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(54) **PROTEINES SECRETEES ET POLYNUCLEOTIDES CODANT CES PROTEINES**

(54) **SECRETED PROTEINS AND POLYNUCLEOTIDES ENCODING THEM**

(57) Cette invention se rapporte à de nouveaux polynucléotides et aux protéines codées par ces polynucléotides.

(57) Novel polynucleotides and the proteins encoded thereby are disclosed.

PCT

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(57) Abstract

Novel polynucleotides and the proteins encoded thereby are disclosed.

SECRETED PROTEINS AND POLYNUCLEOTIDES ENCODING THEM

This application is a continuation-in-part of the following applications: Ser. No. 08/701,931, filed August 23, 1996, which is a continuation-in-part of application Ser. No. 08/702,420, filed August 14, 1996 and now abandoned; Ser. No. 08/721,925, filed September 27, 1996, which is a continuation-in-part of application Ser. No. 08/701,931; and Ser. No. 08/743,690, filed November 6, 1996, which is a continuation-in-part of application Ser. No. 08/702,420; all of which are incorporated by reference herein. Although the '420 application was filed before the '931 application, the '420 application was inexplicably asigned a higher serial number than the '931 application. However, the '420 application was correctly afforded a priority date of August 14, 1996.

FIELD OF THE INVENTION

The present invention provides novel polynucleotides and proteins encoded by such polynucleotides, along with therapeutic, diagnostic and research utilities for these polynucleotides and proteins.

BACKGROUND OF THE INVENTION

Technology aimed at the discovery of protein factors (including e.g., cytokines, such as lymphokines, interferons, CSFs and interleukins) has matured rapidly over the past decade. The now routine hybridization cloning and expression cloning techniques clone novel polynucleotides "directly" in the sense that they rely on information directly related to the discovered protein (i.e., partial DNA/amino acid sequence of the protein in the case of hybridization cloning; activity of the protein in the case of expression cloning). More recent "indirect" cloning techniques such as signal sequence cloning, which isolates DNA sequences based on the presence of a now well-recognized secretory leader sequence motif, as well as various PCR-based or low stringency hybridization cloning techniques, have advanced the state of the art by making available large numbers of DNA/amino acid sequences for proteins that are known to have biological activity by virtue of their secreted nature in the case of leader sequence cloning, or by virtue of the cell or tissue source in the case of PCR-based techniques. It is to these proteins and the polynucleotides encoding them that the present invention is directed.

SUMMARY OF THE INVENTION

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 27 to nucleotide 1652;

(c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 129 to nucleotide 1652;

(d) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 1 to nucleotide 413;

(e) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone AM349_2 deposited under accession number ATCC 98155;

(f) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone AM349_2 deposited under accession number ATCC 98155;

(g) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AM349_2 deposited under accession number ATCC 98155;

(h) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AM349_2 deposited under accession number ATCC 98155;

(i) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:2;

(j) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:2 having biological activity;

(k) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(h) above;

(l) a polynucleotide which encodes a species homologue of the protein of (i) or (j) above ; and

(m) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(j).

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:1 from nucleotide 27 to nucleotide 1652; the nucleotide sequence of SEQ ID NO:1 from nucleotide 129 to nucleotide 1652; the nucleotide sequence of SEQ ID NO:1 from

nucleotide 1 to nucleotide 413; the nucleotide sequence of the full-length protein coding sequence of clone AM349_2 deposited under accession number ATCC 98155; or the nucleotide sequence of the mature protein coding sequence of clone AM349_2 deposited under accession number ATCC 98155. In other preferred embodiments, the polynucleotide encodes the full-length or mature protein encoded by the cDNA insert of clone AM349_2 deposited under accession number ATCC 98155. In yet other preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:2 from amino acid 1 to amino acid 129.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:1.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:2;

(b) the amino acid sequence of SEQ ID NO:2 from amino acid 1 to amino acid 129;

(c) fragments of the amino acid sequence of SEQ ID NO:2; and

(d) the amino acid sequence encoded by the cDNA insert of clone AM349_2 deposited under accession number ATCC 98155;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:2 or the amino acid sequence of SEQ ID NO:2 from amino acid 1 to amino acid 129.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 788 to nucleotide 1153;

(c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 1759 to nucleotide 2146;

(d) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone AR310_3 deposited under accession number ATCC 98155;

(e) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone AR310_3 deposited under accession number ATCC 98155;

(f) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AR310_3 deposited under accession number ATCC 98155;

(g) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AR310_3 deposited under accession number ATCC 98155;

(h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:4;

(i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:4 having biological activity;

(j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;

(k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above ; and

(l) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i).

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:3 from nucleotide 788 to nucleotide 1153; the nucleotide sequence of SEQ ID NO:3 from nucleotide 1759 to nucleotide 2146; the nucleotide sequence of the full-length protein coding sequence of clone AR310_3 deposited under accession number ATCC 98155; or the nucleotide sequence of the mature protein coding sequence of clone AR310_3 deposited under accession number ATCC 98155. In other preferred embodiments, the polynucleotide encodes the full-length or mature protein encoded by the cDNA insert of clone AR310_3 deposited under accession number ATCC 98155.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:3.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:4;

(b) fragments of the amino acid sequence of SEQ ID NO:4; and

(c) the amino acid sequence encoded by the cDNA insert of clone AR310_3 deposited under accession number ATCC 98155;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:4.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 448 to nucleotide 663;

(c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 547 to nucleotide 663;

(d) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 321 to nucleotide 603;

(e) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone AS186_3 deposited under accession number ATCC 98155;

(f) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone AS186_3 deposited under accession number ATCC 98155;

(g) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AS186_3 deposited under accession number ATCC 98155;

(h) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AS186_3 deposited under accession number ATCC 98155;

(i) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:6;

(j) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 having biological activity;

(k) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(h) above;

(l) a polynucleotide which encodes a species homologue of the protein of (i) or (j) above ; and

(m) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(j).

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:5 from nucleotide 448 to nucleotide 663; the nucleotide sequence of SEQ ID NO:5 from

nucleotide 547 to nucleotide 663; the nucleotide sequence of SEQ ID NO:5 from nucleotide 321 to nucleotide 603; the nucleotide sequence of the full-length protein coding sequence of clone AS186_3 deposited under accession number ATCC 98155; or the nucleotide sequence of the mature protein coding sequence of clone AS186_3 deposited under accession number ATCC 98155. In other preferred embodiments, the polynucleotide encodes the full-length or mature protein encoded by the cDNA insert of clone AS186_3 deposited under accession number ATCC 98155. In yet other preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:6 from amino acid 1 to amino acid 52.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:5.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:6;

(b) the amino acid sequence of SEQ ID NO:6 from amino acid 1 to amino acid 52;

(c) fragments of the amino acid sequence of SEQ ID NO:6; and

(d) the amino acid sequence encoded by the cDNA insert of clone AS186_3 deposited under accession number ATCC 98155;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:6 or the amino acid sequence of SEQ ID NO:6 from amino acid 1 to amino acid 52.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 from nucleotide 140 to nucleotide 1498;

(c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 from nucleotide 185 to nucleotide 1498;

(d) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 from nucleotide 132 to nucleotide 457;

(e) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone AY160_2 deposited under accession number ATCC 98155;

(f) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone AY160_2 deposited under accession number ATCC 98155;

(g) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AY160_2 deposited under accession number ATCC 98155;

(h) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AY160_2 deposited under accession number ATCC 98155;

(i) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:8;

(j) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:8 having biological activity;

(k) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(h) above;

(l) a polynucleotide which encodes a species homologue of the protein of (i) or (j) above ; and

(m) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(j).

