used to identify a zacrpl polypeptide or inhibitor. Solid phase systems can also be used to identify a substrate or inhibitor of a zacrpl polypeptide. For example, a zacrpl polypeptide or zacrpl fusion protein can be immobilized onto the surface of a receptor chip of a commercially available biosensor instrument (BIACORE, Biacore AB; Uppsala, Sweden). The use of this instrument is disclosed, for example, by Karlsson, *Immunol. Methods* 145:229 (1991), and Cunningham and Wells, *J. Mol. Biol.* 234:554 (1993).

In brief, a zacrpl polypeptide or fusion protein is covalently attached, using amine or sulfhydryl chemistry, to dextran fibers that are attached to gold film within a flow cell. A test sample is then passed through the cell. If a zacrpl substrate or inhibitor is present in the sample, it will bind to the immobilized polypeptide or fusion protein, causing a change in the refractive index of the medium, which is detected as a change in surface plasmon resonance of the gold film. This system allows the determination on- and off-rates, from which binding affinity can be calculated, and assessment of the stoichiometry of binding, as well as the kinetic effects of zacrpl mutation. This system can also be used to examine antibody-antigen interactions, and the interactions of other complement/anti-complement pairs.

Production of zacrpl Polypeptides in Cultured Cells

The polypeptides of the present invention, including full-length polypeptides, functional fragments, and fusion proteins, can be produced in recombinant host cells following conventional techniques. To express a *zacrpl* gene, a nucleic acid molecule encoding the polypeptide must be operably linked to regulatory sequences that control transcriptional expression in an expression vector and then, introduced into a host cell. In addition to transcriptional regulatory sequences, such as promoters and enhancers, expression vectors can include translational regulatory sequences and a marker gene which is suitable for selection of cells that carry the expression vector.

Expression vectors that are suitable for production of a foreign protein in eukaryotic cells typically contain (1) prokaryotic DNA elements coding for a bacterial replication origin and an antibiotic resistance marker to provide for the growth and selection of the expression vector in a bacterial host; (2) eukaryotic DNA elements that control initiation of transcription, such as a promoter; and (3) DNA elements that control the processing of transcripts, such as a transcription termination/polyadenylation sequence. As discussed above, expression vectors can also include nucleotide sequences encoding a secretory sequence that directs the heterologous polypeptide into the secretory pathway of a host cell. For example, a zacrpl expression vector may
comprise a Zacral gene and a secretory sequence derived from a Zacral gene or another secreted gene.

Zacral proteins of the present invention may be expressed in mammalian cells. Examples of suitable mammalian host cells include African green monkey kidney cells (Vero; ATCC CRL 1587), human embryonic kidney cells (293-HEK; ATCC CRL 1573), baby hamster kidney cells (BHK-21, BHK-570; ATCC CRL 8544, ATCC CRL 10314), canine kidney cells (MDCK; ATCC CCL 34), Chinese hamster ovary cells (CHO-K1; ATCC CCL61; CHO DG44 (Chasin et al., Som. Cell. Molec. Genet. 12:555, 1986)), rat pituitary cells (GH1; ATCC CCL82), HeLa S3 cells (ATCC CCL2.2), rat hepatoma cells (H-4-II-E; ATCC CRL 1548) SV40-transformed monkey kidney cells (COS-1; ATCC CRL 1650) and murine embryonic cells (NIH-3T3; ATCC CRL 1658).

For a mammalian host, the transcriptional and translational regulatory signals may be derived from viral sources, such as adenovirus, bovine papilloma virus, simian virus, or the like, in which the regulatory signals are associated with a particular gene which has a high level of expression. Suitable transcriptional and translational regulatory sequences also can be obtained from mammalian genes, such as actin, collagen, myosin, and metallothionein genes.


Alternatively, a prokaryotic promoter, such as the bacteriophage T3 RNA polymerase promoter, can be used to control Zacral gene expression in mammalian cells if the prokaryotic promoter is regulated by a eukaryotic promoter (Zhou et al., Mol. Cell. Biol. 10:4529 (1990), and Kaufman et al., Nucl. Acids Res. 19:4485 (1991)).

An expression vector can be introduced into host cells using a variety of standard techniques including calcium phosphate transfection, liposome-mediated transfection, microprojectile-mediated delivery, electroporation, and the like. Preferably, the transfected cells are selected and propagated to provide recombinant host cells that
comprise the expression vector stably integrated in the host cell genome. Techniques for introducing vectors into eukaryotic cells and techniques for selecting such stable transformants using a dominant selectable marker are described, for example, by Ausubel (1995) and by Murray (ed.), *Gene Transfer and Expression Protocols* (Humana Press 1991).

For example, one suitable selectable marker is a gene that provides resistance to the antibiotic neomycin. In this case, selection is carried out in the presence of a neomycin-type drug, such as G-418 or the like. Selection systems can also be used to increase the expression level of the gene of interest, a process referred to as "amplification." Amplification is carried out by culturing transfecteds in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high levels of the products of the introduced genes. An exemplary amplifiable selectable marker is dihydrofolate reductase, which confers resistance to methotrexate. Other drug resistance genes (e.g., hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can also be used. Alternatively, markers that introduce an altered phenotype, such as green fluorescent protein, or cell surface proteins (e.g., CD4, CD8, Class I MHC, and placental alkaline phosphatase) may be used to sort transfected cells from untransfected cells by such means as FACS sorting or magnetic bead separation technology.

Zacrp11 polypeptides can also be produced by cultured cells using a viral delivery system. Exemplary viruses for this purpose include adenovirus, herpesvirus, vaccinia virus and adeno-associated virus (AAV). Adenovirus, a double-stranded DNA virus, is currently the best studied gene transfer vector for delivery of heterologous nucleic acid (for a review, see Becker et al., *Meth. Cell Biol.* 43:161 (1994), and Douglas and Curiel, *Science & Medicine* 4:44 (1997)). Advantages of the adenovirus system include the accommodation of relatively large DNA inserts, the ability to grow to high-titer, the ability to infect a broad range of mammalian cell types, and flexibility that allows use with a large number of available vectors containing different promoters.

By deleting portions of the adenovirus genome, larger inserts (up to 7 kb) of heterologous DNA can be accommodated. These inserts can be incorporated into the viral DNA by direct ligation or by homologous recombination with a cotransfected plasmid. An option is to delete the essential EI gene from the viral vector, which results in the inability to replicate unless the EI gene is provided by the host cell. For example, adenovirus vector infected human 293 cells (ATCC Nos. CRL-1573, 45504, 45505) can be grown as adherent cells or in suspension culture at relatively high
cell density to produce significant amounts of protein (see Garnier et al., Cytotechnol. 15:145 (1994)).

 Zacp11 genes may also be expressed in other higher eukaryotic cells, such as avian, fungal, insect, yeast, or plant cells. The baculovirus system provides an efficient means to introduce cloned Zacp11 genes into insect cells. Suitable expression vectors are based upon the Autographa californica multiple nuclear polyhedrosis virus (AcMNPV), and contain well-known promoters such as Drosophila heat shock protein (hsp) 70 promoter, Autographa californica nuclear polyhedrosis virus immediate-early gene promoter (ie-1) and the delayed early 39K promoter, baculovirus p10 promoter, and the Drosophila metallothionein promoter. A second method of making recombinant baculovirus utilizes a transposon-based system described by Luckow (Luckow, et al., J. Virol. 67:4566 (1993)). This system, which utilizes transfer vectors, is sold in the BAC-to-BAC kit (Life Technologies, Rockville, MD). This system utilizes a transfer vector, PFASTBAC (Life Technologies) containing a Tn7 transposon to move the DNA encoding the Zacp11 polypeptide into a baculovirus genome maintained in E. coli as a large plasmid called a “bacmid.” See, Hill-Perkins and Possee, J. Gen. Virol. 71:971 (1990), Bonning, et al., J. Gen. Virol. 75:1551 (1994), and Chazenbalk, and Rapoport, J. Biol. Chem. 270:1543 (1995). In addition, transfer vectors can include an in-frame fusion with DNA encoding an epitope tag at the C- or N-terminus of the expressed Zacp11 polypeptide, for example, a Glu-Glu epitope tag (Grussenmeyer et al., Proc. Nat’l Acad. Sci. 82:7952 (1985)). Using a technique known in the art, a transfer vector containing a Zacp11 gene is transformed into E. coli, and screened for bacmids which contain an interrupted lacZ gene indicative of recombinant baculovirus. The bacmid DNA containing the recombinant baculovirus genome is then isolated using common techniques.

The illustrative PFASTBAC vector can be modified to a considerable degree. For example, the polyhedrin promoter can be removed and substituted with the baculovirus basic protein promoter (also known as Pcor, p6.9 or MP promoter) which is expressed earlier in the baculovirus infection, and has been shown to be advantageous for expressing secreted proteins (see, for example, Hill-Perkins and Possee, J. Gen. Virol. 71:971 (1990), Bonning, et al., J. Gen. Virol. 75:1551 (1994), and Chazenbalk and Rapoport, J. Biol. Chem. 270:1543 (1995). In such transfer vector constructs, a short or long version of the basic protein promoter can be used. Moreover, transfer vectors can be constructed which replace the native Zacp11 secretory signal sequences with secretory signal sequences derived from insect proteins. For example, a secretory signal sequence from Ecdysteroid Glucosyltransferase (EGT), honey bee Melittin
(Invitrogen Corporation; Carlsbad, CA), or baculovirus gp67 (PharMingen: San Diego, CA) can be used in constructs to replace the native zacrp11 secretory signal sequence.

The recombinant virus or bacmid is used to transfect host cells. Suitable insect host cells include cell lines derived from IPLB-Sf-21, a Spodoptera frugiperda pupal ovarian cell line, such as Sf9 (ATCC CRL 1711), Sf21AE, and Sf21 (Invitrogen Corporation; San Diego, CA), as well as Drosophila Schneider-2 cells, and the HIGH FIVEO cell line (Invitrogen) derived from Trichoplusia ni (U.S. Patent No. 5,300,435). Commercially available serum-free media can be used to grow and to maintain the cells. Suitable media are Sf900 II™ (Life Technologies) or ESF 921™ (Expression Systems) for the Sf9 cells; and Ex-cellO405™ (JRH Biosciences, Lenexa, KS) or Express FiveO™ (Life Technologies) for the T. ni cells. When recombinant virus is used, the cells are typically grown up from an inoculation density of approximately 2-5 x 10^5 cells to a density of 1-2 x 10^6 cells at which time a recombinant viral stock is added at a multiplicity of infection (MOI) of 0.1 to 10, more typically near 3.


Fungal cells, including yeast cells, can also be used to express the genes described herein. Yeast species of particular interest in this regard include Saccharomyces cerevisiae, Pichia pastoris, and Pichia methanolica. Suitable promoters for expression in yeast include promoters from GAL1 (galactose), PGK (phosphoglycerate kinase), ADH (alcohol dehydrogenase), AOX1 (alcohol oxidase), HIS4 (histidinol dehydrogenase), and the like. Many yeast cloning vectors have been designed and are readily available. These vectors include YIp-based vectors, such as YIp5, YRp vectors, such as YRp17, YEp vectors such as YEpl3 and YCp vectors, such as YCP19. Methods for transforming S. cerevisiae cells with exogenous DNA and producing recombinant polypeptides there from are disclosed by, for example, Kawasaki, U.S. Patent No. 4,599,311, Kawasaki et al., U.S. Patent No. 4,931,373, Brake, U.S. Patent No. 4,870,008, Welch et al., U.S. Patent No. 5,037,743, and Murray et al., U.S. Patent No. 4,845,075. Transformed cells are selected by phenotype
determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (e.g., leucine). An illustrative vector system for use in Saccharomyces cerevisiae is the POT1 vector system disclosed by Kawasaki et al. (U.S. Patent No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. Additional suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, e.g., Kawasaki, U.S. Patent No. 4,599,311, Kingsman et al., U.S. Patent No. 4,615,974, and Bitter, U.S. Patent No. 4,977,092) and alcohol dehydrogenase genes. See also U.S. Patents Nos. 4,990,446, 5,063,154, 5,139,936, and 4,661,454.

Transformation systems for other yeasts, including Hansenula polymorpha, Schizosaccharomyces pombe, Kluyveromyces lactis, Kluyveromyces fragilis, Ustilago maydis, Pichia pastoris, Pichia methanolica, Pichia guillermontii and Candida maltosa are known in the art. See, for example, Gleeson et al., J. Gen. Microbiol. 132:3459 (1986), and Cregg, U.S. Patent No. 4,882,279. Aspergillus cells may be utilized according to the methods of McKnight et al., U.S. Patent No. 4,935,349. Methods for transforming Acrocomium chrysogenum are disclosed by Sumino et al., U.S. Patent No. 5,162,228. Methods for transforming Neurospora are disclosed by Lambowitz, U.S. Patent No. 4,486,533.

For example, the use of Pichia methanolica as host for the production of recombinant proteins is disclosed by Raymond, U.S. Patent No. 5,716,808, Raymond, U.S. Patent No. 5,736,383, Raymond et al., Yeast 14:11-23 (1998), and in international publication Nos. WO 97/17450, WO 97/17451, WO 98/02536, and WO 98/02565. DNA molecules for use in transforming P. methanolica will commonly be prepared as double-stranded, circular plasmids, which are preferably linearized prior to transformation. For polypeptide production in P. methanolica, it is preferred that the promoter and terminator in the plasmid be that of a P. methanolica gene, such as a P. methanolica alcohol utilization gene (AUG1 or AUG2). Other useful promoters include those of the dihydroxyacetone synthase (DHAS), formate dehydrogenase (FMD), and catalase (CAT) genes. To facilitate integration of the DNA into the host chromosome, it is preferred to have the entire expression segment of the plasmid flanked at both ends by host DNA sequences. An illustrative selectable marker for use in Pichia methanolica is a P. methanolica ADE2 gene, which encodes phosphoribosyl-5-aminomidazole carboxylase (AJRC; EC 4.1.1.21), and which allows ade2 host cells to grow in the absence of adenine. For large-scale, industrial processes where it is desirable to minimize the use of methanol, it is preferred to use host cells in which both methanol utilization genes (AUG1 and AUG2) are deleted. For production of secreted proteins, host cells deficient in vacuolar protease genes (PEP4 and PRB1) are preferred.
Electroporation is used to facilitate the introduction of a plasmid containing DNA encoding a polypeptide of interest into \textit{P. methanolicum} cells. \textit{P. methanolicum} cells can be transformed by electroporation using an exponentially decaying, pulsed electric field having a field strength of from 2.5 to 4.5 kV/cm, preferably about 3.75 kV/cm, and a time constant (\( t \)) of from 1 to 40 milliseconds, most preferably about 20 milliseconds.

Expression vectors can also be introduced into plant protoplasts, intact plant tissues, or isolated plant cells. Methods for introducing expression vectors into plant tissue include the direct infection or co-cultivation of plant tissue with \textit{Agrobacterium tumefaciens}, microprojectile-mediated delivery, DNA injection, electroporation, and the like. See, for example, Horsch \textit{et al.}, \textit{Science} 227:1229 (1985), Klein \textit{et al.}, \textit{Biotechnology} 10:268 (1992), and Miki \textit{et al.}, “Procedures for Introducing Foreign DNA into Plants,” in \textit{Methods in Plant Molecular Biology and Biotechnology}, Glick \textit{et al.} (eds.), pages 67-88 (CRC Press, 1993).

Alternatively, \textit{zacp11} genes can be expressed in prokaryotic host cells. Suitable promoters that can be used to express \textit{zacp11} polypeptides in a prokaryotic host are well-known to those of skill in the art and include promoters capable of recognizing the T4, T3, Sp6 and T7 polymerases, the \( P_R \) and \( P_L \) promoters of bacteriophage lambda, the \textit{trp}, \textit{recA}, heat shock, \textit{lacUV5}, \textit{tac}, \textit{lpp-lacSpr}, \textit{phoA}, and \textit{lacZ} promoters of \textit{E. coli}, promoters of \textit{B. subtilis}, the promoters of the bacteriophages of \textit{Bacillus}, \textit{Streptomyces} promoters, the \textit{int} promoter of bacteriophage lambda, the \textit{bla} promoter of pBR322, and the CAT promoter of the chloramphenicol acetyl transferase gene. Prokaryotic promoters have been reviewed by Glick, \textit{J. Ind. Microbiol.} 1:277 (1987), Watson \textit{et al.}, \textit{Molecular Biology of the Gene, 4th Ed.} (Benjamin Cummins 1987), and by Ausubel \textit{et al.} (1995).

Useful prokaryotic hosts include \textit{E. coli} and \textit{Bacillus subtilis}. Suitable strains of \textit{E. coli} include BL21(DE3), BL21(DE3)pLysS, BL21(DE3)pLysE, DH1, DH4I, DH5, DH5I, DH5IF, DH5IMCR, DH10B, DH10B/p3, DH11S, C600, HB101, JM101, JM105, JM109, JM110, K38, RR1, Y1088, Y1089, CSH18, ER1451, and ER1647 (see, for example, Brown (ed.), \textit{Molecular Biology Labfax} (Academic Press 1991)). Suitable strains of \textit{Bacillus subtilis} include BR151, YB886, MI119, MI120, and B170 (see, for example, Hardy, “Bacillus Cloning Methods,” in \textit{DNA Cloning: A Practical Approach}, Glover (ed.) (IRL Press 1985)).

When expressing a \textit{zacp11} polypeptide in bacteria such as \textit{E. coli}, the polypeptide may be retained in the cytoplasm, typically as insoluble granules, or may be directed to the periplasmic space by a bacterial secretion sequence. In the former case, the cells are lysed, and the granules are recovered and denatured using, for example, guanidine isothiocyanate or urea. The denatured polypeptide can then be refolded and
dimerized by diluting the denaturant, such as by dialysis against a solution of urea and a combination of reduced and oxidized glutathione, followed by dialysis against a buffered saline solution. In the latter case, the polypeptide can be recovered from the periplasmic space in a soluble and functional form by disrupting the cells (by, for example, sonication or osmotic shock) to release the contents of the periplasmic space and recovering the protein, thereby obviating the need for denaturation and refolding.


As an alternative, polypeptides of the present invention can be synthesized by exclusive solid phase synthesis, partial solid phase methods, fragment condensation or classical solution synthesis. These synthesis methods are well-known to those of skill in the art (see, for example, Merrifield, J. Am. Chem. Soc. 85:2149 (1963), Stewart et al., "Solid Phase Peptide Synthesis" (2nd Edition), (Pierce Chemical Co. 1984), Bayer and Rapp, Chem. Pept. Prot. 3:3 (1986), Atherton et al., Solid Phase Peptide Synthesis: A Practical Approach (IRL Press 1989), Fields and Colowick, "Solid-Phase Peptide Synthesis," Methods in Enzymology Volume 289 (Academic Press 1997), and Lloyd-Williams et al., Chemical Approaches to the Synthesis of Peptides and Proteins (CRC Press, Inc. 1997)). Variations in total chemical synthesis strategies, such as “native chemical ligation” and “expressed protein ligation” are also standard

**Isolation of zacrp11 Polypeptides**

The polypeptides of the present invention can be purified to at least about 80% purity, to at least about 90% purity, to at least about 95% purity, or greater than 95% purity with respect to contaminating macromolecules, particularly other proteins and nucleic acids, and free of infectious and pyrogenic agents. The polypeptides of the present invention may also be purified to a pharmaceutically pure state, which is greater than 99.9% pure. Certain purified polypeptide preparations are substantially free of other polypeptides, particularly other polypeptides of animal origin.

Fractionation and/or conventional purification methods can be used to obtain preparations of zacrp11 purified from natural sources, and recombinant zacrp11 polypeptides and fusion zacrp11 polypeptides purified from recombinant host cells. In general, ammonium sulfate precipitation and acid or chaotrope extraction may be used for fractionation of samples. Exemplary purification steps may include hydroxypatite, size exclusion, FPLC and reverse-phase high performance liquid chromatography. Suitable chromatographic media include derivatized dextrans, agarose, cellulose, polyacrylamide, specialty silicas, and the like. PEI, DEAE, QAE and Q derivatives are preferred. Exemplary chromatographic media include those media derivatized with phenyl, butyl, or octyl groups, such as Phenyl-Sepharose FF (Pharmacia), Toyopearl butyl 650 (Toso Haas, Montgomeryville, PA), Octyl-Sepharose (Pharmacia) and the like; or polycrylic resins, such as Amberchrom CG 71 (Toso Haas) and the like. Suitable solid supports include glass beads, silica-based resins, cellulose resins, agarose beads, cross-linked agarose beads, polystyrene beads, cross-linked polycrylamide resins and the like that are insoluble under the conditions in which they are to be used. These supports may be modified with reactive groups that allow attachment of proteins by amino groups, carboxyl groups, sulfhydryl groups, hydroxyl groups and/or carbohydrate moieties.

Examples of coupling chemistries include cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, hydrazide activation, and carboxyl and amino derivatives for carbodiimide coupling chemistries. These and other solid media are well known and widely used in the art, and are available from commercial suppliers. Selection of a particular method for polypeptide isolation and purification is a matter of routine design and is determined in part by the

Additional variations in zacrpi1 isolation and purification can be devised by those of skill in the art. For example, anti-zacrpi1 antibodies, obtained as described below, can be used to isolate large quantities of protein by immunoaffinity purification.

The polypeptides of the present invention can also be isolated by exploitation of particular properties. For example, immobilized metal ion adsorption (IMAC) chromatography can be used to purify histidine-rich proteins, including those comprising polyhistidine tags. Briefly, a gel is first charged with divalent metal ions to form a chelate (Sulkowski, *Trends in Biochem. 3*:1 (1985)). Histidine-rich proteins will be adsorbed to this matrix with differing affinities, depending upon the metal ion used, and will be eluted by competitive elution, lowering the pH, or use of strong chelating agents. Other methods of purification include purification of glycosylated proteins by lectin affinity chromatography and ion exchange chromatography (M. Deutscher, (ed.), *Meth. Enzymol. 182*:529 (1990)). Within additional embodiments of the invention, a fusion of the polypeptide of interest and an affinity tag (*e.g.*, maltose-binding protein, an immunoglobulin domain) may be constructed to facilitate purification.

Zacrpi1 polypeptides or fragments thereof may also be prepared through chemical synthesis, as described above. Zacrpi1 polypeptides may be monomers or multimers; glycosylated or non-glycosylated; PEGylated or non-PEGylated; and may or may not include an initial methionine amino acid residue.

The present invention also contemplates chemically modified zacrpi1 compositions, in which a zacrpi1 polypeptide is linked with a polymer. Typically, the polymer is water soluble so that the zacrpi1 conjugate does not precipitate in an aqueous environment, such as a physiological environment. An example of a suitable polymer is one that has been modified to have a single reactive group, such as an active ester for acylation, or an aldehyde for alkylation. In this way, the degree of polymerization can be controlled. An example of a reactive aldehyde is polyethylene glycol propionaldehyde, or mono-(C1-C10) alkoxy, or aryloxy derivatives thereof (see, for example, Harris, *et al.*, U.S. Patent No. 5,252,714). The polymer may be branched or unbranched. Moreover, a mixture of polymers can be used to produce zacrpi1 conjugates.

Zacrpi1 conjugates used for therapy should preferably comprise pharmaceutically acceptable water-soluble polymer moieties. Suitable water-soluble polymers include polyethylene glycol (PEG), monomethoxy-PEG, mono-(C1-
C10)alkoxy-PEG, aryloxy-PEG, poly-(N-vinyl pyrrolidone)PEG, tressyl monomethoxy PEG, PEG propionaldehyde, bis-succinimidyl carbonate PEG, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, dextran, cellulose, or other carbohydrate-based polymers. Suitable PEG may have a molecular weight from about 600 to about 60,000, including, for example, 5,000, 12,000, 20,000 and 25,000. A zacrp11 conjugate can also comprise a mixture of such water-soluble polymers. Anti-zacrp11 antibodies or anti-idiotype antibodies can also be conjugated with a water-soluble polymer.

The present invention contemplates compositions comprising a peptide or polypeptide described herein. Such compositions can further comprise a carrier. The carrier can be a conventional organic or inorganic carrier. Examples of carriers include water, buffer solution, alcohol, propylene glycol, macrogol, sesame oil, corn oil, and the like.

Peptides and polypeptides of the present invention comprise at least six, at least nine, or at least 15 contiguous amino acid residues of SEQ ID NO:2. Within certain embodiments of the invention, the polypeptides comprise 20, 30, 40, 50, 100, or more contiguous residues of these amino acid sequences. Additional polypeptides can comprise at least 15, at least 30, at least 40, or at least 50 contiguous amino acids of such regions of SEQ ID NO:2. Nucleic acid molecules encoding such peptides and polypeptides are useful as polymerase chain reaction primers and probes.

Production of Antibodies to zacrp11 Proteins

Antibodies to zacrp11 can be obtained, for example, using as an antigen the product of a zacrp11 expression vector or zacrp11 isolated from a natural source. Particularly useful anti-zacrp11 antibodies “bind specifically” with zacrp11. Antibodies are considered to be specifically binding if the antibodies exhibit at least one of the following two properties: (1) antibodies bind to zacrp11 with a threshold level of binding activity, and (2) antibodies do not significantly cross-react with polypeptides related to zacrp11.

With regard to the first characteristic, antibodies specifically bind if they bind to a zacrp11 polypeptide, peptide or epitope with a binding affinity (Kd) of \(10^5 \text{M}^{-1}\) or greater, preferably \(10^7 \text{M}^{-1}\) or greater, more preferably \(10^8 \text{M}^{-1}\) or greater, and most preferably \(10^9 \text{M}^{-1}\) or greater. The binding affinity of an antibody can be readily determined by one of ordinary skill in the art, for example, by Scatchard analysis (Scatchard, *Ann. NY Acad. Sci.* 51:660 (1949)). With regard to the second characteristic, antibodies do not significantly cross-react with related polypeptide
molecules, for example, if they detect zacrpl11, but not known related polypeptides using a standard Western blot analysis. Examples of known related polypeptides are orthologs and proteins from the same species that are members of a protein family.

Anti-zacrpl11 antibodies can be produced using antigenic zacrpl11 epitope-bearing peptides and polypeptides. Antigenic epitope-bearing peptides and polypeptides of the present invention contain a sequence of at least nine, preferably between 15 to about 30 amino acids contained within SEQ ID NO:2. However, peptides or polypeptides comprising a larger portion of an amino acid sequence of the invention, containing from 30 to 50 amino acids, or any length up to and including the entire amino acid sequence of a polypeptide of the invention, also are useful for inducing antibodies that bind with zacrpl11. It is desirable that the amino acid sequence of the epitope-bearing peptide is selected to provide substantial solubility in aqueous solvents (i.e., the sequence includes relatively hydrophilic residues, while hydrophobic residues are preferably avoided). Moreover, amino acid sequences containing proline residues may be also be desirable for antibody production.

As an illustration, potential antigenic sites in zacrpl11 were identified using the Jameson-Wolf method, Jameson and Wolf, *CABIOS* 4:181, (1988), as implemented by the PROTEAN program (version 3.14) of LASERGENE (DNASTAR; Madison, WI). Default parameters were used in this analysis.

The Jameson-Wolf method predicts potential antigenic determinants by combining six major subroutines for protein structural prediction. Briefly, the Hopp-Woods method, Hopp *et al.*, *Proc. Nat'l Acad. Sci. USA* 78:3824 (1981), is first used to identify amino acid sequences representing areas of greatest local hydrophilicity (parameter: seven residues averaged). In the second step, Emini's method, Emini *et al.*, *J. Virolology* 55:836 (1985), is used to calculate surface probabilities (parameter: surface decision threshold (0.6) = 1). Third, the Karplus-Schultz method, Karplus and Schultz, *Naturwissenschaften* 72:212 (1985), is used to predict backbone chain flexibility (parameter: flexibility threshold (0.2) = 1). In the fourth and fifth steps of the analysis, secondary structure predictions are applied to the data using the methods of Chou-Fasman, Chou, “Prediction of Protein Structural Classes from Amino Acid Composition,” in *Prediction of Protein Structure and the Principles of Protein Conformation*, Fasman (ed.), pages 549-586 (Plenum Press 1990), and Garnier-Robson, Garnier *et al.*, *J. Mol. Biol.* 120:97 (1978) (Chou-Fasman parameters: conformation table = 64 proteins; α region threshold = 103; β region threshold = 105; Garnier-Robson parameters: α and β decision constants = 0). In the sixth subroutine, flexibility parameters and hydropathy/solvent accessibility factors are combined to determine a surface contour value, designated as the “antigenic index.” Finally, a peak broadening
function is applied to the antigenic index, which broadens major surface peaks by adding 20, 40, 60, or 80% of the respective peak value to account for additional free energy derived from the mobility of surface regions relative to interior regions. This calculation is not applied, however, to any major peak that resides in a helical region, since helical regions tend to be less flexible.


The immunogenicity of a zacrp11 polypeptide can be increased through the use of an adjuvant, such as alum (aluminum hydroxide) or Freund's complete or incomplete adjuvant. Polypeptides useful for immunization also include fusion polypeptides, such as fusions of zacrp11 or a portion thereof with an immunoglobulin polypeptide or with maltose binding protein. The polypeptide immunogen may be a full-length molecule or a portion thereof. If the polypeptide portion is "hapten-like," such portion may be advantageously joined or linked to a macromolecular carrier (such as keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or tetanus toxoid) for immunization.

Although polyclonal antibodies are typically raised in animals such as horse, cow, dog, chicken, rat, mouse, rabbit, goat, guinea pig, or sheep, an anti-zacrp11 antibody of the present invention may also be derived from a subhuman primate antibody. General techniques for raising diagnostically and therapeutically useful antibodies in baboons may be found, for example, in Goldenberg *et al.*, international patent publication No. WO 91/11465, and in Losman *et al.*, *Int. J. Cancer* 46:310 (1990).