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:7 from nucleotide 140 to nucleotide 1498; the nucleotide sequence of SEQ ID NO:7 from nucleotide 185 to nucleotide 1498; the nucleotide sequence of SEQ ID NO:7 from nucleotide 132 to nucleotide 457; the nucleotide sequence of the full-length protein coding sequence of clone AY160_2 deposited under accession number ATCC 98155; or the nucleotide sequence of the mature protein coding sequence of clone AY160_2 deposited under accession number ATCC 98155. In other preferred embodiments, the polynucleotide encodes the full-length or mature protein encoded by the cDNA insert of clone AY160_2 deposited under accession number ATCC 98155. In yet other preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:8 from amino acid 1 to amino acid 106.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:7.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:8;

(b) the amino acid sequence of SEQ ID NO:8 from amino acid 1 to amino acid 106;

(c) fragments of the amino acid sequence of SEQ ID NO:8; and

(d) the amino acid sequence encoded by the cDNA insert of clone AY160_2 deposited under accession number ATCC 98155;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:8 or the amino acid sequence of SEQ ID NO:8 from amino acid 1 to amino acid 106.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 84 to nucleotide 548;

(c) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone BD127_16 deposited under accession number ATCC 98155;

(d) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone BD127_16 deposited under accession number ATCC 98155;

(e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone BD127_16 deposited under accession number ATCC 98155;

(f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone BD127_16 deposited under accession number ATCC 98155;

(g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:10;

(h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:10 having biological activity;

(i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(f) above;

(j) a polynucleotide which encodes a species homologue of the protein of (g) or (h) above ; and

(k) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(h).

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:9 from nucleotide 84 to nucleotide 548; the nucleotide sequence of the full-length protein coding sequence of clone BD127_16 deposited under accession number ATCC 98155; or the nucleotide sequence of the mature protein coding sequence of clone BD127_16 deposited under accession number ATCC 98155. In other preferred embodiments, the polynucleotide encodes the full-length or mature protein encoded by the cDNA insert of clone BD127_16 deposited under accession number ATCC 98155.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:9 or SEQ ID NO:11.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:10;

(b) fragments of the amino acid sequence of SEQ ID NO:10; and

(c) the amino acid sequence encoded by the cDNA insert of clone BD127_16 deposited under accession number ATCC 98155;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:10.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:12;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:12 from nucleotide 236 to nucleotide 1480;

(c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:12 from nucleotide 450 to nucleotide 800;

(d) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone BL205_14 deposited under accession number ATCC 98155;

(e) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone BL205_14 deposited under accession number ATCC 98155;

(f) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone BL205_14 deposited under accession number ATCC 98155;

(g) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone BL205_14 deposited under accession number ATCC 98155;

(h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:13;

(i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:13 having biological activity;

(j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;

(k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above ; and

(l) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i).

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:12 from nucleotide 236 to nucleotide 1480; the nucleotide sequence of SEQ ID NO:12 from nucleotide 450 to nucleotide 800; the nucleotide sequence of the full-length protein coding sequence of clone BL205_14 deposited under accession number ATCC 98155; or the nucleotide sequence of the mature protein coding sequence of clone BL205_14 deposited under accession number ATCC 98155. In other preferred embodiments, the polynucleotide encodes the full-length or mature protein encoded by the cDNA insert of clone BL205_14 deposited under accession number ATCC 98155. In yet other preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:13 from amino acid 89 to amino acid 188.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:12.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:13;

(b) the amino acid sequence of SEQ ID NO:13 from amino acid 89 to amino acid 188;

(c) fragments of the amino acid sequence of SEQ ID NO:13; and

(d) the amino acid sequence encoded by the cDNA insert of clone BL205_14 deposited under accession number ATCC 98155;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:13 or the amino acid sequence of SEQ ID NO:13 from amino acid 89 to amino acid 188.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:14;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:14 from nucleotide 69 to nucleotide 371;

(c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:14 from nucleotide 109 to nucleotide 350;

(d) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone H438_1 deposited under accession number ATCC 98140;

(e) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone H438_1 deposited under accession number ATCC 98140;

(f) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone H438_1 deposited under accession number ATCC 98140;

(g) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone H438_1 deposited under accession number ATCC 98140;

(h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:15;

(i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:15 having biological activity;

(j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;

(k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above ; and

(l) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i).

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:14 from nucleotide 69 to nucleotide 371; the nucleotide sequence of SEQ ID NO:14 from nucleotide 109 to nucleotide 350; the nucleotide sequence of the full-length protein coding sequence of clone H438_1 deposited under accession number ATCC 98140; or the nucleotide sequence of the mature protein coding sequence of clone H438_1 deposited under accession number ATCC 98140. In other preferred embodiments, the polynucleotide encodes the full-length or mature protein encoded by the cDNA insert of clone H438_1 deposited under accession number ATCC 98140. In yet other preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:15 from amino acid 17 to amino acid 94.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:14.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:15;

(b) the amino acid sequence of SEQ ID NO:15 from amino acid 17 to amino acid 94;

(c) fragments of the amino acid sequence of SEQ ID NO:15; and

(d) the amino acid sequence encoded by the cDNA insert of clone H438_1 deposited under accession number ATCC 98140;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:15 or the amino acid sequence of SEQ ID NO:15 from amino acid 17 to amino acid 94.

In certain preferred embodiments, the polynucleotide is operably linked to an expression control sequence. The invention also provides a host cell, including bacterial, yeast, insect and mammalian cells, transformed with such polynucleotide compositions.

Processes are also provided for producing a protein, which comprise:

(a) growing a culture of the host cell transformed with such polynucleotide compositions in a suitable culture medium; and

(b) purifying the protein from the culture.

The protein produced according to such methods is also provided by the present invention. Preferred embodiments include those in which the protein produced by such process is a mature form of the protein.

Protein compositions of the present invention may further comprise a pharmaceutically acceptable carrier. Compositions comprising an antibody which specifically reacts with such protein are also provided by the present invention.

Methods are also provided for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition comprising a protein of the present invention and a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B are schematic representations of the pED6 and pNOTs vectors, respectively, used for deposit of clones disclosed herein.

Fig. 2 is an autoradiograph evidencing the expression of the following clone disclosed herein, H438_1, in COS cells.

DETAILED DESCRIPTION

ISOLATED PROTEINS AND POLYNUCLEOTIDES

Nucleotide and amino acid sequences, as presently determined, are reported below for each clone and protein disclosed in the present application. The nucleotide sequence of each clone can readily be determined by sequencing of the deposited clone in accordance with known methods. The predicted amino acid sequence (both full-length and mature) can then be determined from such nucleotide sequence. The amino acid sequence of the protein encoded by a particular clone can also be determined by expression of the clone in a suitable host cell, collecting the protein and determining its sequence. For each disclosed protein applicants have identified what they have determined to be the reading frame best identifiable with sequence information available at the time of filing.

As used herein a "secreted" protein is one which, when expressed in a suitable host cell, is transported across or through a membrane, including transport as a result of signal sequences in its amino acid sequence. "Secreted" proteins include without limitation proteins secreted wholly (e.g., soluble proteins) or partially (e.g. , receptors) from the cell

in which they are expressed. "Secreted" proteins also include without limitation proteins which are transported across the membrane of the endoplasmic reticulum.

Clone "AM349_2"

A polynucleotide of the present invention has been identified as clone "AM349_2". AM349_2 was isolated from a human fetal kidney cDNA library using methods which are selective for cDNAs encoding secreted proteins (see U.S. Pat. No. 5,536,637), or was identified as encoding a secreted or transmembrane protein on the basis of computer analysis of the amino acid sequence of the encoded protein. AM349_2 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "AM349_2 protein").

The nucleotide sequence of AM349_2 as presently determined is reported in SEQ ID NO:1. What applicants presently believe to be the proper reading frame and the predicted amino acid sequence of the AM349_2 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:2. Amino acids 22 to 34 are a predicted leader/signal sequence, with the predicted mature amino acid sequence beginning at amino acid 35, or are a transmembrane domain.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone AM349_2 should be approximately 3400 bp.