Alternatively, monoclonal anti-zacrp11 antibodies can be generated. Rodent monoclonal antibodies to specific antigens may be obtained by methods known to those skilled in the art (see, for example, Kohler *et al.*, *Nature* 256:495 (1975), Coligan *et al.* (eds.), *Current Protocols in Immunology*, Vol. 1, pages 2.5.1-2.6.7 (John Wiley & Sons 1991) ["Coligan"], Picklesy *et al.*, "Production of monoclonal antibodies

Briefly, monoclonal antibodies can be obtained by injecting mice with a composition comprising a *zacrp11* gene product, verifying the presence of antibody production by removing a serum sample, removing the spleen to obtain B-lymphocytes, fusing the B-lymphocytes with myeloma cells to produce hybridomas, cloning the hybridomas, selecting positive clones which produce antibodies to the antigen, culturing the clones that produce antibodies to the antigen, and isolating the antibodies from the hybridoma cultures.

In addition, an anti-*zacrp11* antibody of the present invention may be derived from a human monoclonal antibody. Human monoclonal antibodies are obtained from transgenic mice that have been engineered to produce specific human antibodies in response to antigenic challenge. In this technique, elements of the heavy and light chain locus are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy chain and light chain loci. The transgenic mice can synthesize human antibodies specific for human antigens, and the mice can be used to produce human antibody-secreting hybridomas. Methods for obtaining human antibodies from transgenic mice are described, for example, by Green *et al.*, *Nature Genet.* 7:13 (1994), Lonberg *et al.*, *Nature* 368:856 (1994), and Taylor *et al.*, *Int. Immun.* 6:579 (1994).

Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography (see, for example, Coligan at pages 2.7.1-2.7.12 and pages 2.9.1-2.9.3; Baines *et al.*, “Purification of Immunoglobulin G (IgG),” in *Methods in Molecular Biology, Vol. 10*, pages 79-104 (The Humana Press, Inc. 1992)).

For particular uses, it may be desirable to prepare fragments of anti-*zacrp11* antibodies. Such antibody fragments can be obtained, for example, by proteolytic hydrolysis of the antibody. Antibody fragments can be obtained by papain digestion of whole antibodies by conventional methods. As an illustration, antibody fragments can be produced by enzymatic cleavage of antibodies with papain to provide a 5S fragment denoted F(ab')2. This fragment can be further cleaved using a thiol reducing agent to produce 3.5S Fab' monovalent fragments. Optionally, the cleavage reaction can be performed using a blocking group for the sulphydryl groups that result from cleavage of disulfide linkages. As an alternative, an enzymatic cleavage using pepsin produces two monovalent Fab fragments and an Fc fragment directly. These methods are described, for example, by Goldenberg, U.S. patent No.

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

For example, Fv fragments comprise an association of V\(_H\) and V\(_L\) chains. This association can be noncovalent, as described by Inbar *et al.*, *Proc. Nat'l Acad. Sci. USA* 69:2659 (1972). Alternatively, the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde (see, for example, Sandhu, *Crit. Rev. Biotech.* 12:437 (1992)).

The Fv fragments may comprise V\(_H\) and V\(_L\) chains which are connected by a peptide linker. These single-chain antigen binding proteins (scFv) are prepared by constructing a structural gene comprising DNA sequences encoding the V\(_H\) and V\(_L\) domains which are connected by an oligonucleotide. The structural gene is inserted into an expression vector which is subsequently introduced into a host cell, such as *E. coli*. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing scFvs are described, for example, by Whitlow *et al.*, *Methods: A Companion to Methods in Enzymology* 2:97 (1991) (also see, Bird *et al.*, *Science* 242:423 (1988), Ladner *et al.*, U.S. Patent No. 4,946,778, Pack *et al.*, *Bio/Technology* 11:1271 (1993), and Sandhu, *supra*).

As an illustration, a scFv can be obtained by exposing lymphocytes to zacrpl1 polypeptide *in vitro*, and selecting antibody display libraries in phage or similar vectors (for instance, through use of immobilized or labeled zacrpl1 protein or peptide). Genes encoding polypeptides having potential zacrpl1 polypeptide binding domains can be obtained by screening random peptide libraries displayed on phage (phage display) or on bacteria, such as *E. coli*. Nucleotide sequences encoding the polypeptides can be obtained in a number of ways, such as through random mutagenesis and random polynucleotide synthesis. These random peptide display libraries can be used to screen for peptides which interact with a known target which can be a protein or polypeptide, such as a ligand or receptor, a biological or synthetic macromolecule, or organic or inorganic substances. Techniques for creating and screening such random peptide display libraries are known in the art (Ladner *et al.*, U.S. Patent No. 5,223,409, Ladner *et al.*, U.S. Patent No. 4,946,778, Ladner *et al.*, U.S. Patent No. 5,403,484, Ladner *et al.*, U.S. Patent No. 5,571,698, and Kay *et al.*, *Phage Display of Peptides and Proteins* (Academic Press, Inc. 1996)) and random peptide display libraries and kits for
screening such libraries are available commercially, for instance from CLONTECH Laboratories, Inc. (Palo Alto, CA), Invitrogen Inc. (San Diego, CA), New England Biolabs, Inc. (Beverly, MA), and Pharmacia LKB Biotechnology Inc. (Piscataway, NJ). Random peptide display libraries can be screened using the zacrp11 sequences disclosed herein to identify proteins which bind to zacrp11.

Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition units") can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells (see, for example, Larrick et al., Methods: A Companion to Methods in Enzymology 2:106 (1991), Courtenay-Luck, "Genetic Manipulation of Monoclonal Antibodies," in Monoclonal Antibodies: Production, Engineering and Clinical Application, Ritter et al. (eds.), page 166 (Cambridge University Press 1995), and Ward et al., "Genetic Manipulation and Expression of Antibodies," in Monoclonal Antibodies: Principles and Applications, Birch et al. (eds.), page 137 (Wiley-Liss, Inc. 1995)).


Polyclonal anti-idiotypic antibodies can be prepared by immunizing animals with anti-zacrp11 antibodies or antibody fragments, using standard techniques. See, for example, Green et al., "Production of Polyclonal Antisera," in Methods In Molecular Biology: Immunochemical Protocols, Manson (ed.), pages 1-12 (Humana Press 1992). Also, see Coligan at pages 2.4.1-2.4.7. Alternatively, monoclonal anti-
idiotype antibodies can be prepared using anti-zacrpl11 antibodies or antibody fragments as immunogens with the techniques, described above. As another alternative, humanized anti-idiotype antibodies or subhuman primate anti-idiotype antibodies can be prepared using the above-described techniques. Methods for producing anti-idiotype antibodies are described, for example, by Iric, U.S. Patent No. 5,208,146, Greene, et al., U.S. Patent No. 5,637,677, and Varthakavi and Minocha, J. Gen. Virol. 77:1875 (1996).

Anti-idiotype zacrpl11 antibodies, as well as zacrpl11 polypeptides, can be used to identify and to isolate zacrpl11 substrates and inhibitors. For example, proteins and peptides of the present invention can be immobilized on a column and used to bind substrate and inhibitor proteins from biological samples that are run over the column (Hermanson et al. (eds.), Immobilized Affinity Ligand Techniques, pages 195-202 (Academic Press 1992)). Radiolabeled or affinity labeled zacrpl11 polypeptides can also be used to identify or to localize zacrpl11 substrates and inhibitors in a biological sample (see, for example, Deutscher (ed.), Methods in Enzymol., vol. 182, pages 721-37 (Academic Press 1990); Brunner et al., Ann. Rev. Biochem. 62:483 (1993); Fedan et al., Biochem. Pharmacol. 33:1167 (1984)).

Use of Zacrp11 Nucleotide Sequences to Detect Zacrp11 Gene Expression and to Examine Zacrp11 Gene Structure

Nucleic acid molecules can be used to detect the expression of a zacrpl11 gene in a biological sample. Such probe molecules include double-stranded nucleic acid molecules comprising the nucleotide sequence of SEQ ID NO:1, or a fragment thereof, as well as single-stranded nucleic acid molecules having the complement of the nucleotide sequence of SEQ ID NO:1, or a fragment thereof. Probe molecules may be DNA, RNA, oligonucleotides, and the like.

In a basic assay, a single-stranded probe molecule is incubated with RNA, isolated from a biological sample, under conditions of temperature and ionic strength that promote base pairing between the probe and target zacrpl11 RNA species. After separating unbound probe from hybridized molecules, the amount of hybrids is detected.

Well-established hybridization methods of RNA detection include northern analysis and dot/slot blot hybridization (see, for example, Ausubel (1995) at pages 4-1 to 4-27, and Wu et al. (eds.), "Analysis of Gene Expression at the RNA Level," in Methods in Gene Biotechnology, pages 225-239 (CRC Press, Inc. 1997)). Nucleic acid probes can be detectably labeled with radioisotopes such as $^{32}$P or $^{35}$S. Alternatively, zacrpl11 RNA can be detected with a nonradioactive hybridization method
(see, for example, Isaac (ed.), *Protocols for Nucleic Acid Analysis by Nonradioactive Probes* (Humana Press, Inc. 1993)). Typically, nonradioactive detection is achieved by enzymatic conversion of chromogenic or chemiluminescent substrates. Illustrative nonradioactive moieties include biotin, fluorescein, and digoxigenin.

Zacrp11 oligonucleotide probes are also useful for *in vivo* diagnosis. As an illustration, $^{18}$F-labeled oligonucleotides can be administered to a subject and visualized by positron emission tomography (Tavitian et al., *Nature Medicine* 4:467 (1998)).

Numerous diagnostic procedures take advantage of the polymerase chain reaction (PCR) to increase sensitivity of detection methods. Standard techniques for performing PCR are well-known (see, generally, Mathew (ed.), *Protocols in Human Molecular Genetics* (Humana Press, Inc. 1991), White (ed.), *PCR Protocols: Current Methods and Applications* (Humana Press, Inc. 1993), Cotter (ed.), *Molecular Diagnosis of Cancer* (Humana Press, Inc. 1996), Hanasek and Walaszek (eds.), *Tumor Marker Protocols* (Humana Press, Inc. 1998), Lo (ed.), *Clinical Applications of PCR* (Humana Press, Inc. 1998), and Meltzer (ed.), *PCR in Bioanalysis* (Humana Press, Inc. 1998)).

One variation of PCR for diagnostic assays is reverse transcriptase-PCR (RT-PCR). In the RT-PCR technique, RNA is isolated from a biological sample, reverse transcribed to cDNA, and the cDNA is incubated with zacrp11 primers (see, for example, Wu et al. (eds.), “Rapid Isolation of Specific cDNAs or Genes by PCR,” in *Methods in Gene Biotechnology*, pages 15-28 (CRC Press, Inc. 1997)). PCR is then performed and the products are analyzed using standard techniques.

As an illustration, RNA is isolated from biological sample using, for example, the guanidinium-thiocyanate cell lysis procedure described above. Alternatively, a solid-phase technique can be used to isolate mRNA from a cell lysate. A reverse transcription reaction can be primed with the isolated RNA using random oligonucleotides, short homopolymers of dT, or zacrp11 anti-sense oligomers. Oligo-dT primers offer the advantage that various mRNA nucleotide sequences are amplified that can provide control target sequences. zacrp11 sequences are amplified by the polymerase chain reaction using two flanking oligonucleotide primers that are typically 20 bases in length.

PCR amplification products can be detected using a variety of approaches. For example, PCR products can be fractionated by gel electrophoresis, and visualized by ethidium bromide staining. Alternatively, fractionated PCR products can be transferred to a membrane, hybridized with a detectably-labeled zacrp11 probe, and examined by autoradiography. Additional alternative approaches include the use of
digoxigenin-labeled deoxyribonucleic acid triphosphates to provide chemiluminescence detection, and the C-TRAK colorimetric assay.

Another approach for detection of zacrp11 expression is cycling probe technology (CPT), in which a single-stranded DNA target binds with an excess of DNA-RNA-DNA chimeric probe to form a complex, the RNA portion is cleaved with RNAse H, and the presence of cleaved chimeric probe is detected (see, for example, Beggs et al., J. Clin. Microbiol. 34:2985 (1996), Bekkaoui et al., Biotechniques 20:240 (1996)). Alternative methods for detection of zacrp11 sequences can utilize approaches such as nucleic acid sequence-based amplification (NASBA), cooperative amplification of templates by cross-hybridization (CATCH), and the ligase chain reaction (LCR) (see, for example, Marshall et al., U.S. Patent No. 5,686,272 (1997), Dyer et al., J. Virol. Methods 60:161 (1996), Ehrlich et al., Eur. J. Biochem. 243:358 (1997), and Chadwick et al., J. Virol. Methods 70:59 (1998)). Other standard methods are known to those of skill in the art.

Zacrp11 probes and primers can also be used to detect and to localize zacrp11 gene expression in tissue samples. Methods for such in situ hybridization are well-known to those of skill in the art (see, for example, Choo (ed.), In Situ Hybridization Protocols (Humana Press, Inc. 1994), Wu et al. (eds.), “Analysis of Cellular DNA or Abundance of mRNA by Radioactive In Situ Hybridization (RISH),” in Methods in Gene Biotechnology, pages 259-278 (CRC Press, Inc. 1997), and Wu et al. (eds.), “Localization of DNA or Abundance of mRNA by Fluorescence In Situ Hybridization (RISH),” in Methods in Gene Biotechnology, pages 279-289 (CRC Press, Inc. 1997)). Various additional diagnostic approaches are well-known to those of skill in the art (see, for example, Mathew (ed.), Protocols in Human Molecular Genetics (Humana Press, Inc. 1991), Coleman and Tsongalis, Molecular Diagnostics (Humana Press, Inc. 1996), and Elles, Molecular Diagnosis of Genetic Diseases (Humana Press, Inc., 1996)).

Zacrp11 nucleotide sequences can be used in linkage-based testing for various diseases, and to determine whether a subject’s chromosomes contain a mutation in the zacrp11 gene. Detectable chromosomal aberrations at the zacrp11 gene locus include, but are not limited to, aneuploidy, gene copy number changes, insertions, deletions, restriction site changes and rearrangements. Of particular interest are genetic alterations that inactivate a zacrp11 gene. Aberrations associated with a zacrp11 locus can be detected using nucleic acid molecules of the present invention by employing molecular genetic techniques, such as restriction fragment length polymorphism (RFLP) analysis, short tandem repeat (STR) analysis employing PCR techniques, amplification-refractory mutation system analysis (ARMS), single-strand conformation

The protein truncation test is also useful for detecting the inactivation of a gene in which translation-terminating mutations produce only portions of the encoded protein (see, for example, Stoppa-Lyonnet et al., Blood 91:3920 (1998)). According to this approach, RNA is isolated from a biological sample, and used to synthesize cDNA. PCR is then used to amplify the zacrpl1 target sequence and to introduce an RNA polymerase promoter, a translation initiation sequence, and an in-frame ATG triplet. PCR products are transcribed using an RNA polymerase, and the transcripts are translated in vitro with a T7-coupled reticulocyte lysate system. The translation products are then fractionated by SDS-PAGE to determine the lengths of the translation products. The protein truncation test is described, for example, by Dracopoli et al. (eds.), Current Protocols in Human Genetics, pages 9.11.1 - 9.11.18 (John Wiley & Sons 1998).