The nucleotide sequence disclosed herein for AM349_2 was searched against the GenBank and GeneSeq nucleotide sequence databases using BLASTN/BLASTX and FASTA search protocols. AM349_2 demonstrated at least some similarity with sequences identified as AA078927 (zm92a08.s1 Stratagene ovarian cancer (#937219) Homo sapiens cDNA clone 545366 3'), H06061 (yl72e10.s1 Homo sapiens cDNA clone 43276 3'), U46493 (Cloning vector pFlp recombinase gene, complete cds), W81648 (zd84d09.r1 Soares fetal heart NbHH19W Homo sapiens cDNA clone 347345 5'), and W81649 (zd84d09.s1 Soares fetal heart NbHH19W Homo sapiens cDNA clone 347345 3'). The predicted amino acid sequence disclosed herein for AM349_2 was searched against the GenPept and GeneSeq amino acid sequence databases using the BLASTX search protocol. The predicted AM349_2 protein demonstrated at least some similarity to sequences identified as J01969 (DNA polymerase [Human adenovirus type 5]) and X59599 (protein-tyrosine phosphatase). Based upon sequence similarity, AM349_2 proteins and each similar protein or peptide may share at least some activity.

Clone "AR310_3"

A polynucleotide of the present invention has been identified as clone "AR310_3". AR310_3 was isolated from a human adult retina cDNA library using methods which are selective for cDNAs encoding secreted proteins (see U.S. Pat. No. 5,536,637), or was identified as encoding a secreted or transmembrane protein on the basis of computer analysis of the amino acid sequence of the encoded protein. AR310_3 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "AR310_3 protein").

The nucleotide sequence of AR310_3 as presently determined is reported in SEQ ID NO:3. What applicants presently believe to be the proper reading frame and the predicted amino acid sequence of the AR310_3 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:4.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone AR310_3 should be approximately 3800 bp.

The nucleotide sequence disclosed herein for AR310_3 was searched against the GenBank and GeneSeq nucleotide sequence databases using BLASTN/BLASTX and FASTA search protocols. AR310_3 demonstrated at least some similarity with sequences identified as AA313755 (EST185840 Colon carcinoma (HCC) cell line II Homo sapiens cDNA 5' end), N35123 (yy20b01.s1 Homo sapiens cDNA clone 271753 3'), N36408 (yy33f03.s1 Homo sapiens cDNA clone 273053 3'), W61057 (zc54a11.r1 Soares senescent fibroblasts NbHSF Homo sapiens cDNA clone 326108 5' similar to contains element MSR1 repetitive element), and X16706 (Human fra-2 mRNA). Based upon sequence similarity, AR310_3 proteins and each similar protein or peptide may share at least some activity. The TopPredII computer program predicts a potential transmembrane domain within the AR310_3 protein sequence centered around amino acid 66 of SEQ ID NO:4; this region is also a possible signal sequence.

Clone "AS186_3"

A polynucleotide of the present invention has been identified as clone "AS186_3". AS186_3 was isolated from a human fetal brain cDNA library using methods which are selective for cDNAs encoding secreted proteins (see U.S. Pat. No. 5,536,637), or was identified as encoding a secreted or transmembrane protein on the basis of computer analysis of the amino acid sequence of the encoded protein. AS186_3 is a full-length clone,

including the entire coding sequence of a secreted protein (also referred to herein as "AS186_3 protein").

The nucleotide sequence of AS186_3 as presently determined is reported in SEQ ID NO:5. What applicants presently believe to be the proper reading frame and the predicted amino acid sequence of the AS186_3 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:6. Amino acids 21 to 33 are a predicted leader/signal sequence, with the predicted mature amino acid sequence beginning at amino acid 34, or are a transmembrane domain.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone AS186_3 should be approximately 1200 bp.

The nucleotide sequence disclosed herein for AS186_3 was searched against the GenBank and GeneSeq nucleotide sequence databases using BLASTN/BLASTX and FASTA search protocols. AS186_3 demonstrated at least some similarity with sequences identified as J00083 (Human Alu family interspersed repeat; clone BLUR11) and U14574 (***ALU WARNING Human Alu-Sx subfamily consensus sequence). The predicted amino acid sequence disclosed herein for AS186_3 was searched against the GenPept and GeneSeq amino acid sequence databases using the BLASTX search protocol. The predicted AS186_3 protein demonstrated at least some similarity to sequences identified as S58722 (X-linked retinopathy protein {C-terminal, clone XEH.8c} [human]). Based upon sequence similarity, AS186_3 proteins and each similar protein or peptide may share at least some activity. The nucleotide sequence of AS186_3 indicates that it may contain an Alu repetitive element.

Clone "AY160_2"

A polynucleotide of the present invention has been identified as clone "AY160_2". AY160_2 was isolated from a human adult retina cDNA library using methods which are selective for cDNAs encoding secreted proteins (see U.S. Pat. No. 5,536,637), or was identified as encoding a secreted or transmembrane protein on the basis of computer analysis of the amino acid sequence of the encoded protein. AY160_2 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "AY160_2 protein").

The nucleotide sequence of AY160_2 as presently determined is reported in SEQ ID NO:7. What applicants presently believe to be the proper reading frame and the predicted amino acid sequence of the AY160_2 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:8. Amino acids 3 to 15 are a predicted leader/signal sequence, with the predicted mature amino acid sequence beginning at amino acid 16, or are a transmembrane domain.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone AY160_2 should be approximately 1900 bp.

The nucleotide sequence disclosed herein for AY160_2 was searched against the GenBank and GeneSeq nucleotide sequence databases using BLASTN/BLASTX and FASTA search protocols. AY160_2 demonstrated at least some similarity with sequences identified as D12485 (Human mRNA for nucleotide pyrophosphatase, complete cds), D30649 (Rat mRNA for phosphodiesterase I, complete cds), N77069 (yz84h12.r1 Homo sapiens cDNA clone 289799 5'), and Z47987 (R.norvegicus mRNA for RB13-6 antigen). The predicted amino acid sequence disclosed herein for AY160_2 was searched against the GenPept and GeneSeq amino acid sequence databases using the BLASTX search protocol. The predicted AY160_2 protein demonstrated at least some similarity to sequences identified as D12485 (HUMNPP_2 NPPase [Homo sapiens]), D30649 (phosphodiesterase I [Rattus rattus]), and Z47987 (RNRB13X6_1 RB13-6 antigen [Rattus norvegicus]). The AY160_2 protein also has some domains of sequence similarity to a variety of integral membrane proteins including glycoprotein PC-1. Based upon sequence similarity, AY160_2 proteins and each similar protein or peptide may share at least some activity. The TopPredII computer program predicts a potential transmembrane domain near the carboxy terminus of the AY160_2 protein, around amino acid 418 of SEQ ID NO:8.

Clone "BD127_16"

A polynucleotide of the present invention has been identified as clone "BD127_16". BD127_16 was isolated from a human fetal kidney cDNA library using methods which are selective for cDNAs encoding secreted proteins (see U.S. Pat. No. 5,536,637), or was identified as encoding a secreted or transmembrane protein on the basis of computer analysis of the amino acid sequence of the encoded protein. BD127_16 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "BD127_16 protein").

The nucleotide sequence of the 5' portion of BD127_16 as presently determined is reported in SEQ ID NO:9. What applicants presently believe is the proper reading frame for the coding region is indicated in SEQ ID NO:10. The predicted amino acid sequence of the BD127_16 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:10. Additional nucleotide sequence from the 3' portion of BD127_16, including the polyA tail, is reported in SEQ ID NO:11.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone BD127_16 should be approximately 1080 bp.