The present invention also contemplates kits for performing a diagnostic assay for zacrpl1 gene expression or to analyze the zacrpl1 locus of a subject. Such kits comprise nucleic acid probes, such as double-stranded nucleic acid molecules comprising the nucleotide sequence of SEQ ID NO:1, or a fragment thereof, as well as single-stranded nucleic acid molecules having the complement of the nucleotide sequence of SEQ ID NO:1, or a fragment thereof. Probe molecules may be DNA, RNA, oligonucleotides, and the like. Kits may comprise nucleic acid primers for performing PCR. Such a kit can contain all the necessary elements to perform a nucleic acid diagnostic assay described above. A kit will comprise at least one container comprising a zacrpl1 probe or primer. The kit may also comprise a second container comprising one or more reagents capable of indicating the presence of zacrpl1 sequences. Examples of such indicator reagents include detectable labels such as radioactive labels, fluorochromes, chemiluminescent agents, and the like. A kit may also comprise a means for conveying to the user that the zacrpl1 probes and primers are
used to detect zacrp11 gene expression. For example, written instructions may state that the enclosed nucleic acid molecules can be used to detect either a nucleic acid molecule that encodes zacrp11, or a nucleic acid molecule having a nucleotide sequence that is complementary to a zacrp11-encoding nucleotide sequence, or to analyze chromosomal sequences associated with the zacrp11 locus. The written material can be applied directly to a container, or the written material can be provided in the form of a packaging insert.

The present invention also provides reagents which will find use in diagnostic applications. For example, the zacrp11 gene, a probe comprising zacrp11 DNA or RNA or a subsequence thereof can be used to determine if the zacrp11 gene is present on a human chromosome, such as chromosome 10, or if a gene mutation has occurred. Based on annotation of a fragment of human genomic DNA containing a part of zacrp11 genomic DNA (Genbank Accession No.'s AL353576, and AL360230), zacrp11 is located at the 10p13 region of chromosome 10. Detectable chromosomal aberrations at the zacrp11 gene locus include, but are not limited to, aneuploidy, gene copy number changes, loss of heterogeneity (LOH), translocations, insertions, deletions, restriction site changes and rearrangements. Such aberrations can be detected using polynucleotides of the present invention by employing molecular genetic techniques, such as restriction fragment length polymorphism (RFLP) analysis, short tandem repeat (STR) analysis employing PCR techniques, and other genetic linkage analysis techniques known in the art (Sambrook et al., ibid.; Ausubel et. al., ibid.; Marian, Chest 108:255-65, 1995).

The precise knowledge of a gene’s position can be useful for a number of purposes, including: 1) determining if a sequence is part of an existing contig and obtaining additional surrounding genetic sequences in various forms, such as YACs, BACs or cDNA clones; 2) providing a possible candidate gene for an inheritable disease which shows linkage to the same chromosomal region; and 3) cross-referencing model organisms, such as mouse, which may aid in determining what function a particular gene might have.

The zacrp11 gene is located at the 10p13 region of chromosome 10. Several genes of known function map to this region that are linked to human disease. For example, loss of deletion and partial monosomy of chromosome 10p13-14 is a
chromosomal abnormality observed in DiGeorge Syndrome (DGS)/velocardiofacial syndrome, HDR (hypoparathyroidism, deafness, renal dysplasia) Syndrome and midline defects (Daw, SCM et al. Nature Genet. 13:458-461, 1996; Lichtner, P. et al. J. Med. Genet. 37:33-37, 2000; and Schuffenhauer, S. et al. Europ. J. Hum. Genet. 6:213-225, 1998; Hasegawar, T. et al. Am. J. Med. Genet. 73:416-418, 1997). Thus, since the zacrp11 gene maps to chromosome 10p13, the zacrp11 polynucleotide probes of the present invention can be used to detect and diagnose the presence of chromosome 10 monosomy and other chromosome 10p13 loss, and particularly chromosome 10p13 monosomy and loss associated with DGS, HDR, and other human disease. Moreover, translocation between chromosome 10p13 and chromosome 11q14 ((t(10;11)(p13;q14)) is associated with acute ALL and AML leukemias (Dreyling, MH et al., Proc. Nat. Acad. Sci. 93:4804-4809, 1996). Thus, the zacrp11 polynucleotide probes of the present invention can be used to detect and diagnose chromosome 10p13 translocation associated with acute leukemias. Moreover, several chromosomal aberrations at 10p13 including deletions, rearrangements, and chromosomal breakpoints, and translocations are seen in humans with disease as described above; since the zacrp11 gene maps to this critical region, the zacrp11 polynucleotide probes of the present invention can be used to detect chromosome deletions, translocations and rearrangements associated with those diseases. Similarly, zacrp11 polynucleotide probes of the present invention can be used to detect chromosome deletions, translocations and rearrangements associated with MPS VII. Moreover, amongst other genetic loci, those for Alzheimer’s susceptibility (AD7) gene (10p13) all manifest themselves in human disease states as well as map to this region of the human genome. See the Online Mendelian Inheritance of Man (OMIM™, National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD) gene map, and references therein, for this region of human chromosome 10 on a publicly available world wide web server. All of these serve as possible candidate genes for an inheritable disease that show linkage to the same chromosomal region as the zacrp11 gene. Thus, zacrp11 polynucleotide probes can be used to detect abnormalities or genotypes associated with these defects.

A diagnostic could assist physicians in determining the type of disease and appropriate associated therapy, or assistance in genetic counseling. As such, the
inventive anti-zacrp11 antibodies, polynucleotides, and polypeptides can be used for the
detection of zacrp11 polypeptide, mRNA or anti-zacrp11 antibodies, thus serving as
markers and be directly used for detecting or genetic diseases or cancers, as described
herein, using methods known in the art and described herein. Further, zacrp11
polynucleotide probes can be used to detect abnormalities or genotypes associated with
chromosome 10p13 deletions, monosomy and translocations associated with human
diseases, such as described above, or other translocations and LOH involved with
malignant progression of tumors or other 10p13 mutations, which are expected to be
involved in chromosome rearrangements in malignancy; or in other cancers. Similarly,
zacrp11 polynucleotide probes can be used to detect abnormalities or genotypes
associated with chromosome 10p13 trisomy and chromosome loss associated with
human diseases or spontaneous abortion. All of these serve as possible candidate genes
for an inheritable disease which show linkage to the same chromosomal region as the
zacrp11 gene. Thus, zacrp11 polynucleotide probes can be used to detect abnormalities
or genotypes associated with these defects.

One of skill in the art would recognize that of zacrp11 polynucleotide
probes are particularly useful for diagnosis of gross chromosomal abnormalities
associated with loss of heterogeneity (LOH), chromosome gain (e.g. trisomy),
translocation, chromosome loss (monosomy), DNA amplification, and the like.
Translocations within chromosomal locus 10p13 wherein the zacrp11 gene is located
are known to be associated with human disease. For example, 10p13 deletions,
monosomy and translocations are associated with specific human diseases as discussed
above. Thus, since the zacrp11 gene maps to this critical region, zacrp11 polynucleotide
probes of the present invention can be used to detect abnormalities or genotypes
associated with 10p13 translocation, deletion and trisomy, and the like, described
above.

As discussed above, defects in the zacrp11 gene itself may result in a
heritable human disease state. Molecules of the present invention, such as the
polypeptides, antagonists, agonists, polynucleotides and antibodies of the present
invention would aid in the detection, diagnosis prevention, and treatment associated
with a zacrp11 genetic defect. In addition, zacrp11 polynucleotide probes can be used
to detect allelic differences between diseased or non-diseased individuals at the zacrpl11 chromosomal locus. As such, the zacrpl11 sequences can be used as diagnostics in forensic DNA profiling.

In general, the diagnostic methods used in genetic linkage analysis, to detect a genetic abnormality or aberration in a patient, are known in the art. Analytical probes will be generally at least 20 nt in length, although somewhat shorter probes can be used (e.g., 14-17 nt). PCR primers are at least 5 nt in length, preferably 15 or more, more preferably 20-30 nt. For gross analysis of genes, or chromosomal DNA, a zacrpl11 polynucleotide probe may comprise an entire exon or more. Exons are readily determined by one of skill in the art by comparing zacrpl11 sequences (SEQ ID NO:1) with the genomic DNA for zacrpl11 (Genbank Accession No.'s AL353576, and AL360230). In general, the diagnostic methods used in genetic linkage analysis, to detect a genetic abnormality or aberration in a patient, are known in the art. Most diagnostic methods comprise the steps of (a) obtaining a genetic sample from a potentially diseased patient, diseased patient or potential non-diseased carrier of a recessive disease allele; (b) producing a first reaction product by incubating the genetic sample with a zacrpl11 polynucleotide probe wherein the polynucleotide will hybridize to complementary polynucleotide sequence, such as in RFLP analysis or by incubating the genetic sample with sense and antisense primers in a PCR reaction under appropriate PCR reaction conditions; (iii) Visualizing the first reaction product by gel electrophoresis and/or other known method such as visualizing the first reaction product with a zacrpl11 polynucleotide probe wherein the polynucleotide will hybridize to the complementary polynucleotide sequence of the first reaction; and (iv) comparing the visualized first reaction product to a second control reaction product of a genetic sample from wild type patient. A difference between the first reaction product and the control reaction product is indicative of a genetic abnormality in the diseased or potentially diseased patient, or the presence of a heterozygous recessive carrier phenotype for a non-diseased patient, or the presence of a genetic defect in a tumor from a diseased patient, or the presence of a genetic abnormality in a fetus or pre-implantation embryo. For example, a difference in restriction fragment pattern, length of PCR products, length of repetitive sequences at the zacrpl11 genetic locus, and the
like, are indicative of a genetic abnormality, genetic aberration, or allelic difference in comparison to the normal wild type control. Controls can be from unaffected family members, or unrelated individuals, depending on the test and availability of samples. Genetic samples for use within the present invention include genomic DNA, mRNA, and cDNA isolated from any tissue or other biological sample from a patient, such as but not limited to, blood, saliva, semen, embryonic cells, amniotic fluid, and the like. The polynucleotide probe or primer can be RNA or DNA, and will comprise a portion of SEQ ID NO:1, the complement of SEQ ID NO:1, or an RNA equivalent thereof. Such methods of showing genetic linkage analysis to human disease phenotypes are well known in the art. For reference to PCR based methods in diagnostics see see, generally, Mathew (ed.), Protocols in Human Molecular Genetics (Humana Press, Inc. 1991), White (ed.), PCR Protocols: Current Methods and Applications (Humana Press, Inc. 1993), Cotter (ed.), Molecular Diagnosis of Cancer (Humana Press, Inc. 1996), Hanausek and Walaszek (eds.), Tumor Marker Protocols (Humana Press, Inc. 1998), Lo (ed.), Clinical Applications of PCR (Humana Press, Inc. 1998), and Meltzer (ed.), PCR in Bioanalysis (Humana Press, Inc. 1998)).

Mutations associated with the zacrp11 locus can be detected using nucleic acid molecules of the present invention by employing standard methods for direct mutation analysis, such as restriction fragment length polymorphism analysis, short tandem repeat analysis employing PCR techniques, amplification-refractory mutation system analysis, single-strand conformation polymorphism detection, RNase cleavage methods, denaturing gradient gel electrophoresis, fluorescence-assisted mismatch analysis, and other genetic analysis techniques known in the art (see, for example, Mathew (ed.), Protocols in Human Molecular Genetics (Humana Press, Inc. 1991), Marian, Chest 108:255 (1995), Coleman and Tsongalis, Molecular Diagnostics (Human Press, Inc. 1996), Elles (ed.) Molecular Diagnosis of Genetic Diseases (Humana Press, Inc. 1996), Landegren (ed.), Laboratory Protocols for Mutation Detection (Oxford University Press 1996), Birren et al. (eds.), Genome Analysis, Vol. 2: Detecting Genes (Cold Spring Harbor Laboratory Press 1998), Dracopoli et al. (eds.), Current Protocols in Human Genetics (John Wiley & Sons 1998), and Richards and Ward, "Molecular Diagnostic Testing," in Principles of Molecular Medicine, pages 83-
88 (Humana Press, Inc. 1998)). Direct analysis of an zacrp11 gene for a mutation can be performed using a subject’s genomic DNA. Methods for amplifying genomic DNA, obtained for example from peripheral blood lymphocytes, are well-known to those of skill in the art (see, for example, Dracopoli et al. (eds.), Current Protocols in Human Genetics, at pages 7.1.6 to 7.1.7 (John Wiley & Sons 1998)).

Use of Anti-Zacrp11 Antibodies to Detect Zacrp11 Protein

The present invention contemplates the use of anti-zacrp11 antibodies to screen biological samples *in vitro* for the presence of zacrp11. In one type of *in vitro* assay, anti-zacrp11 antibodies are used in liquid phase. For example, the presence of zacrp11 in a biological sample can be tested by mixing the biological sample with a trace amount of labeled zacrp11 and an anti-zacrp11 antibody under conditions that promote binding between zacrp11 and its antibody. Complexes of zacrp11 and anti-zacrp11 in the sample can be separated from the reaction mixture by contacting the complex with an immobilized protein which binds with the antibody, such as an Fc antibody or *Staphylococcus* protein A. The concentration of zacrp11 in the biological sample will be inversely proportional to the amount of labeled zacrp11 bound to the antibody and directly related to the amount of free labeled zacrp11.

Alternatively, *in vitro* assays can be performed in which anti-zacrp11 antibody is bound to a solid-phase carrier. For example, antibody can be attached to a polymer, such as aminodextran, in order to link the antibody to an insoluble support such as a polymer-coated bead, a plate or a tube. Other suitable *in vitro* assays will be readily apparent to those of skill in the art.

In another approach, anti-zacrp11 antibodies can be used to detect zacrp11 in tissue sections prepared from a biopsy specimen. Such immunochemical detection can be used to determine the relative abundance of zacrp11 and to determine the distribution of zacrp11 in the examined tissue. General immunochemistry techniques are well established (see, for example, Ponder, “Cell Marking Techniques and Their Application,” in Mammalian Development: A Practical Approach, Monk (ed.), pages 115-38 (IRL Press 1987), Coligan at pages 5.8.1-5.8.8, Ausubel (1995) at pages 14.6.1 to 14.6.13 (Wiley Interscience 1990), and Manson (ed.), Methods In Molecular Biology, Vol.10: Immunochemical Protocols (The Humana Press, Inc. 1992)).

Immmunochemical detection can be performed by contacting a biological sample with an anti-zacrp11 antibody, and then contacting the biological sample with a detectably labeled molecule which binds to the antibody. For example, the detectably
labeled molecule can comprise an antibody moiety that binds to anti-zacrp11 antibody. Alternatively, the anti-zacrp11 antibody can be conjugated with avidin/streptavidin (or biotin) and the detectably labeled molecule can comprise biotin (or avidin/streptavidin). Numerous variations of this basic technique are well-known to those of skill in the art.

Alternatively, an anti-zacrp11 antibody can be conjugated with a detectable label to form an anti-zacrp11 immunoconjugate. Suitable detectable labels include, for example, a radioisotope, a fluorescent label, a chemiluminescent label, an enzyme label, a bioluminescent label or colloidal gold. Methods of making and detecting such detectably-labeled immunoconjugates are well-known to those of ordinary skill in the art, and are described in more detail below.

The detectable label can be a radioisotope that is detected by autoradiography. Isotopes that are particularly useful for the purpose of the present invention are $^3\text{H}$, $^{125}\text{I}$, $^{131}\text{I}$, $^{35}\text{S}$ and $^{14}\text{C}$.

Anti-zacrp11 immunoconjugates can also be labeled with a fluorescent compound. The presence of a fluorescently-labeled antibody is determined by exposing the immunoconjugate to light of the proper wavelength and detecting the resultant fluorescence. Fluorescent labeling compounds include fluorescein isothiocyanate, rhodamine, phycoerytherin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

Alternatively, anti-zacrp11 immunoconjugates can be detectably labeled by coupling an antibody component to a chemiluminescent compound. The presence of the chemiluminescent-tagged immunoconjugate is determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of chemiluminescent labeling compounds include luminol, isoluminol, an aromatic acridinium ester, an imidazole, an acridinium salt and an oxalate ester.

Similarly, a bioluminescent compound can be used to label anti-zacrp11 immunoconjugates of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Bioluminescent compounds that are useful for labeling include luciferin, luciferase and aequorin.

Alternatively, anti-zacrp11 immunoconjugates can be detectably labeled by linking an anti-zacrp11 antibody component to an enzyme. When the anti-zacrp11-enzyme conjugate is incubated in the presence of the appropriate substrate, the enzyme moiety reacts with the substrate to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorometric or visual means. Examples of enzymes that can be used to detectably label polyspecific immunoconjugates include $\beta$-galactosidase, glucose oxidase, peroxidase and alkaline phosphatase.
Those of skill in the art will know of other suitable labels which can be employed in accordance with the present invention. The binding of marker moieties to anti-zacrp11 antibodies can be accomplished using standard techniques known to the art. Typical methodology in this regard is described by Kennedy et al., *Clin. Chim. Acta* 70:1 (1976), Schurs et al., *Clin. Chim. Acta* 81:1 (1977), Shih et al., *Int'l J. Cancer* 46:1101 (1990), Stein et al., *Cancer Res.* 50:1330 (1990), and Coligan, *supra*.

Moreover, the convenience and versatility of immunochemical detection can be enhanced by using anti-zacrp11 antibodies that have been conjugated with avidin, streptavidin, and biotin (see, for example, Wilchek et al. (eds.), “Avidin-Biotin Technology,” *Methods In Enzymology*, Vol. 184 (Academic Press 1990), and Bayer et al., “Immunochemical Applications of Avidin-Biotin Technology,” in *Methods In Molecular Biology*, Vol. 10, Manson (ed.), pages 149-162 (The Humana Press, Inc. 1992).


In a related approach, biotin- or FITC-labeled zacrp11 can be used to identify cells that bind zacrp11. Such can binding can be detected, for example, using flow cytometry.

The present invention also contemplates kits for performing an immunological diagnostic assay for zacrp11 gene expression. Such kits comprise at least one container comprising an anti-zacrp11 antibody, or antibody fragment. A kit may also comprise a second container comprising one or more reagents capable of indicating the presence of zacrp11 antibody or antibody fragments. Examples of such indicator reagents include detectable labels such as a radioactive label, a fluorescent label, a chemiluminescent label, an enzyme label, a bioluminescent label, colloidal gold, and the like. A kit may also comprise a means for conveying to the user that zacrp11 antibodies or antibody fragments are used to detect zacrp11 protein. For example, written instructions may state that the enclosed antibody or antibody fragment can be used to detect zacrp11. The written material can be applied directly to a container, or the written material can be provided in the form of a packaging insert.
Use of zacrpl1 polypeptides and polypeptides

Zacrpl1 polypeptides, fragments, fusions, agonists or antagonists can be used to modulate energy balance in mammals or to protect endothelial cells from injury. With regard to modulating energy balance, Zacrp11 polypeptides could find use to modulate cellular metabolic reactions. Such metabolic reactions include adipogenesis, gluconeogenesis, glycogenolysis, lipogenesis, glucose uptake, protein synthesis, thermogenesis, oxygen utilization and the like. Zacrp11 may also be evaluated for antimicrobial activity. Zacrp11 polypeptide may be used for surgical pretreatment to prevent injury due to ischemia and/or inflammation or in like procedures. Zacrp11 polypeptides may also find use as neurotransmitters or as modulators of neurotransmission. In this regard, zacrpl1 polypeptides may find utility in modulating nutrient uptake, as demonstrated, for example, by 2-deoxy-glucose uptake in the brain or the like.

Among other methods known in the art or described herein, mammalian energy balance may be evaluated by monitoring one or more of the following metabolic functions: adipogenesis, gluconeogenesis, glycogenolysis, lipogenesis, glucose uptake, protein synthesis, thermogenesis, oxygen utilization or the like. These metabolic functions are monitored by techniques (assays or animal models) known to one of ordinary skill in the art, as is more fully set forth below. For example, the glucoregulatory effects of insulin are predominantly exerted in the liver, skeletal muscle and adipose tissue. Insulin binds to its cellular receptor in these three tissues and initiates tissue-specific actions that result in, for example, the inhibition of glucose production and the stimulation of glucose utilization. In the liver, insulin stimulates glucose uptake and inhibits gluconeogenesis and glycogenolysis. In skeletal muscle and adipose tissue, insulin acts to stimulate the uptake, storage and utilization of glucose.

Art-recognized methods exist for monitoring all of the metabolic functions recited above. Thus, one of ordinary skill in the art is able to evaluate zacrpl1 polypeptides, fragments, fusion proteins, antibodies, agonists and antagonists for metabolic modulating functions. Exemplary modulating techniques are set forth below.

Adipogenesis, gluconeogenesis and glycogenolysis are interrelated components of mammalian energy balance, which may be evaluated by known techniques using, for example, ob/ob mice or db/db mice. The ob/ob mice are inbred mice that are homozygous for an inactivating mutation at the ob (obese) locus. Such ob/ob mice are hyperphagic and hypometabolic, and are believed to be deficient in production of circulating OB protein. The db/db mice are inbred mice that are homozygous for an inactivating mutation at the db (diabetes) locus. The db/db mice
display a phenotype similar to that of ob/ob mice, except db/db mice also display a
diabetic phenotype. Such db/db mice are believed to be resistant to the effects of
circulating OB protein. Also, various in vitro methods of assessing these parameters
are known in the art.

Insulin-stimulated lipogenesis, for example, may be monitored by
measuring the incorporation of $^{14}$C-acetate into triglyceride (Mackall et al. J. Biol.
Chem. 251:6462-4, 1976) or triglyceride accumulation (Kletzien et al., Mol.
Pharmacol. 41:393-8, 1992).

Glucose uptake may be evaluated, for example, in an assay for insulin-
stimulated glucose transport. Non-transfected, differentiated L6 myotubes (maintained
in the absence of G418) are placed in DMEM containing 1 g/l glucose, 0.5 or 1.0%
BSA, 20 mM Hepes, and 2 mM glutamine. After two to five hours of culture, the
medium is replaced with fresh, glucose-free DMEM containing 0.5 or 1.0% BSA, 20
mM Hepes, 1 mM pyruvate, and 2 mM glutamine. Appropriate concentrations of
insulin or IGF-1, or a dilution series of the test substance, are added, and the cells are
incubated for 20-30 minutes. $^3$H or $^{14}$C-labeled deoxyglucose is added to $\approx$50 nM final
concentration, and the cells are incubated for approximately 10-30 minutes. The cells
are then quickly rinsed with cold buffer (e.g. PBS), then lysed with a suitable lysing
agent (e.g. 1% SDS or 1 N NaOH). The cell lysate is then evaluated by counting in a
scintillation counter. Cell-associated radioactivity is taken as a measure of glucose
transport after subtracting non-specific binding as determined by incubating cells in the
presence of cytocholasin b, an inhibitor of glucose transport. Other methods include
those described by, for example, Manchester et al., Am. J. Physiol. 266 (Endocrinol.

Protein synthesis may be evaluated, for example, by comparing
precipitation of $^{35}$S-methionine-labeled proteins following incubation of the test cells
with $^{35}$S-methionine and $^{38}$S-methionine and a putative modulator of protein synthesis.

Thermogenesis may be evaluated as described by B. Stanley in The
Biology of Neuropeptide Y and Related Peptides, W. Colmers and C. Wahlestedt (eds.),
Humana Press, Ottawa, 1993, pp. 457-509; C. Billington et al., Am. J. Physiol.
260:R321, 1991; N. Zarjevski et al., Endocrinology 133:1753, 1993; C. Billington et
al., Am. J. Physiol. 266:R1765, 1994; Heller et al., Am. J. Physiol. 252(4 Pt 2): R661-7,
1987; and Heller et al., Am. J. Physiol. 245: R321-8, 1983. Also, metabolic rate, which
may be measured by a variety of techniques, is an indirect measurement of
thermogenesis.

Oxygen utilization may be evaluated as described by Heller et al.,
Pflugers Arch 369: 55-9, 1977. This method also involved an analysis of hypothalmic
temperature and metabolic heat production. Oxygen utilization and thermoregulation have also been evaluated in humans as described by Haskell et al., *J. Appl. Physiol.* 51: 948-54, 1981.

Among other methods known in the art or described herein, neurotransmission functions may be evaluated by monitoring 2-deoxy-glucose uptake in the brain. This parameter is monitored by techniques (assays or animal models) known to one of ordinary skill in the art, for example, autoradiography. Useful monitoring techniques are described, for example, by Kilduff et al., *J. Neurosci.* 10: 2463-75, 1990, with related techniques used to evaluate the “hibernating heart” as described in Gerber et al. *Circulation* 94: 651-8, 1996, and Fallavollita et al., *Circulation* 95: 1900-9, 1997.

In addition, zacp11 polypeptides, fragments, fusions agonists or antagonists thereof may be therapeutically useful for anti-microbial applications. For example, complement component C1q plays a role in host defense against infectious agents, such as bacteria and viruses. C1q is known to exhibit several specialized functions. For example, C1q triggers the complement cascade via interaction with bound antibody or C-reactive protein (CRP). Also, C1q interacts directly with certain bacteria, RNA viruses, mycoplasma, uric acid crystals, the lipid A component of bacterial endotoxin and membranes of certain intracellular organelles. C1q binding to the C1q receptor is believed to promote phagocytosis. C1q also appears to enhance the antibody formation aspect of the host defense system. See, for example, Johnston, *Pediatr. Infect. Dis. J.* 12(11): 933-41, 1993. Thus, soluble C1q-like molecules may be useful as anti-microbial agents, promoting lysis or phagocytosis of infectious agents.

The collagogenous domains of proteins such as C1q and macrophage scavenger receptor are known to bind acidic phospholipids such as LPA. The interaction of zacp11 polypeptides, fragments, fusions, agonists or antagonists with mitogenic anions such as LPA can be determined using assays known in the art, see for example, Acton et al., *ibid.* Inhibition of inflammatory processes by polypeptides and antibodies of the present invention would also be useful in preventing infection at the wound site.

Anti-microbial protective agents may be directly acting or indirectly acting. Such agents operating via membrane association or pore forming mechanisms of action directly attach to the offending microbe. Anti-microbial agents can also act via an enzymatic mechanism, breaking down microbial protective substances or the cell wall/membrane thereof. Anti-microbial agents, capable of inhibiting microorganism proliferation or action or of disrupting microorganism integrity by either mechanism set forth above, are useful in methods for preventing contamination in cell culture by microbes susceptible to that anti-microbial activity. Such techniques involve culturing
cells in the presence of an effective amount of said zacrpl1 polypeptide or an agonist or antagonist thereof.

Also, zacrpl1 polypeptides or agonists thereof may be used as cell culture reagents in in vitro studies of exogenous microorganism infection, such as bacterial, viral or fungal infection. Such moieties may also be used in in vivo animal models of infection.

Zacrpl1 fragments as well as zacrpl1 polypeptides, fusion proteins, agonists, antagonists or antibodies may be evaluated with respect to their anti-microbial properties according to procedures known in the art. See, for example, Barsum et al., Eur. Respir. J. 8(5): 709-14, 1995; Sandovsky-Losica et al., J. Med. Vet. Mycol (England) 28(4): 279-87, 1990; Mehentee et al., J. Gen. Microbiol. (England) 135 (Pt. 8): 2181-8, 1989; Segal and Savage, J. Med. Vet. Mycol. 24: 477-9, 1986 and the like. If desired, the performance of zacrpl1 in this regard can be compared to proteins known to be functional in this regard, such as proline-rich proteins, lysozyme, histatins, lactoperoxidase or the like. In addition, zacrpl1 fragments, polypeptides, fusion proteins, agonists, antagonists or antibodies may be evaluated in combination with one or more anti-microbial agents to identify synergistic effects. One of ordinary skill in the art will recognize that the anti-microbial properties of zacrpl1 polypeptides, fragments, fusion proteins, agonists, antagonists and antibodies may be similarly evaluated.

As neurotransmitters or neurotransmission modulators, zacrpl1 polypeptide fragments as well as zacrpl1 polypeptides, fusion proteins, agonists, antagonists or antibodies of the present invention may also modulate calcium ion concentration, muscle contraction, hormone secretion, DNA synthesis or cell growth, inositol phosphate turnover, arachidonate release, phospholipase-C activation, gastric emptying, human neutrophil activation or ADCC capability, superoxide anion production and the like. Evaluation of these properties can be conducted by known methods, such as those set forth herein.

The impact of zacrpl1 polypeptide, fragment, fusion, antibody, agonist or antagonist on intracellular calcium level may be assessed by methods known in the art, such as those described by Dobrzanski et al., Regulatory Peptides 45: 341-52, 1993, and the like. The impact of zacrpl1 polypeptide, fragment, fusion, agonist or antagonist on muscle contraction may be assessed by methods known in the art, such as those described by Smits & Lebebvre, J. Auton. Pharmacol. 14: 383-92, 1994, Belloli et al., J. Vet. Pharmacol. Therap. 17: 379-83, 1994, Maggi et al., Regulatory Peptides 53: 259-74, 1994, and the like. The impact of zacrpl1 polypeptide, fragment, fusion, agonist or antagonist on hormone secretion may be assessed by methods known in the art, such as those for prolactin release described by Henriksen et al., J. Recep. Sig.
*Transd. Res. 15(1-4): 529-41, 1995*, and the like. The impact of zacrp11 polypeptide, fragment, fusion, agonist or antagonist on DNA synthesis or cell growth may be assessed by methods known in the art, such as those described by Dobrzanski et al., *Regulatory Peptides 45*: 341-52, 1993, and the like. The impact of zacrp11 polypeptide, fragment, fusion, agonist or antagonist on inositol phosphate turnover may be assessed by methods known in the art, such as those described by Dobrzanski et al., *Regulatory Peptides 45*: 341-52, 1993, and the like.