The nucleotide sequence disclosed herein for BD127_16 was searched against the GenBank and GeneSeq nucleotide sequence databases using BLASTN/BLASTX and FASTA search protocols. BD127_16 demonstrated at least some similarity with sequences identified as M55683 (Human cartilage matrix protein). The predicted amino acid sequence disclosed herein for BD127_16 was searched against the GenPept and GeneSeq amino acid sequence databases using the BLASTX search protocol. The predicted BD127_16 protein demonstrated at least some similarity to sequences identified as U03272 (HSU03272_1 fibrillin-2 [Homo sapiens]) and X04571 (HSEGFRER_1 Human mRNA for kidney epidermal growth factor (EGF) precursor [Homo sapiens]). Based upon sequence similarity, BD127_16 proteins and each similar protein or peptide may share at least some activity. The nucleotide sequence of BD127_16 indicates that it may contain a PTR7 repetitive element.

Clone "BL205_14"

A polynucleotide of the present invention has been identified as clone "BL205_14". BL205_14 was isolated from a human adult testes cDNA library using methods which are selective for cDNAs encoding secreted proteins (see U.S. Pat. No. 5,536,637), or was identified as encoding a secreted or transmembrane protein on the basis of computer analysis of the amino acid sequence of the encoded protein. BL205_14 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "BL205_14 protein").

The nucleotide sequence of BL205_14 as presently determined is reported in SEQ ID NO:12. What applicants presently believe to be the proper reading frame and the predicted amino acid sequence of the BL205_14 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:13.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone BL205_14 should be approximately 1500 bp.

The nucleotide sequence disclosed herein for BL205_14 was searched against the GenBank and GeneSeq nucleotide sequence databases using BLASTN/BLASTX and FASTA search protocols. BL205_14 demonstrated at least some similarity with sequences identified as T08683 (EST06575 Homo sapiens cDNA clone HIBBI30 5' end) and U55178 (Mus musculus TIL mRNA from progressing tumor site, clone NFB#3). Based upon sequence similarity, BL205_14 proteins and each similar protein or peptide may share at least some activity.

Clone "H438_1"

A polynucleotide of the present invention has been identified as clone "H438_1". H438_1 was isolated from a human adult blood (peripheral blood mononuclear cells treated with phytohemagglutinin and phorbol myristate acetate and mixed lymphocyte reaction) cDNA library using methods which are selective for cDNAs encoding secreted proteins (see U.S. Pat. No. 5,536,637), or was identified as encoding a secreted or transmembrane protein on the basis of computer analysis of the amino acid sequence of the encoded protein. H438_1 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "H438_1 protein").

The nucleotide sequence of H438_1 as presently determined is reported in SEQ ID NO:14. What applicants presently believe to be the proper reading frame and the predicted amino acid sequence of the H438_1 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:15.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone H438_1 should be approximately 2100 bp.

The nucleotide sequence disclosed herein for H438_1 was searched against the GenBank and GeneSeq nucleotide sequence databases using BLASTN/BLASTX and FASTA search protocols. H438_1 demonstrated at least some similarity with sequences identified as H06234 (yl78e09.r1 Homo sapiens cDNA clone 44074 5') and R56040 (yg91a04.s1 Homo sapiens cDNA clone 40669 3'). Based upon sequence similarity, H438_1 proteins and each similar protein or peptide may share at least some activity. The TopPredII computer program predicts a potential transmembrane domain within the H438_1 protein sequence, centered around amino acid 25 of SEQ ID NO:15.

Fig. 2 is an autoradiograph evidencing expression of clone H438_1 in COS cells.

Deposit of Clones

Clones AM349_1, AR310_2, AS186_2, AY160_1, BD127_11, BL205_7, and H438_1 were deposited on August 14, 1996 with the American Type Culture Collection as an original deposit under the Budapest Treaty and were given the accession number ATCC 98140, from which each clone comprising a particular polynucleotide is obtainable. An additional isolate of each of clones AM349_1, AR310_2, AS186_2, AY160_1, BD127_11, BL205_7 (namely AM349_2, AR310_3, AS186_3, AY160_2, BD127_16, and BL205_14, respectively) was deposited on August 23, 1996 with the American Type Culture Collection as an original deposit under the Budapest Treaty under accession number ATCC 98155. All restrictions on the availability to the public of the deposited material will be irrevocably removed upon the granting of the patent, except for the requirements specified in 37 C.F.R. § 1.808(b).

Each clone has been transfected into separate bacterial cells (*E. coli*) in this composite deposit. Each clone can be removed from the vector in which it was deposited by performing an EcoRI/NotI digestion (5' site, EcoRI; 3' site, NotI) to produce the appropriate fragment for such clone. Each clone was deposited in either the pED6 or pNOTs vector depicted in Fig. 1. The pED6dpc2 vector ("pED6") was derived from pED6dpc1 by insertion of a new polylinker to facilitate cDNA cloning (Kaufman *et al.*, 1991, *Nucleic Acids Res.* **19**: 4485-4490); the pNOTs vector was derived from pMT2 (Kaufman *et al.*, 1989, *Mol. Cell. Biol.* **9**: 946-958) by deletion of the DHFR sequences, insertion of a new polylinker, and insertion of the M13 origin of replication in the ClaI site. In some instances, the deposited clone can become "flipped" (i.e., in the reverse orientation) in the deposited isolate. In such instances, the cDNA insert can still be isolated by digestion with EcoRI and NotI. However, NotI will then produce the 5' site and EcoRI will produce the 3' site for placement of the cDNA in proper orientation for expression in a suitable vector. The cDNA may also be expressed from the vectors in which they were deposited.

Bacterial cells containing a particular clone can be obtained from the composite deposit as follows:

An oligonucleotide probe or probes should be designed to the sequence that is known for that particular clone. This sequence can be derived from the sequences provided herein, or from a combination of those sequences. The sequence of the oligonucleotide probe that was used to isolate each full-length clone is identified below, and should be most reliable in isolating the clone of interest.

Clone	Probe Sequence
AM349_2	SEQ ID NO:16
AR310_3	SEQ ID NO:17
AS186_3	SEQ ID NO:18
AY160_2	SEQ ID NO:19
BD127_16	SEQ ID NO:20
BL205_14	SEQ ID NO:21
H438_1	SEQ ID NO:22

In the sequences listed above which include an N at position 2, that position is occupied in preferred probes/primers by a biotinylated phosphoaramidite residue rather than a nucleotide (such as , for example, that produced by use of biotin phosphoramidite (1-dimethoxytrityloxy-2-(N-biotinyl-4-aminobutyl)-propyl-3-O-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramadite) (Glen Research, cat. no. 10-1953)).

The design of the oligonucleotide probe should preferably follow these parameters:

(a) It should be designed to an area of the sequence which has the fewest ambiguous bases ("N's"), if any;

(b) It should be designed to have a T_m of approx. 80 ° C (assuming 2° for each A or T and 4 degrees for each G or C).

The oligonucleotide should preferably be labeled with g-^{32}P ATP (specific activity 6000 Ci/mmole) and T4 polynucleotide kinase using commonly employed techniques for labeling oligonucleotides. Other labeling techniques can also be used. Unincorporated label should preferably be removed by gel filtration chromatography or other established methods. The amount of radioactivity incorporated into the probe should be quantitated by measurement in a scintillation counter. Preferably, specific activity of the resulting probe should be approximately 4e+6 dpm/pmole.

The bacterial culture containing the pool of full-length clones should preferably be thawed and 100 µl of the stock used to inoculate a sterile culture flask containing 25 ml of sterile L-broth containing ampicillin at 100 µg/ml. The culture should preferably be grown to saturation at 37°C, and the saturated culture should preferably be diluted in fresh L-broth. Aliquots of these dilutions should preferably be plated to determine the dilution and volume which will yield approximately 5000 distinct and well-separated colonies on solid bacteriological media containing L-broth containing ampicillin at 100

μg/ml and agar at 1.5% in a 150 mm petri dish when grown overnight at 37°C. Other known methods of obtaining distinct, well-separated colonies can also be employed.

Standard colony hybridization procedures should then be used to transfer the colonies to nitrocellulose filters and lyse, denature and bake them.