Also, the impact of zacrp11 polypeptide, fragment, fusion, agonist or antagonist on arachidonate release may be assessed by methods known in the art, such as those described by Dobrzanski et al., *Regulatory Peptides 45*: 341-52, 1993, and the like. The impact of zacrp11 polypeptide, fragment, fusion, agonist or antagonist on phospholipase-C activation may be assessed by methods known in the art, such as those described by Dobrzanski et al., *Regulatory Peptides 45*: 341-52, 1993, and the like. The impact of zacrp11 polypeptide, fragment, fusion, agonist or antagonist on gastric emptying may be assessed by methods known in the art, such as those described by Varga et al., *Eur. J. Pharmacol. 286*: 109-112, 1995, and the like. The impact of zacrp11 polypeptide, fragment, fusion, agonist or antagonist on human neutrophil activation and ADCC capability may be assessed by methods known in the art, such as those described by Wozniak et al., *Immunology 78*: 629-34, 1993, and the like. The impact of zacrp11 polypeptide, fragment, fusion, agonist or antagonist on superoxide anion production may be assessed by methods known in the art, such as those described by Wozniak et al., *Immunology 78*: 629-34, 1993, and the like.

The effect of zacrp11 on expression of cell surface adhesion molecules such as E-selectin (endothelial leukocyte adhesion molecule), V-CAM (vascular cell adhesion molecule), and I-CAM (intercellular adhesion molecule) can be measured using microvascular bone marrow cells (TRBMEC) in a cell ELISA according to Ouchi et al., (*Circulation 100*:2473-7, 1999). This activity can be compared to the stimulation from inflammatory cytokines such as TNF (tumor necrosis factor). A THP-1 monocyte adherence assay according to Ouchi et al., (*ibid.*) and Cybulsky and Gimbrone, (*Science 251*:788-91, 1991) may be used to measure zacrp11 activity as well.

Collagen is a potent inducer of platelet aggregation. Platelets interact with damaged vessel walls to form a thrombus. The degree of response is graded due to the subendothelium tissue exposed and the blood flow in the injured area. This poses risks to patients recovering from vascular injuries. Inhibitors of collagen-induced platelet aggregation would be useful for blocking the binding of platelets to collagen-coated surfaces and reducing associated collagen-induced platelet aggregation. C1q is a component of the complement pathway and has been found to stimulate defense
mechanisms as well as trigger the generation of toxic oxygen species that can cause tissue damage (Tenner, Behring Inst. Mitt. 93:241-53, 1993). C1q binding sites are found on platelets. C1q, independent of an immune binding partner, has been found to inhibit platelet aggregation but not platelet adhesion or shape change. The amino terminal region of C1q shares homology with collagen (Peerschke and Ghebrehiwet, J. Immunol. 145:2984-88, 1990). Inhibition of C1q and the complement pathway can be determined using methods disclosed herein or known in the art, such as described in Suba and Csako, J. Immunol. 117:304-9, 1976. In this regard, zacrpol11 polypeptides would be useful in modulating hemostasis, increasing blood flow flowing vascular injury and pacifying collagenous surfaces.

The activity of zacrpol11 polypeptide, fragments, fusions, agonists or antagonists on collagen-mediated platelet adhesion, activation and aggregation may be measured using methods described herein or known in the art, such as the platelet aggregation assay (Chiang et al., Thrombosis Res. 37:605-12, 1985) and platelet adhesion assays (Peerschke and Ghebrehiwet, J. Immunol. 144:221-25, 1990). Assays for platelet adhesion to collagen and inhibition of collagen-induced platelet aggregation can be measured using methods described in Keller et al., J. Biol. Chem. 268:5450-6, 1993; Waxman and Connolly, J. Biol. Chem. 268:5445-9, 1993; Noeske-Jungblut et al., J. Biol. Chem. 269:5050-3 or 1994 Deckmyn et al., Blood 85:712-9, 1995.

Zacrpol11 polypeptides, fragments, fusion proteins, antibodies, agonists or antagonists of the present invention can be used in methods for promoting blood flow within the vasculature of a mammal by reducing the number of platelets that adhere and are activated and the size of platelet aggregates. Such methods would comprise administration of a therapeutically effective amount of zacrpol11 polypeptides, fragments, fusions, antibodies, agonists or antagonists to a mammal in need of such treatment, whereby zacrpol11 reduces thrombogenic and complement activity within the vasculature of the mammal. Zacrpol11 polypeptides, fragments, fusions, antibodies, agonists or antagonists used in such methods can be administered prior to, during or following an acute vascular injury in the mammal.

In one such method, the vascular injury is due to vascular reconstruction, including but not limited to, angioplasty, endarterectomy, coronary artery bypass graft, microvascular repair or anastomosis of a vascular graft. Also contemplated are vascular injuries due to trauma, stroke or aneurysm. In other preferred methods the vascular injury is due to plaque rupture, degradation of the vasculature, complications associated with diabetes and atherosclerosis. Plaque rupture in the coronary artery induces heart attack and in the cerebral artery induces stroke. Use of zacrpol11 polypeptides, fragments, fusion proteins, antibodies, agonists or antagonists in such methods would
also be useful for ameliorating whole system diseases of the vasculature associated with the immune system, such as disseminated intravascular coagulation (DIC) and SIDs. Additionally the complement inhibiting activity would be useful for treating non-vasculature immune diseases such as arteriolosclerosis.

A correlation has been found between the presence of C1q in localized ischemic myocardium and the accumulation of leukocytes following coronary occlusion and reperfusion. Release of cellular components following tissue damage triggers complement activation which results in toxic oxygen products that may be the primary cause of myocardial damage (Rossen et al., Circ. Res. 62:572-84, 1998 and Tenner, *ibid*). Blocking the complement pathway was found to protect ischemic myocardium from reperfusion injury (Buerke et al., J. Pharm. Exp. Therap. 286:429-38, 1998). The complement inhibition and C1q binding activity of zacrpl polypeptides would be useful for such purposes.

The activity of zacrpl polypeptide, fragments, fusions, agonists or antagonists on vasodilation of aortic rings can be measured according to the methods of Dainty et al., *J. Pharmacol. 100*:767, 1990 and Rhee et al., *Neurotox. 16*:179, 1995.

Various *in vitro* and *in vivo* models are available for measuring the effect of zacrpl polypeptides, fragments, fusion proteins, antibodies, agonists and antagonists on ischemia and reperfusion injury. See for example, Shandelya et al., Circulation 88:2812-26, 1993; Weisman et al., Science 249:146-151, 1991; Buerke et al., Circulation 91:393-402, 1995; Horstick et al., Circulation 95:701-8, 1997 and Burke et al., J. Pharm. Exp. Therap. 286:429-38, 1998. An *ex vivo* hamster platelet aggregation assay is described by Deckmyn et al. *ibid*. Bleeding times in hamsters and baboons can be measured following injection of zacrpl polypeptides using the model described by Deckmyn et al., *ibid*. The formation of thrombus in response to administration of proteins of the present invention can be measured using the hamster femoral vein thrombosis model is provided by Deckmyn et al., *ibid*. Changes in platelet adhesion under flow conditions following administration of zacrpl1 can be measured using the method described in Harsfalvi et al., Blood 85:705-11, 1995.

Complement inhibition and wound healing activity of zacrpl polypeptides, fragments, fusion proteins, antibodies, agonists or antagonists can be assayed alone or in combination with other known inhibitors of collagen-induced platelet activation and aggregation, such as pallepiper, moubatin or calin, for example.

Zacrpl1 polypeptides, fragments, fusion proteins, antibodies, agonists or antagonists can be evaluated using methods described herein or known in the art, such as healing of dermal layers in pigs (Lynch et al., Proc. Natl. Acad. Sci. USA 84: 7696-700, 1987) and full-thickness skin wounds in genetically diabetic mice (Greenhalgh et
al., *Am. J. Pathol.* 136: 1235-46, 1990), for example. The polypeptides of the present invention can be assayed alone or in combination with other known complement inhibitors as described above.

Proteins that bind collagen are useful to pacify damaged collagenous tissues preventing platelet adhesion, activation or aggregation, and the activation of inflammatory processes which lead to the release of toxic oxygen products. By rendering the exposed tissue inert towards such processes as complement activity, thrombotic activity and immune activation, zacrp11 polypeptides, fragments, fusions, antibodies, agonists or antagonists would be useful in reducing the injurious effects of ischemia and reperfusion. In particular, such injuries would include trauma injury ischemia, intestinal strangulation, and injury associated with pre- and post-establishment of blood flow. Zacrp11 would be useful in the treatment of cardiopulmonary bypass ischemia and recesitation, myocardial infarction and post trauma vasospasm, such as stroke or percutaneous transluminal angioplasty as well as accidental or surgical-induced vascular trauma.

Zacrp11 polypeptides, fragments, fusions, antibodies, agonists or antagonists would also be useful to pacify prosthetic biomaterials and surgical equipment to render the surface of the materials inert towards complement activation, thrombotic activity or immune activation. Such materials include, but are not limited to, collagen or collagen fragment-coated biomaterials, gelatin-coated biomaterials, fibrin-coated biomaterials, fibronectin-coated biomaterials, heparin-coated biomaterials, collagen and gel-coated stents, arterial grafts, synthetic heart valves, artificial organs or any prosthetic application exposed to blood that will bind zacrp11 at greater than $1 \times 10^8$. Coating such materials can be done using methods known in the art, see for example, Rubens, US Patent No. 5,272,074.

Complement and C1q play a role in inflammation. The complement activation is initiated by binding of C1q to immunoglobulins (Johnston, *Pediatr. Infect. Dis. J.* 12:933-41, 1993; Ward and Ghetie, *Therap. Immunol.* 2:77-94, 1995). Inhibitors of C1q and complement would be useful as anti-inflammatory agents. Such application can be made to prevent infection. Additionally, such inhibitors can be administered to an individual suffering from inflammation mediated by complement activation and binding of immune complexes to C1q. Zacrp11 polypeptides, fragments, fusion proteins, antibodies, agonists or antagonists would be useful in methods of mediating wound repair, enhancing progression in wound healing by overcoming impaired wound healing. Progression in wound healing would include, for example, such elements as a reduction in inflammation, fibroblasts recruitment, wound retraction and reduction in infection.
Ability of tumor cells to bind to collagen may contribute to the metastasis of tumors. Inhibitors of collagen binding are also useful for mediating the adhesive interactions and metastatic spread of tumors (Noeske-Jungblut et al., US Patent No. 5,723,312).

Platelet adhesion, activation and aggregation can be evaluated using methods described herein or known in the art, such as the platelet aggregation assay (Chiang et al., Thrombosis Res. 37:605-12, 1985) and platelet adhesion assays (Peerschke and Ghebrehiwet, J. Immunol. 144:221-25, 1990) Inhibition of C1q and the complement pathway can be determined using methods disclosed herein or know in the art, such as described in Suba and Csako, J. Immunol. 117:304-9, 1976. Assays for platelet adhesion to collagen and inhibition of collagen-induced platelet aggregation can be measured using methods described in Keller et al., J. Biol. Chem. 268:5450-6, 1993; Waxman and Connolly, J. Biol. Chem. 268:5445-9, 1993; Noeske-Jungblut et al., J. Biol. Chem. 269:5050-3 or 1994 Deckmyn et al., Blood 85:712-9, 1995.

The positively charged, extracellular, triple helix, collagenous domains of C1q and macrophage scavenger receptor were determined to play a role in ligand binding and were shown to have a broad binding specificity for polyanions (Acton et al., J. Biol. Chem. 268:3530-37, 1993). Lysophospholipid growth factor (lysophosphaticid acid, LPA) and other mitogenic anions localize at the site of damaged tissues and assist in wound repair. LPA exerts many biological effects including activation of platelets and up-regulation of matrix assembly. It is thought that LPA synergizes with other blood coagulation factors and mediates wound healing.

Therapeutic Uses of Polypeptides Having zacrpl11 Activity

The present invention includes the use of proteins, polypeptides, and peptides having zacrpl11 activity (such as zacrpl11 polypeptides, anti-idiotpe anti-zacrpl11 antibodies, and zacrpl11 fusion proteins) to a subject in need of a zacrpl11 protein.

Generally, the dosage of administered polypeptide, protein or peptide will vary depending upon such factors as the patient's age, weight, height, sex, general medical condition and previous medical history. Typically, it is desirable to provide the recipient with a dosage of a molecule having zacrpl11 activity which is in the range of from about 1 pg/kg to 10 mg/kg (amount of agent/body weight of patient), although a lower or higher dosage also may be administered as circumstances dictate.

Administration of a molecule having zacrpl11 activity to a subject can be intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, intrapleural,
intrathecal, by perfusion through a regional catheter, or by direct intralesional injection. When administering therapeutic proteins by injection, the administration may be by continuous infusion or by single or multiple boluses. One form of administration is made at or near the site of vascular injury.

A pharmaceutical composition comprising a protein, polypeptide, or peptide having zacrp11 activity can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the therapeutic proteins are combined in a mixture with a pharmaceutically acceptable carrier. A composition is said to be a “pharmaceutically acceptable carrier” if its administration can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other suitable carriers are well-known to those in the art. See, for example, Gennaro (ed.), Remington's Pharmaceutical Sciences, 19th Edition (Mack Publishing Company 1995).

For purposes of therapy, molecules having zacrp11 activity and a pharmaceutically acceptable carrier are administered to a patient in a therapeutically effective amount. A combination of a protein, polypeptide, or peptide having zacrp11 activity and a pharmaceutically acceptable carrier is said to be administered in a “therapeutically effective amount” if the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient as noted by the clinician or other qualified observer.

A pharmaceutical composition comprising molecules having zacrp11 activity can be furnished in liquid form, or in solid form. Liquid forms, including liposome-encapsulated formulations, are illustrated by injectable solutions and oral suspensions. Exemplary solid forms include capsules, tablets, and controlled-release forms, such as a miniosmotic pump or an implant. Other dosage forms can be devised by those skilled in the art, as shown, for example, by Ansel and Popovich, Pharmaceutical Dosage Forms and Drug Delivery Systems, 5th Edition (Lea & Febiger 1990), Gennaro (ed.), Remington's Pharmaceutical Sciences, 19th Edition (Mack Publishing Company 1995), and by Ranade and Hollinger, Drug Delivery Systems (CRC Press 1996).

As an illustration, zacrp11 pharmaceutical compositions may be supplied as a kit comprising a container that comprises zacrp11. zacrp11 can be provided in the form of an injectable solution for single or multiple doses, or as a sterile powder that will be reconstituted before injection. Such a kit may further comprise written information on indications and usage of the pharmaceutical composition.
Moreover, such information may include a statement that the zacrpl11 composition is contraindicated in patients with known hypersensitivity to zacrpl11.

Educational Uses

Polynucleotides and polypeptides of the present invention will be useful as educational tools in laboratory practicum kits for courses related to genetics and molecular biology, protein chemistry, and antibody production and analysis. Due to its unique polynucleotide and polypeptide sequences, molecules of zacrpl11 can be used as standards or as “unknowns” for testing purposes. For example, zacrpl11 polynucleotides can be used as an aid, such as, for example, to teach a student how to prepare expression constructs for bacterial, viral, or mammalian expression, including fusion constructs, wherein zacrpl11 is the gene to be expressed; for determining the restriction endonuclease cleavage sites of the polynucleotides; determining mRNA and DNA localization of zacrpl11 polynucleotides in tissues (i.e., by northern and Southern blotting as well as polymerase chain reaction); and for identifying related polynucleotides and polypeptides by nucleic acid hybridization.