The filter is then preferably incubated at 65°C for 1 hour with gentle agitation in 6X SSC (20X stock is 175.3 g NaCl/liter, 88.2 g Na citrate/liter, adjusted to pH 7.0 with NaOH) containing 0.5% SDS, 100 μg/ml of yeast RNA, and 10 mM EDTA (approximately 10 mL per 150 mm filter). Preferably, the probe is then added to the hybridization mix at a concentration greater than or equal to 1e+6 dpm/mL. The filter is then preferably incubated at 65°C with gentle agitation overnight. The filter is then preferably washed in 500 mL of 2X SSC/0.5% SDS at room temperature without agitation, preferably followed by 500 mL of 2X SSC/0.1% SDS at room temperature with gentle shaking for 15 minutes. A third wash with 0.1X SSC/0.5% SDS at 65°C for 30 minutes to 1 hour is optional. The filter is then preferably dried and subjected to autoradiography for sufficient time to visualize the positives on the X-ray film. Other known hybridization methods can also be employed.

The positive colonies are picked, grown in culture, and plasmid DNA isolated using standard procedures. The clones can then be verified by restriction analysis, hybridization analysis, or DNA sequencing.

Fragments of the proteins of the present invention which are capable of exhibiting biological activity are also encompassed by the present invention. Fragments of the protein may be in linear form or they may be cyclized using known methods, for example, as described in H.U. Saragovi, *et al.*, Bio/Technology 10, 773-778 (1992) and in R.S. McDowell, *et al.*, J. Amer. Chem. Soc. 114, 9245-9253 (1992), both of which are incorporated herein by reference. Such fragments may be fused to carrier molecules such as immunoglobulins for many purposes, including increasing the valency of protein binding sites. For example, fragments of the protein may be fused through "linker" sequences to the Fc portion of an immunoglobulin. For a bivalent form of the protein, such a fusion could be to the Fc portion of an IgG molecule. Other immunoglobulin isotypes may also be used to generate such fusions. For example, a protein - IgM fusion would generate a decavalent form of the protein of the invention.

The present invention also provides both full-length and mature forms of the disclosed proteins. The full-length form of the such proteins is identified in the sequence listing by translation of the nucleotide sequence of each disclosed clone. The mature form

of such protein may be obtained by expression of the disclosed full-length polynucleotide (preferably those deposited with ATCC) in a suitable mammalian cell or other host cell. The sequence of the mature form of the protein may also be determinable from the amino acid sequence of the full-length form.

The present invention also provides genes corresponding to the cDNA sequences disclosed herein. "Corresponding genes" are the regions of the genome that are transcribed to produce the mRNAs from which the cDNA sequences are derived and any contiguous regions of the genome necessary for the regulated expression of such genes, including but not limited to coding sequences, 5' and 3' untranslated regions, alternatively spliced exons, introns, promoters, enhancers, and silencer or suppressor elements. The corresponding genes can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include the preparation of probes or primers from the disclosed sequence information for identification and/or amplification of genes in appropriate genomic libraries or other sources of genomic materials.

Where the protein of the present invention is membrane-bound (e.g., is a receptor), the present invention also provides for soluble forms of such protein. In such forms part or all of the intracellular and transmembrane domains of the protein are deleted such that the protein is fully secreted from the cell in which it is expressed. The intracellular and transmembrane domains of proteins of the invention can be identified in accordance with known techniques for determination of such domains from sequence information.

Proteins and protein fragments of the present invention include proteins with amino acid sequence lengths that are at least 25%(more preferably at least 50%, and most preferably at least 75%) of the length of a disclosed protein and have at least 60% sequence identity (more preferably, at least 75% identity; most preferably at least 90% or 95% identity) with that disclosed protein, where sequence identity is determined by comparing the amino acid sequences of the proteins when aligned so as to maximize overlap and identity while minimizing sequence gaps. Also included in the present invention are proteins and protein fragments that contain a segment preferably comprising 8 or more (more preferably 20 or more, most preferably 30 or more) contiguous amino acids that shares at least 75% sequence identity (more preferably, at least 85% identity; most preferably at least 95% identity) with any such segment of any of the disclosed proteins.

Species homologs of the disclosed polynucleotides and proteins are also provided by the present invention. As used herein, a "species homologue" is a protein or

polynucleotide with a different species of origin from that of a given protein or polynucleotide, but with significant sequence similarity to the given protein or polynucleotide, as determined by those of skill in the art. Species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source from the desired species.

The invention also encompasses allelic variants of the disclosed polynucleotides or proteins; that is, naturally-occurring alternative forms of the isolated polynucleotide which also encode proteins which are identical, homologous, or related to that encoded by the polynucleotides .

The invention also includes polynucleotides with sequences complementary to those of the polynucleotides disclosed herein.

The present invention also includes polynucleotides capable of hybridizing under reduced stringency conditions, more preferably stringent conditions, and most preferably highly stringent conditions, to polynucleotides described herein. Examples of stringency conditions are shown in the table below: highly stringent conditions are those that are at least as stringent as, for example, conditions A-F; stringent conditions are at least as stringent as, for example, conditions G-L; and reduced stringency conditions are at least as stringent as, for example, conditions M-R.

Stringency Condition	Polynucleotide Hybrid	Hybrid Length (bp)‡	Hybridization Temperature and Buffer†	Wash Temperature and Buffer†
A	DNA:DNA	≥ 50	65°C; 1xSSC -or- 42°C; 1xSSC, 50% formamide	65°C; 0.3xSSC
B	DNA:DNA	<50	T_B*; 1xSSC	T_B*; 1xSSC
C	DNA:RNA	≥ 50	67°C; 1xSSC -or- 45°C; 1xSSC, 50% formamide	67°C; 0.3xSSC
D	DNA:RNA	<50	T_D*; 1xSSC	T_D*; 1xSSC
E	RNA:RNA	≥ 50	70°C; 1xSSC -or- 50°C; 1xSSC, 50% formamide	70°C; 0.3xSSC
F	RNA:RNA	<50	T_F*; 1xSSC	T_F*; 1xSSC
G	DNA:DNA	≥ 50	65°C; 4xSSC -or- 42°C; 4xSSC, 50% formamide	65°C; 1xSSC
H	DNA:DNA	<50	T_H*; 4xSSC	T_H*; 4xSSC
I	DNA:RNA	≥ 50	67°C; 4xSSC -or- 45°C; 4xSSC, 50% formamide	67°C; 1xSSC
J	DNA:RNA	<50	T_J*; 4xSSC	T_J*; 4xSSC
K	RNA:RNA	≥ 50	70°C; 4xSSC -or- 50°C; 4xSSC, 50% formamide	67°C; 1xSSC
L	RNA:RNA	<50	T_L*; 2xSSC	T_L*; 2xSSC
M	DNA:DNA	≥ 50	50°C; 4xSSC -or- 40°C; 6xSSC, 50% formamide	50°C; 2xSSC
N	DNA:DNA	<50	T_N*; 6xSSC	T_N*; 6xSSC
O	DNA:RNA	≥ 50	55°C; 4xSSC -or- 42°C; 6xSSC, 50% formamide	55°C; 2xSSC
P	DNA:RNA	<50	T_P*; 6xSSC	T_P*; 6xSSC
Q	RNA:RNA	≥ 50	60°C; 4xSSC -or- 45°C; 6xSSC, 50% formamide	60°C; 2xSSC
R	RNA:RNA	<50	T_R*; 4xSSC	T_R*; 4xSSC

‡: The hybrid length is that anticipated for the hybridized region(s) of the hybridizing polynucleotides. When hybridizing a polynucleotide to a target polynucleotide of unknown sequence, the hybrid length is assumed to be that of the hybridizing polynucleotide. When polynucleotides of known sequence are hybridized, the hybrid length can be determined by aligning the sequences of the polynucleotides and identifying the region or regions of optimal sequence complementarity.

†: SSPE (1xSSPE is 0.15M NaCl, 10mM NaH_2PO_4, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (1xSSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes after hybridization is complete.