Zacrpl11 polypeptides can be used as an aid to teach preparation of antibodies; identifying proteins by western blotting; protein purification; determining the weight of produced zacrpl11 polypeptides as a ratio to total protein produced; identifying peptide cleavage sites; coupling amino and carboxyl terminal tags; amino acid sequence analysis, as well as, but not limited to monitoring biological activities of both the native and tagged protein in vitro and in vivo.

Zacrpl11 polypeptides can also be used to teach analytical skills such as mass spectrometry, circular dichroism to determine conformation, especially of the four alpha helices, x-ray crystallography to determine the three-dimensional structure in atomic detail, nuclear magnetic resonance spectroscopy to reveal the structure of proteins in solution. For example, a kit containing the zacrpl11 can be given to the student to analyze. Since the amino acid sequence would be known by the instructor, the protein can be given to the student as a test to determine the skills or develop the skills of the student, the instructor would then know whether or not the student has correctly analyzed the polypeptide. Since every polypeptide is unique, the educational utility of zacrpl11 would be unique unto itself.

The antibodies which bind specifically to zacrpl11 can be used as a teaching aid to instruct students how to prepare affinity chromatography columns to purify zacrpl11, cloning and sequencing the polynucleotide that encodes an antibody and thus as a practicum for teaching a student how to design humanized antibodies. The zacrpl11 gene, polypeptide, or antibody would then be packaged by reagent companies
and sold to educational institutions so that the students gain skill in art of molecular biology. Because each gene and protein is unique, each gene and protein creates unique challenges and learning experiences for students in a lab practicum. Such educational kits containing the Zacrp11 gene, polypeptide, or antibody are considered within the scope of the present invention.

**Therapeutic Uses of Zacrp11 Nucleotide Sequences**

The present invention includes the use of Zacrp11 nucleotide sequences to provide Zacrp11 to a subject in need of such treatment. In addition, a therapeutic expression vector can be provided that inhibits Zacrp11 gene expression, such as an anti-sense molecule, a ribozyme, or an external guide sequence molecule.

There are numerous approaches to introduce a Zacrp11 gene to a subject, including the use of recombinant host cells that express Zacrp11, delivery of naked nucleic acid encoding Zacrp11, use of a cationic lipid carrier with a nucleic acid molecule that encodes Zacrp11, and the use of viruses that express Zacrp11, such as recombinant retroviruses, recombinant adeno-associated viruses, recombinant adenoviruses, and recombinant Herpes simplex viruses (see, for example, Mulligan, *Science* 260:926 (1993), Rosenberg et al., *Science* 242:1575 (1988), LaSalle et al., *Science* 259:988 (1993), Wolff et al., *Science* 247:1465 (1990), Breakfield and Deluca, *The New Biologist* 3:203 (1991)). In an *ex vivo* approach, for example, cells are isolated from a subject, transfected with a vector that expresses a Zacrp11 gene, and then transplanted into the subject.

In order to effect expression of a Zacrp11 gene, an expression vector is constructed in which a nucleotide sequence encoding a Zacrp11 gene is operably linked to a core promoter, and optionally a regulatory element, to control gene transcription. The general requirements of an expression vector are described above.


As an illustration of one system, adenovirus, a double-stranded DNA virus, is a well-characterized gene transfer vector for delivery of a heterologous nucleic acid molecule (for a review, see Becker et al., Meth. Cell Biol. 43:161 (1994); Douglas and Curiel, Science & Medicine 4:44 (1997)). The adenovirus system offers several advantages including: (i) the ability to accommodate relatively large DNA inserts, (ii) the ability to be grown to high-titer, (iii) the ability to infect a broad range of mammalian cell types, and (iv) the ability to be used with many different promoters including ubiquitous, tissue specific, and regulatable promoters. In addition, adenoviruses can be administered by intravenous injection, because the viruses are stable in the bloodstream.

Using adenovirus vectors where portions of the adenovirus genome are deleted, inserts are incorporated into the viral DNA by direct ligation or by homologous recombination with a co-transfected plasmid. In an exemplary system, the essential E1 gene is deleted from the viral vector, and the virus will not replicate unless the E1 gene is provided by the host cell. When intravenously administered to intact animals, adenovirus primarily targets the liver. Although an adenoviral delivery system with an E1 gene deletion cannot replicate in the host cells, the host’s tissue will express and process an encoded heterologous protein. Host cells will also secrete the heterologous protein if the corresponding gene includes a secretory signal sequence. Secreted proteins will enter the circulation from tissue that expresses the heterologous gene (e.g., the highly vascularized liver).

Moreover, adenoviral vectors containing various deletions of viral genes can be used to reduce or eliminate immune responses to the vector. Such adenoviruses are E1-deleted, and in addition, contain deletions of E2A or E4 (Lusky et al., J. Virol. 72:2022 (1998); Raper et al., Human Gene Therapy 9:671 (1998)). The deletion of E2b has also been reported to reduce immune responses (Amalfitano et al., J. Virol. 72:926 (1998)). By deleting the entire adenovirus genome, very large inserts of heterologous DNA can be accommodated. Generation of so called “gutless” adenoviruses, where all
viral genes are deleted, are particularly advantageous for insertion of large inserts of heterologous DNA (for a review, see Yeh and Perricaudet, *FASEB J.* 11:615 (1997)).


Alternatively, an expression vector comprising a zacrpl1 gene can be introduced into a subject's cells by lipofection in vivo using liposomes. Synthetic cationic lipids can be used to prepare liposomes for in vivo transfection of a gene encoding a marker (Felgner et al., *Proc. Nat'l Acad. Sci. USA* 84:7413 (1987); Mackey et al., *Proc. Nat'l Acad. Sci. USA* 85:8027 (1988)). The use of lipofection to introduce exogenous genes into specific organs in vivo has certain practical advantages. Liposomes can be used to direct transfection to particular cell types, which is particularly advantageous in a tissue with cellular heterogeneity, such as the pancreas, liver, kidney, and brain. Lipids may be chemically coupled to other molecules for the purpose of targeting. Targeted peptides (e.g., hormones or neurotransmitters), proteins such as antibodies, or non-peptide molecules can be coupled to liposomes chemically.

Electroporation is another alternative mode of administration of a zacrpl1 nucleic acid molecules. For example, Aihara and Miyazaki, *Nature Biotechnology* 16:867 (1998), have demonstrated the use of in vivo electroporation for gene transfer into muscle.

In an alternative approach to gene therapy, a therapeutic gene may encode a zacrpl1 anti-sense RNA that inhibits the expression of zacrpl1. Methods of preparing anti-sense constructs are known to those in the art. See, for example, Erickson et al., *Dev. Genet.* 14:274 (1993) [transgenic mice], Augustine et al., *Dev. Genet.* 14:500 (1993) [murine whole embryo culture], and Olson and Gibo, *Exp. Cell Res.* 241:134 (1998) [cultured cells]. Suitable sequences for zacrpl1 anti-sense molecules can be derived from the nucleotide sequences of zacrpl1 disclosed herein.

Alternatively, an expression vector can be constructed in which a regulatory element is operably linked to a nucleotide sequence that encodes a ribozyme. Ribozymes can be designed to express endonuclease activity that is directed to a certain target sequence in a mRNA molecule (see, for example, Draper and Macejak, U.S. Patent No. 5,496,698, McSwiggen, U.S. Patent No. 5,525,468, Chowrirara and McSwiggen, U.S. Patent No. 5,631,359, and Robertson and Goldberg, U.S. Patent No.
5,225,337). In the context of the present invention, ribozymes include nucleotide sequences that bind with zacrp11 mRNA.

In another approach, expression vectors can be constructed in which a regulatory element directs the production of RNA transcripts capable of promoting RNase P-mediated cleavage of mRNA molecules that encode a zacrp11 gene. According to this approach, an external guide sequence can be constructed for directing the endogenous ribozyme, RNase P, to a particular species of intracellular mRNA, which is subsequently cleaved by the cellular ribozyme (see, for example, Altman et al., U.S. Patent No. 5,168,053, Yuan et al., Science 263:1269 (1994), Pace et al., international publication No. WO 96/18733, George et al., international publication No. WO 96/21731, and Werner et al., international publication No. WO 97/33991). Preferably, the external guide sequence comprises a ten to fifteen nucleotide sequence complementary to zacrp11 mRNA, and a 3'-NCCA nucleotide sequence, wherein N is preferably a purine. The external guide sequence transcripts bind to the targeted mRNA species by the formation of base pairs between the mRNA and the complementary external guide sequences, thus promoting cleavage of mRNA by RNase P at the nucleotide located at the 5'-side of the base-paired region.

In general, the dosage of a composition comprising a therapeutic vector having a zacrp11 nucleotide acid sequence, such as a recombinant virus, will vary depending upon such factors as the subject's age, weight, height, sex, general medical condition and previous medical history. Suitable routes of administration of therapeutic vectors include intravenous injection, intraarterial injection, intraperitoneal injection, intramuscular injection, intratumoral injection, and injection into a cavity that contains a tumor.

A composition comprising viral vectors, non-viral vectors, or a combination of viral and non-viral vectors of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby vectors or viruses are combined in a mixture with a pharmaceutically acceptable carrier. As noted above, a composition, such as phosphate-buffered saline is said to be a "pharmaceutically acceptable carrier" if its administration can be tolerated by a recipient subject. Other suitable carriers are well-known to those in the art (see, for example, Remington's Pharmaceutical Sciences, 19th Ed. (Mack Publishing Co. 1995), and Gilman's the Pharmacological Basis of Therapeutics, 7th Ed. (MacMillan Publishing Co. 1985)).

For purposes of therapy, a therapeutic gene expression vector, or a recombinant virus comprising such a vector, and a pharmaceutically acceptable carrier are administered to a subject in a therapeutically effective amount. A combination of
an expression vector (or virus) and a pharmaceutically acceptable carrier is said to be administered in a “therapeutically effective amount” if the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient subject.

When the subject treated with a therapeutic gene expression vector or a recombinant virus is a human, then the therapy is preferably somatic cell gene therapy. That is, the preferred treatment of a human with a therapeutic gene expression vector or a recombinant virus does not entail introducing into cells a nucleic acid molecule that can form part of a human germ line and be passed onto successive generations (i.e., human germ line gene therapy).

Production of Transgenic Mice

Transgenic mice can be engineered to over-express the zacrplI gene in all tissues or under the control of a tissue-specific or tissue-preferred regulatory element. These over-producers of zacrpl can be used to characterize the phenotype that results from over-expression, and the transgenic animals can serve as models for human disease caused by excess zacrplI. Transgenic mice that over-express zacrplI also provide model bioreactors for production of zacrplI in the milk or blood of larger animals. Methods for producing transgenic mice are well-known to those of skill in the art (see, for example, Jacob, “Expression and Knockout of Interferons in Transgenic Mice,” in Overexpression and Knockout of Cytokines in Transgenic Mice, Jacob (ed.), pages 111-124 (Academic Press, Ltd. 1994), Monastersky and Robl (eds.), Strategies in Transgenic Animal Science (ASM Press 1995), and Abbud and Nilson, “Recombinant Protein Expression in Transgenic Mice,” in Gene Expression Systems: Using Nature for the Art of Expression, Fernandez and Hoeffler (eds.), pages 367-397 (Academic Press, Inc. 1999)).

For example, a method for producing a transgenic mouse that expresses a zacrplI gene can begin with adult, fertile males (studs) (B6C3f1, 2-8 months of age (Taconic Farms, Germantown, NY)), vasectomized males (duds) (B6D2f1, 2-8 months, (Taconic Farms)), prepubescent fertile females (donors) (B6C3f1, 4-5 weeks, (Taconic Farms)) and adult fertile females (recipients) (B6D2f1, 2-4 months, (Taconic Farms)). The donors are acclimated for one week and then injected with approximately 8 IU/mouse of Pregnant Mare’s Serum gonadotrophin (Sigma Chemical Company; St. Louis, MO) L.P., and 46-47 hours later, 8 IU/mouse of human Chorionic Gonadotropin (hCG (Sigma)) L.P. to induce superovulation. Donors are mated with studs subsequent to hormone injections. Ovulation generally occurs within 13 hours of hCG injection.
Copulation is confirmed by the presence of a vaginal plug the morning following mating.

Fertilized eggs are collected under a surgical scope. The oviducts are collected and eggs are released into urinanalysis slides containing hyaluronidase (Sigma). Eggs are washed once in hyaluronidase, and twice in Whitten’s W640 medium (described, for example, by Menino and O’Clary, Biol. Reprod. 77:159 (1986), and Dienhart and Downs, Zygote 4:129 (1996)) that has been incubated with 5% CO₂, 5% O₂, and 90% N₂ at 37°C. The eggs are then stored in a 37°C/5% CO₂ incubator until microinjection.

Ten to twenty micrograms of plasmid DNA containing a zacrp11 encoding sequence is linearized, gel-purified, and resuspended in 10 mM Tris-HCl (pH 7.4), 0.25 mM EDTA (pH 8.0), at a final concentration of 5-10 nanograms per microliter for microinjection. For example, the zacrp11 encoding sequences can encode the amino acid residues of SEQ ID NO:2.

Plasmid DNA is microinjected into harvested eggs contained in a drop of W640 medium overlaid by warm, CO₂-equilibrated mineral oil. The DNA is drawn into an injection needle (pulled from a 0.75mm ID, 1mm OD borosilicate glass capillary), and injected into individual eggs. Each egg is penetrated with the injection needle, into one or both of the haploid pronuclei.

Picoliters of DNA are injected into the pronuclei, and the injection needle withdrawn without coming into contact with the nucleoli. The procedure is repeated until all the eggs are injected. Successfully microinjected eggs are transferred into an organ tissue-culture dish with pre-gassed W640 medium for storage overnight in a 37°C/5% CO₂ incubator.

The following day, two-cell embryos are transferred into pseudopregnant recipients. The recipients are identified by the presence of copulation plugs, after copulating with vasectomized duds. Recipients are anesthetized and shaved on the dorsal left side and transferred to a surgical microscope. A small incision is made in the skin and through the muscle wall in the middle of the abdominal area outlined by the ribcage, the saddle, and the hind leg, midway between knee and spleen. The reproductive organs are exteriorized onto a small surgical drape. The fat pad is stretched out over the surgical drape, and a baby serrefine (Roboz, Rockville, MD) is attached to the fat pad and left hanging over the back of the mouse, preventing the organs from sliding back in.

With a fine transfer pipette containing mineral oil followed by alternating W640 and air bubbles, 12-17 healthy two-cell embryos from the previous day’s injection are transferred into the recipient. The swollen ampulla is located and
holding the oviduct between the ampulla and the bursa, a nick in the oviduct is made with a 28 g needle close to the bursa, making sure not to tear the ampulla or the bursa.

The pipette is transferred into the nick in the oviduct, and the embryos are blown in, allowing the first air bubble to escape the pipette. The fat pad is gently pushed into the peritoneum, and the reproductive organs allowed to slide in. The peritoneal wall is closed with one suture and the skin closed with a wound clip. The mice recuperate on a 37°C slide warmer for a minimum of four hours.

The recipients are returned to cages in pairs, and allowed 19-21 days gestation. After birth, 19-21 days postpartum is allowed before weaning. The weanlings are sexed and placed into separate sex cages, and a 0.5 cm biopsy (used for genotyping) is snipped off the tail with clean scissors.

Genomic DNA is prepared from the tail snips using, for example, a QIAGEN DNEASY kit following the manufacturer’s instructions. Genomic DNA is analyzed by PCR using primers designed to amplify a *zacrpl1* gene or a selectable marker gene that was introduced in the same plasmid. After animals are confirmed to be transgenic, they are back-crossed into an inbred strain by placing a transgenic female with a wild-type male, or a transgenic male with one or two wild-type female(s). As pups are born and weaned, the sexes are separated, and their tails snipped for genotyping.

To check for expression of a transgene in a live animal, a partial hepatectomy is performed. A surgical prep is made of the upper abdomen directly below the zyphoid process. Using sterile technique, a small 1.5-2 cm incision is made below the sternum and the left lateral lobe of the liver exteriorized. Using 4-0 silk, a tie is made around the lower lobe securing it outside the body cavity. Anatraumatic clamp is used to hold the tie while a second loop of absorbable Dexon (American Cyanamid; Wayne, N.J.) is placed proximal to the first tie. A distal cut is made from the Dexon tie and approximately 100 mg of the excised liver tissue is placed in a sterile petri dish. The excised liver section is transferred to a 14 ml polypropylene round bottom tube and snap frozen in liquid nitrogen and then stored on dry ice. The surgical site is closed with suture and wound clips, and the animal’s cage placed on a 37°C heating pad for 24 hours post operatively. The animal is checked daily post operatively and the wound clips removed 7-10 days after surgery. The expression level of *zacrpl1* mRNA is examined for each transgenic mouse using an RNA solution hybridization assay or polymerase chain reaction.

In addition to producing transgenic mice that over-express *zacrpl1*, it is useful to engineer transgenic mice with either abnormally low or no expression of the gene. Such transgenic mice provide useful models for diseases associated with a lack
of zacrp11. As discussed above, zacrp11 gene expression can be inhibited using anti-
sense genes, ribozyme genes, or external guide sequence genes. To produce transgenic
mice that under-express the zacrp11 gene, such inhibitory sequences are targeted to
zacrp11 mRNA. Methods for producing transgenic mice that have abnormally low
expression of a particular gene are known to those in the art (see, for example, Wu et
al., “Gene Underexpression in Cultured Cells and Animals by Antisense DNA and
RNA Strategies,” in Methods in Gene Biotechnology, pages 205-224 (CRC Press
1997)).

An alternative approach to producing transgenic mice that have little or
no zacrp11 gene expression is to generate mice having at least one normal zacrp11
allele replaced by a nonfunctional zacrp11 gene. One method of designing a
nonfunctional zacrp11 gene is to insert another gene, such as a selectable marker gene,
within a nucleic acid molecule that encodes zacrp11. Standard methods for producing
these so-called “knockout mice” are known to those skilled in the art (see, for example,
Jacob, “Expression and Knockout of Interferons in Transgenic Mice,” in
Overexpression and Knockout of Cytokines in Transgenic Mice, Jacob (ed.), pages 111-
124 (Academic Press, Ltd. 1994), and Wu et al., “New Strategies for Gene Knockout,”
in Methods in Gene Biotechnology, pages 339-365 (CRC Press 1997)).

From the foregoing, it will be appreciated that, although specific
embodiments of the invention have been described herein for purposes of illustration,
various modifications may be made without deviating from the spirit and scope of the
invention. Accordingly, the invention is not limited except as by the appended claims.
CLAIMS

What is claimed is:

1. A polypeptide selected from the group consisting of:
   (a) a polypeptide comprising amino acid residues 61-111 of SEQ ID NO:2;
   (b) a polypeptide comprising amino acid residues 21-111 of SEQ ID NO:2;
   (c) a polypeptide comprising amino acid residues 1-111 of SEQ ID NO:2;
   (d) a polypeptide comprising amino acid residues 112-219 of SEQ ID NO:2;
   (e) a polypeptide comprising amino acid residues 112-268 of SEQ ID NO:2;
   (f) a polypeptide comprising amino acid residues 61-219 of SEQ ID NO:2;
   (g) a polypeptide comprising amino acid residues 61-268 of SEQ ID NO:2;
   (h) a polypeptide comprising amino acid residues 21-219 of SEQ ID NO:2;
   (i) a polypeptide comprising amino acid residues 21-268 of SEQ ID NO:2;
   (j) a polypeptide comprising amino acid residues 1-219 of SEQ ID NO:2; and
   (k) a polypeptide comprising amino acid residues 1-268 of SEQ ID NO:2.

2. A polypeptide according to claim 1, wherein the polypeptide further
   comprises a moiety selected from the group consisting of: affinity tags, toxins,
   radionucleotides, enzymes, and fluorophores.

3. A fusion protein comprising a first portion and a second portion joined
   by a peptide bond, the first portion consisting of a polypeptide selected from the group
   consisting of:
   (a) a polypeptide comprising amino acid residues 61-111 of SEQ ID NO:2;
   (b) a polypeptide comprising amino acid residues 21-111 of SEQ ID NO:2;
   (c) a polypeptide comprising amino acid residues 1-111 of SEQ ID NO:2;
   (d) a polypeptide comprising amino acid residues 112-219 of SEQ ID NO:2;
   (e) a polypeptide comprising amino acid residues 112-268 of SEQ ID NO:2;
   (f) a polypeptide comprising amino acid residues 61-219 of SEQ ID NO:2;
   (g) a polypeptide comprising amino acid residues 61-268 of SEQ ID NO:2;
   (h) a polypeptide comprising amino acid residues 21-219 of SEQ ID NO:2;
   (i) a polypeptide comprising amino acid residues 21-268 of SEQ ID NO:2;
   (j) a polypeptide comprising amino acid residues 1-219 of SEQ ID NO:2; and
   (k) a polypeptide comprising amino acid residues 1-268 of SEQ ID NO:2; and
   the second portion comprising another polypeptide.
4. A fusion protein according to claim 3, wherein the second portion is a collagen-like domain or a CIQ domain from an adipocyte complement related protein.

5. A polypeptide according to claim 1, wherein the polypeptide is selected from the group consisting of:
   (a) a polypeptide consisting of amino acid residues 61-111 of SEQ ID NO:2;
   (b) a polypeptide consisting of amino acid residues 21-111 of SEQ ID NO:2;
   (c) a polypeptide consisting of amino acid residues 1-111 of SEQ ID NO:2;
   (d) a polypeptide consisting of amino acid residues 112-219 of SEQ ID NO:2;
   (e) a polypeptide consisting of amino acid residues 112-268 of SEQ ID NO:2;
   (f) a polypeptide consisting of amino acid residues 61-219 of SEQ ID NO:2;
   (g) a polypeptide consisting of amino acid residues 61-268 of SEQ ID NO:2;
   (h) a polypeptide consisting of amino acid residues 21-219 of SEQ ID NO:2;
   (i) a polypeptide consisting of amino acid residues 21-268 of SEQ ID NO:2;
   (j) a polypeptide consisting of amino acid residues 1-219 of SEQ ID NO:2;

   and

   (k) a polypeptide consisting of amino acid residues 1-268 of SEQ ID NO:2

6. An isolated nucleic acid molecule encoding a polypeptide selected from the group consisting of:
   (a) a polypeptide comprising amino acid residues 61-111 of SEQ ID NO:2;
   (b) a polypeptide comprising amino acid residues 21-111 of SEQ ID NO:2;
   (c) a polypeptide comprising amino acid residues 1-111 of SEQ ID NO:2;
   (d) a polypeptide comprising amino acid residues 112-219 of SEQ ID NO:2;
   (e) a polypeptide comprising amino acid residues 112-268 of SEQ ID NO:2;
   (f) a polypeptide comprising amino acid residues 61-219 of SEQ ID NO:2;
   (g) a polypeptide comprising amino acid residues 61-268 of SEQ ID NO:2;
   (h) a polypeptide comprising amino acid residues 21-219 of SEQ ID NO:2;
   (i) a polypeptide comprising amino acid residues 21-268 of SEQ ID NO:2;
   (j) a polypeptide comprising amino acid residues 1-219 of SEQ ID NO:2; and
   (k) a polypeptide comprising amino acid residues 1-268 of SEQ ID NO:2.

7. An isolated nucleic acid molecule encoding a polypeptide according to claim 6, wherein the polypeptide further comprises a moiety selected from the group consisting of: affinity tags, toxins, radionucleotides, enzymes, and fluorophores.
8. A nucleic acid molecule encoding a fusion protein comprising a first portion and a second portion joined by a peptide bond, the first portion consisting of a polypeptide selected from the group consisting of:

(a) a polypeptide comprising amino acid residues 61-111 of SEQ ID NO:2;
(b) a polypeptide comprising amino acid residues 21-111 of SEQ ID NO:2;
(c) a polypeptide comprising amino acid residues 1-111 of SEQ ID NO:2;
(d) a polypeptide comprising amino acid residues 112-219 of SEQ ID NO:2;
(e) a polypeptide comprising amino acid residues 112-268 of SEQ ID NO:2;
(f) a polypeptide comprising amino acid residues 61-219 of SEQ ID NO:2;
(g) a polypeptide comprising amino acid residues 61-268 of SEQ ID NO:2;
(h) a polypeptide comprising amino acid residues 21-219 of SEQ ID NO:2;
(i) a polypeptide comprising amino acid residues 21-268 of SEQ ID NO:2;
(j) a polypeptide comprising amino acid residues 1-219 of SEQ ID NO:2; and
(k) a polypeptide comprising amino acid residues 1-268 of SEQ ID NO:2; and the second portion comprising another polypeptide.

9. A nucleic acid molecule encoding a fusion protein according to claim 8, wherein the second portion is a collagen-like domain or a C1Q domain from an adipocyte complement related protein.

10. An isolated nucleic acid molecule according to claim 6, wherein the nucleic acid encodes a polypeptide selected from the group consisting of:

(a) a polypeptide consisting of amino acid residues 61-111 of SEQ ID NO:2;
(b) a polypeptide consisting of amino acid residues 21-111 of SEQ ID NO:2;
(c) a polypeptide consisting of amino acid residues 1-111 of SEQ ID NO:2;
(d) a polypeptide consisting of amino acid residues 112-219 of SEQ ID NO:2;
(e) a polypeptide consisting of amino acid residues 112-268 of SEQ ID NO:2;
(f) a polypeptide consisting of amino acid residues 61-219 of SEQ ID NO:2;
(g) a polypeptide consisting of amino acid residues 61-268 of SEQ ID NO:2;
(h) a polypeptide consisting of amino acid residues 21-219 of SEQ ID NO:2;
(i) a polypeptide consisting of amino acid residues 21-268 of SEQ ID NO:2;
(j) a polypeptide consisting of amino acid residues 1-219 of SEQ ID NO:2; and
(k) a polypeptide consisting of amino acid residues 1-268 of SEQ ID NO:2

11. An isolated nucleic acid molecule selected from the group consisting
of:

a) a nucleic acid molecule consisting of nucleotides 181-333 of SEQ ID NO:1;
b) a nucleic acid molecule consisting of nucleotides 61-333 of SEQ ID NO:1;
c) a nucleic acid molecule consisting of nucleotides 1-333 of SEQ ID NO:1;
d) a nucleic acid molecule consisting of nucleotides 334-657 of SEQ ID NO:1;
e) a nucleic acid molecule consisting of nucleotides 334-804 of SEQ ID NO:1;
f) a nucleic acid molecule consisting of nucleotides 118-657 of SEQ ID NO:1;
g) a nucleic acid molecule consisting of nucleotides 118-804 of SEQ ID NO:1;
h) a nucleic acid molecule consisting of nucleotides 64-657 of SEQ ID NO:1;
i) a nucleic acid molecule consisting of nucleotides 64-804 of SEQ ID NO:1;
j) a nucleic acid molecule consisting of nucleotides 1-657 of SEQ ID NO:1;
k) a nucleic acid molecule consisting of nucleotides 1-804 of SEQ ID NO:1; and
l) a nucleic acid molecule consisting of SEQ ID NO:3.

12. An expression vector comprising the following operably linked elements:

a transcription promoter;
a DNA segment encoding a polypeptide with an amino acid sequence consisting of:

(a) amino acid residues 61-111 of SEQ ID NO:2;
(b) amino acid residues 21-111 of SEQ ID NO:2;
(c) amino acid residues 1-111 of SEQ ID NO:2;
(d) acid residues 112-219 of SEQ ID NO:2;
(e) amino acid residues 112-268 of SEQ ID NO:2;
(f) amino acid residues 61-219 of SEQ ID NO:2;
(g) amino acid residues 61-268 of SEQ ID NO:2;
(h) amino acid residues 21-219 of SEQ ID NO:2;
(i) amino acid residues 21-268 of SEQ ID NO:2;
(j) amino acid residues 1-219 of SEQ ID NO:2; and
(k) amino acid residues 1-268 of SEQ ID NO:2; and

a transcription terminator.

13. An expression vector according to claim 12, further comprising a secretory signal sequence operably linked to the DNA segment.
14. A cultured cell into which has been introduced an expression vector according to claim 12, wherein the cell expresses a polypeptide encoded by the DNA segment.

15. A method of producing a polypeptide comprising:
culturing a cell according to claim 14; and isolating the polypeptide produced by the cell.

16. A method of producing an antibody to a polypeptide comprising:
inoculating an animal with a polypeptide selected from the group consisting of:

(a) a polypeptide consisting of 9 to 252 amino acids, wherein the polypeptide is a contiguous sequence of amino acids in SEQ ID NO:2 from amino acid residue 1 to amino acid residue 268;
(b) a polypeptide consisting of amino acid residues 61-111 of SEQ ID NO:2;
(c) a polypeptide consisting of amino acid residues 21-111 of SEQ ID NO:2;
(d) a polypeptide consisting of amino acid residues 1-111 of SEQ ID NO:2;
(e) a polypeptide consisting of amino acid residues 112-219 of SEQ ID NO:2;
(f) a polypeptide consisting of amino acid residues 112-268 of SEQ ID NO:2;
(g) a polypeptide consisting of amino acid residues 61-219 of SEQ ID NO:2;
(h) a polypeptide consisting of amino acid residues 61-268 of SEQ ID NO:2;
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(j) a polypeptide consisting of amino acid residues 21-268 of SEQ ID NO:2;
(k) a polypeptide consisting of amino acid residues 1-219 of SEQ ID NO:2;

and

(l) a polypeptide consisting of amino acid residues 1-268 of SEQ ID NO:2;

and wherein the polypeptide elicits an immune response in the animal to produce the antibody; and isolating the antibody from the animal.

17. An antibody produced by the method of claim 16, which binds to a polypeptide of SEQ ID NO:2.

18. An antibody according to claim 17, wherein the antibody is selected from the group consisting of:
(a) polyclonal antibody;
(b) murine monoclonal antibody;
(c) humanized antibody derived from (b);
(d) an antibody fragment; and
(e) human monoclonal antibody.

19. An antibody fragment according to claim 18, wherein the antibody fragment is selected from the group consisting of F(ab’), F(ab), Fab’, Fab, Fv, scFv, and minimal recognition unit.

20. An anti-idiotypic antibody that specifically binds to the antibody of claim 17.

21. An antibody that specifically binds to a polypeptide of claim 1.
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