*T_B - T_R: The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature (T_m) of the hybrid, where T_m is determined according to the following equations. For hybrids less than 18 base pairs in length, T_m(°C) = 2(# of A + T bases) + 4(# of G + C bases). For hybrids between 18 and 49 base pairs in length, T_m(°C) = 81.5 + 16.6($\log_{10}$[Na^+]) + 0.41(%G+C) - (600/N), where N is the number of bases in the hybrid, and [Na^+] is the concentration of sodium ions in the hybridization buffer ([Na^+] for 1xSSC = 0.165 M).

Additional examples of stringency conditions for polynucleotide hybridization are provided in Sambrook, J., E.F. Fritsch, and T. Maniatis, 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, chapters 9 and 11, and *Current Protocols in Molecular Biology*, 1995, F.M. Ausubel et al., eds., John Wiley & Sons, Inc., sections 2.10 and 6.3-6.4, incorporated herein by reference.

Preferably, each such hybridizing polynucleotide has a length that is at least 25%(more preferably at least 50%, and most preferably at least 75%) of the length of the polynucleotide of the present invention to which it hybridizes, and has at least 60% sequence identity (more preferably, at least 75% identity; most preferably at least 90% or 95% identity) with the polynucleotide of the present invention to which it hybridizes, where sequence identity is determined by comparing the sequences of the hybridizing polynucleotides when aligned so as to maximize overlap and identity while minimizing sequence gaps.

The isolated polynucleotide of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman *et al.*, Nucleic Acids Res. 19, 4485-4490 (1991), in order to produce the protein recombinantly. Many suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, Methods in Enzymology 185, 537-566 (1990). As defined herein "operably linked" means that the isolated polynucleotide of the invention and an expression control sequence are situated within a vector or cell in such a way that the protein is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/expression control sequence.

A number of types of cells may act as suitable host cells for expression of the protein. Mammalian host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK or Jurkat cells.

Alternatively, it may be possible to produce the protein in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Potentially suitable yeast strains include *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces* strains, *Candida,* or any yeast strain capable of expressing heterologous proteins. Potentially suitable bacterial strains include *Escherichia coli, Bacillus subtilis, Salmonella typhimurium,* or any bacterial

strain capable of expressing heterologous proteins. If the protein is made in yeast or bacteria, it may be necessary to modify the protein produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain the functional protein. Such covalent attachments may be accomplished using known chemical or enzymatic methods.

The protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *e.g.*, Invitrogen, San Diego, California, U.S.A. (the MaxBac® kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. As used herein, an insect cell capable of expressing a polynucleotide of the present invention is "transformed."

The protein of the invention may be prepared by culturing transformed host cells under culture conditions suitable to express the recombinant protein. The resulting expressed protein may then be purified from such culture (i.e., from culture medium or cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification of the protein may also include an affinity column containing agents which will bind to the protein; one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl® or Cibacrom blue 3GA Sepharose®; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

Alternatively, the protein of the invention may also be expressed in a form which will facilitate purification. For example, it may be expressed as a fusion protein, such as those of maltose binding protein (MBP), glutathione-S-transferase (GST) or thioredoxin (TRX). Kits for expression and purification of such fusion proteins are commercially available from New England BioLab (Beverly, MA), Pharmacia (Piscataway, NJ) and InVitrogen, respectively. The protein can also be tagged with an epitope and subsequently purified by using a specific antibody directed to such epitope. One such epitope ("Flag") is commercially available from Kodak (New Haven, CT).

Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant

methyl or other aliphatic groups, can be employed to further purify the protein. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a substantially homogeneous isolated recombinant protein. The protein thus purified is substantially free of other mammalian proteins and is defined in accordance with the present invention as an "isolated protein."

The protein of the invention may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a nucleotide sequence encoding the protein.

The protein may also be produced by known conventional chemical synthesis. Methods for constructing the proteins of the present invention by synthetic means are known to those skilled in the art. The synthetically-constructed protein sequences, by virtue of sharing primary, secondary or tertiary structural and/or conformational characteristics with proteins may possess biological properties in common therewith, including protein activity. Thus, they may be employed as biologically active or immunological substitutes for natural, purified proteins in screening of therapeutic compounds and in immunological processes for the development of antibodies.

The proteins provided herein also include proteins characterized by amino acid sequences similar to those of purified proteins but into which modification are naturally provided or deliberately engineered. For example, modifications in the peptide or DNA sequences can be made by those skilled in the art using known techniques. Modifications of interest in the protein sequences may include the alteration, substitution, replacement, insertion or deletion of a selected amino acid residue in the coding sequence. For example, one or more of the cysteine residues may be deleted or replaced with another amino acid to alter the conformation of the molecule. Techniques for such alteration, substitution, replacement, insertion or deletion are well known to those skilled in the art (see, e.g., U.S. Patent No. 4,518,584). Preferably, such alteration, substitution, replacement, insertion or deletion retains the desired activity of the protein.

Other fragments and derivatives of the sequences of proteins which would be expected to retain protein activity in whole or in part and may thus be useful for screening or other immunological methodologies may also be easily made by those skilled in the art given the disclosures herein. Such modifications are believed to be encompassed by the present invention.

USES AND BIOLOGICAL ACTIVITY

The polynucleotides and proteins of the present invention are expected to exhibit one or more of the uses or biological activities (including those associated with assays cited herein) identified below. Uses or activities described for proteins of the present invention may be provided by administration or use of such proteins or by administration or use of polynucleotides encoding such proteins (such as, for example, in gene therapies or vectors suitable for introduction of DNA).

Research Uses and Utilities

The polynucleotides provided by the present invention can be used by the research community for various purposes. The polynucleotides can be used to express recombinant protein for analysis, characterization or therapeutic use; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in disease states); as molecular weight markers on Southern gels; as chromosome markers or tags (when labeled) to identify chromosomes or to map related gene positions; to compare with endogenous DNA sequences in patients to identify potential genetic disorders; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; as a probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides; for selecting and making oligomers for attachment to a "gene chip" or other support, including for examination of expression patterns; to raise anti-protein antibodies using DNA immunization techniques; and as an antigen to raise anti-DNA antibodies or elicit another immune response. Where the polynucleotide encodes a protein which binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the polynucleotide can also be used in interaction trap assays (such as, for example, that described in Gyuris et al., Cell 75:791-803 (1993)) to identify polynucleotides encoding the other protein with which binding occurs or to identify inhibitors of the binding interaction.

The proteins provided by the present invention can similarly be used in assay to determine biological activity, including in a panel of multiple proteins for high-throughput screening; to raise antibodies or to elicit another immune response; as a reagent (including the labeled reagent) in assays designed to quantitatively determine levels of the protein (or its receptor) in biological fluids; as markers for tissues in which

the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Where the protein binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the protein can be used to identify the other protein with which binding occurs or to identify inhibitors of the binding interaction. Proteins involved in these binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

Any or all of these research utilities are capable of being developed into reagent grade or kit format for commercialization as research products.

Methods for performing the uses listed above are well known to those skilled in the art. References disclosing such methods include without limitation "Molecular Cloning: A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory Press, Sambrook, J., E.F. Fritsch and T. Maniatis eds., 1989, and "Methods in Enzymology: Guide to Molecular Cloning Techniques", Academic Press, Berger, S.L. and A.R. Kimmel eds., 1987.

Nutritional Uses

Polynucleotides and proteins of the present invention can also be used as nutritional sources or supplements. Such uses include without limitation use as a protein or amino acid supplement, use as a carbon source, use as a nitrogen source and use as a source of carbohydrate. In such cases the protein or polynucleotide of the invention can be added to the feed of a particular organism or can be administered as a separate solid or liquid preparation, such as in the form of powder, pills, solutions, suspensions or capsules. In the case of microorganisms, the protein or polynucleotide of the invention can be added to the medium in or on which the microorganism is cultured.

Cytokine and Cell Proliferation/Differentiation Activity

A protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays

for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+ (preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e and CMK.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for T-cell or thymocyte proliferation include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Bertagnolli et al., J. Immunol. 145:1706-1712, 1990; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Bertagnolli, et al., J. Immunol. 149:3778-3783, 1992; Bowman et al., J. Immunol. 152: 1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell stimulation, Kruisbeek, A.M. and Shevach, E.M. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto. 1994; and Measurement of mouse and human Interferon γ, Schreiber, R.D. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L.S. and Lipsky, P.E. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries et al., J. Exp. Med. 173:1205-1211, 1991; Moreau et al., Nature 336:690-692, 1988; Greenberger et al., Proc. Natl. Acad. Sci. U.S.A. 80:2931-2938, 1983; Measurement of mouse and human interleukin 6 - Nordan, R. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith et al., Proc. Natl. Acad. Sci. U.S.A. 83:1857-1861, 1986; Measurement of human Interleukin 11 - Bennett, F., Giannotti, J., Clark, S.C. and Turner, K. J. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991; Measurement of mouse and human Interleukin 9 - Ciarletta, A., Giannotti, J., Clark, S.C. and Turner, K.J. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.

Immune Stimulating or Suppressing Activity

A protein of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases causes by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpesviruses, mycobacteria, Leishmania spp., malaria spp. and various fungal infections such as candidiasis. Of course, in this regard, a protein of the present invention may also be useful where a boost to the immune system generally may be desirable, *i.e.*, in the treatment of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitis, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein of the present invention may also to be useful in the treatment of allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for

example, organ transplantation), may also be treatable using a protein of the present invention.

Using the proteins of the invention it may also be possible to immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T cells may be inhibited by suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active, non-antigen-specific, process which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or anergy in T cells, is distinguishable from immunosuppression in that it is generally antigen-specific and persists after exposure to the tolerizing agent has ceased. Operationally, tolerance can be demonstrated by the lack of a T cell response upon reexposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as , for example, B7)), *e.g.*, preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a molecule which inhibits or blocks interaction of a B7 lymphocyte antigen with its natural ligand(s) on immune cells (such as a soluble, monomeric form of a peptide having B7-2 activity alone or in conjunction with a monomeric form of a peptide having an activity of another B lymphocyte antigen (*e.g.*, B7-1, B7-3) or blocking antibody), prior to transplantation can lead to the binding of the molecule to the natural ligand(s) on the immune cells without transmitting the corresponding costimulatory signal. Blocking B lymphocyte antigen function in this matter prevents cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, the lack of costimulation may also be sufficient to anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or

tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens.

The efficacy of particular blocking reagents in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins *in vivo* as described in Lenschow *et al.*, Science 257:789-792 (1992) and Turka *et al.*, Proc. Natl. Acad. Sci USA, *89*:11102-11105 (1992). In addition, murine models of GVHD (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of blocking B lymphocyte antigen function *in vivo* on the development of that disease.

Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms. Administration of reagents which block costimulation of T cells by disrupting receptor:ligand interactions of B lymphocyte antigens can be used to inhibit T cell activation and prevent production of autoantibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from the disease. The efficacy of blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythmatosis in MRL/*lpr/lpr* mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 840-856).

Upregulation of an antigen function (preferably a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy. Upregulation of immune responses may be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response through stimulating B lymphocyte antigen function may be useful in cases of

viral infection. In addition, systemic viral diseases such as influenza, the common cold, and encephalitis might be alleviated by the administration of stimulatory forms of B lymphocyte antigens systemically.

Alternatively, anti-viral immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells *in vitro* with viral antigen-pulsed APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and reintroducing the *in vitro* activated T cells into the patient. Another method of enhancing anti-viral immune responses would be to isolate infected cells from a patient, transfect them with a nucleic acid encoding a protein of the present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to, and thereby activate, T cells *in vivo*.

In another application, up regulation or enhancement of antigen function (preferably B lymphocyte antigen function) may be useful in the induction of tumor immunity. Tumor cells (*e.g.*, sarcoma, melanoma, lymphoma, leukemia, neuroblastoma, carcinoma) transfected with a nucleic acid encoding at least one peptide of the present invention can be administered to a subject to overcome tumor-specific tolerance in the subject. If desired, the tumor cell can be transfected to express a combination of peptides. For example, tumor cells obtained from a patient can be transfected *ex vivo* with an expression vector directing the expression of a peptide having B7-2-like activity alone, or in conjunction with a peptide having B7-1-like activity and/or B7-3-like activity. The transfected tumor cells are returned to the patient to result in expression of the peptides on the surface of the transfected cell. Alternatively, gene therapy techniques can be used to target a tumor cell for transfection *in vivo*.

The presence of the peptide of the present invention having the activity of a B lymphocyte antigen(s) on the surface of the tumor cell provides the necessary costimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to reexpress sufficient amounts of MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (*e.g.*, a cytoplasmic-domain truncated portion) of an MHC class I α chain protein and β_2 microglobulin protein or an MHC class II α chain protein and an MHC class II β chain protein to thereby express MHC class I or MHC class II proteins on the cell surface.

Expression of the appropriate class I or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (*e.g.*, B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Bowmanet al., J. Virology 61:1992-1998; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell function: *In vitro* antibody production, Mond, J.J. and Brunswick, M. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto. 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter

7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by dendritic cells that activate naive T-cells) include, without limitation, those described in: Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal of Experimental Medicine 173:549-559, 1991; Macatonia et al., Journal of Immunology 154:5071-5079, 1995; Porgador et al., Journal of Experimental Medicine 182:255-260, 1995; Nair et al., Journal of Virology 67:4062-4069, 1993; Huang et al., Science 264:961-965, 1994; Macatonia et al., Journal of Experimental Medicine 169:1255-1264, 1989; Bhardwaj et al., Journal of Clinical Investigation 94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., Cytometry 13:795-808, 1992; Gorczyca et al., Leukemia 7:659-670, 1993; Gorczyca et al., Cancer Research 53:1945-1951, 1993; Itoh et al., Cell 66:233-243, 1991; Zacharchuk, Journal of Immunology 145:4037-4045, 1990; Zamai et al., Cytometry 14:891-897, 1993; Gorczyca et al., International Journal of Oncology 1:639-648, 1992.

Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine et al., Cellular Immunology 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., Proc. Nat. Acad Sci. USA 88:7548-7551, 1991.

Hematopoiesis Regulating Activity

A protein of the present invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis, e.g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent

myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either *in-vivo* or *ex-vivo* (i.e., in conjunction with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous)) as normal cells or genetically manipulated for gene therapy.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson et al. Cellular Biology 15:141-151, 1995; Keller et al., Molecular and Cellular Biology 13:473-486, 1993; McClanahan et al., Blood 81:2903-2915, 1993.

Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, M.G. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, NY. 1994; Hirayama et al., Proc. Natl. Acad. Sci. USA 89:5907-5911, 1992; Primitive hematopoietic colony forming cells with high proliferative potential, McNiece, I.K. and Briddell, R.A. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, NY. 1994; Neben et al., Experimental Hematology 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R.E. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 1-21, Wiley-Liss, Inc.., New York, NY. 1994; Long term bone marrow cultures in the presence of stromal cells, Spooncer, E., Dexter, M. and Allen, T. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, NY. 1994; Long term culture initiating cell assay, Sutherland,

H.J. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al*. eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, NY. 1994.

Tissue Growth Activity

A protein of the present invention also may have utility in compositions used for bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as well as for wound healing and tissue repair and replacement, and in the treatment of burns, incisions and ulcers.

A protein of the present invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a preparation employing a protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. *De novo* bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

A protein of this invention may also be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. A protein of the invention may also be useful in the treatment of osteoporosis or osteoarthritis, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes.

Another category of tissue regeneration activity that may be attributable to the protein of the present invention is tendon/ligament formation. A protein of the present invention, which induces tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by a composition of the present invention contributes to the repair of

congenital, trauma induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions of the present invention may provide an environment to attract tendon- or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament cells or progenitors *ex vivo* for return *in vivo* to effect tissue repair. The compositions of the invention may also be useful in the treatment of tendinitis, carpal tunnel syndrome and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

The protein of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue, *i.e.* for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, a protein may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which may be treated in accordance with the present invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a protein of the invention.

Proteins of the invention may also be useful to promote better or faster closure of non-healing wounds, including without limitation pressure ulcers, ulcers associated with vascular insufficiency, surgical and traumatic wounds, and the like.

It is expected that a protein of the present invention may also exhibit activity for generation or regeneration of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and vascular (including vascular endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition or modulation of fibrotic scarring to allow normal tissue to regenerate. A protein of the invention may also exhibit angiogenic activity.

A protein of the present invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

A protein of the present invention may also be useful for promoting or inhibiting differentiation of tissues described above from precursor tissues or cells; or for inhibiting the growth of tissues described above.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for tissue generation activity include, without limitation, those described in: International Patent Publication No. WO95/16035 (bone, cartilage, tendon); International Patent Publication No. WO95/05846 (nerve, neuronal); International Patent Publication No. WO91/07491 (skin, endothelium).

Assays for wound healing activity include, without limitation, those described in: Winter, Epidermal Wound Healing, pps. 71-112 (Maibach, HI and Rovee, DT, eds.), Year Book Medical Publishers, Inc., Chicago, as modified by Eaglstein and Mertz, J. Invest. Dermatol 71:382-84 (1978).

Activin/Inhibin Activity

A protein of the present invention may also exhibit activin- or inhibin-related activities. Inhibins are characterized by their ability to inhibit the release of follicle stimulating hormone (FSH), while activins and are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH). Thus, a protein of the present invention, alone or in heterodimers with a member of the inhibin α family, may be useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals. Administration of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the protein of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin-β group, may be useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See, for example, United States Patent 4,798,885. A protein of the invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as cows, sheep and pigs.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for activin/inhibin activity include, without limitation, those described in: Vale et al., Endocrinology 91:562-572, 1972; Ling et al., Nature 321:779-782, 1986; Vale et al., Nature 321:776-779, 1986; Mason et al., Nature 318:659-663, 1985; Forage et al., Proc. Natl. Acad. Sci. USA 83:3091-3095, 1986.

Chemotactic/Chemokinetic Activity

A protein of the present invention may have chemotactic or chemokinetic activity (e.g., act as a chemokine) for mammalian cells, including, for example, monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells. Chemotactic and chemokinetic proteins can be used to mobilize or attract a desired cell population to a desired site of action. Chemotactic or chemokinetic proteins provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis) consist of assays that measure the ability of a protein to induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell population to another cell population. Suitable assays for movement and adhesion include, without limitation, those described in: Current Protocols in Immunology, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W.Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 6.12, Measurement of alpha and beta Chemokines 6.12.1-6.12.28; Taub et al. J. Clin. Invest. 95:1370-1376, 1995; Lind et al. APMIS 103:140-146, 1995; Muller et al Eur. J. Immunol. 25: 1744-1748; Gruber et al. J. of Immunol. 152:5860-5867, 1994; Johnston et al. J. of Immunol. 153: 1762-1768, 1994.

Hemostatic and Thrombolytic Activity

A protein of the invention may also exhibit hemostatic or thrombolytic activity. As a result, such a protein is expected to be useful in treatment of various coagulation disorders (including hereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds resulting from trauma, surgery or other causes. A protein of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of conditions resulting therefrom (such as, for example, infarction of cardiac and central nervous system vessels (e.g., stroke).

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assay for hemostatic and thrombolytic activity include, without limitation, those described in: Linet et al., J. Clin. Pharmacol. 26:131-140, 1986; Burdick et al., Thrombosis Res. 45:413-419, 1987; Humphrey et al., Fibrinolysis 5:71-79 (1991); Schaub, Prostaglandins 35:467-474, 1988.

Receptor/Ligand Activity

A protein of the present invention may also demonstrate activity as receptors, receptor ligands or inhibitors or agonists of receptor/ligand interactions. Examples of such receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell-cell interactions and their ligands (including without limitation, cellular adhesion molecules (such as selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses). Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. A protein of the present invention (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for receptor-ligand activity include without limitation those described in:Current Protocols in Immunology, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W.Strober, Pub. Greene Publishing Associates and

Wiley-Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static conditions 7.28.1-7.28.22), Takai et al., Proc. Natl. Acad. Sci. USA 84:6864-6868, 1987; Bierer et al., J. Exp. Med. 168:1145-1156, 1988; Rosenstein et al., J. Exp. Med. 169:149-160 1989; Stoltenborg et al., J. Immunol. Methods 175:59-68, 1994; Stitt et al., Cell 80:661-670, 1995.

Anti-Inflammatory Activity

Proteins of the present invention may also exhibit anti-inflammatory activity. The anti-inflammatory activity may be achieved by providing a stimulus to cells involved in the inflammatory response, by inhibiting or promoting cell-cell interactions (such as, for example, cell adhesion), by inhibiting or promoting chemotaxis of cells involved in the inflammatory process, inhibiting or promoting cell extravasation, or by stimulating or suppressing production of other factors which more directly inhibit or promote an inflammatory response. Proteins exhibiting such activities can be used to treat inflammatory conditions including chronic or acute conditions), including without limitation inflammation associated with infection (such as septic shock, sepsis or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting from over production of cytokines such as TNF or IL-1. Proteins of the invention may also be useful to treat anaphylaxis and hypersensitivity to an antigenic substance or material.

Cadherin/Tumor Invasion Suppressor Activity

Cadherins are calcium-dependent adhesion molecules that appear to play major roles during development, particularly in defining specific cell types. Loss or alteration of normal cadherin expression can lead to changes in cell adhesion properties linked to tumor growth and metastasis. Cadherin malfunction is also implicated in other human diseases, such as pemphigus vulgaris and pemphigus foliaceus (auto-immune blistering skin diseases), Crohn's disease, and some developmental abnormalities.

The cadherin superfamily includes well over forty members, each with a distinct pattern of expression. All members of the superfamily have in common conserved extracellular repeats (cadherin domains), but structural differences are found in other parts of the molecule. The cadherin domains bind calcium to form their tertiary structure and thus calcium is required to mediate their adhesion. Only a few amino acids in the

first cadherin domain provide the basis for homophilic adhesion; modification of this recognition site can change the specificity of a cadherin so that instead of recognizing only itself, the mutant molecule can now also bind to a different cadherin. In addition, some cadherins engage in heterophilic adhesion with other cadherins.

E-cadherin, one member of the cadherin superfamily, is expressed in epithelial cell types. Pathologically, if E-cadherin expression is lost in a tumor, the malignant cells become invasive and the cancer metastasizes. Transfection of cancer cell lines with polynucleotides expressing E-cadherin has reversed cancer-associated changes by returning altered cell shapes to normal, restoring cells' adhesiveness to each other and to their substrate, decreasing the cell growth rate, and drastically reducing anchorage-independent cell growth. Thus, reintroducing E-cadherin expression reverts carcinomas to a less advanced stage. It is likely that other cadherins have the same invasion suppressor role in carcinomas derived from other tissue types. Therefore, proteins of the present invention with cadherin activity, and polynucleotides of the present invention encoding such proteins, can be used to treat cancer. Introducing such proteins or polynucleotides into cancer cells can reduce or eliminate the cancerous changes observed in these cells by providing normal cadherin expression.

Cancer cells have also been shown to express cadherins of a different tissue type than their origin, thus allowing these cells to invade and metastasize in a different tissue in the body. Proteins of the present invention with cadherin activity, and polynucleotides of the present invention encoding such proteins, can be substituted in these cells for the inappropriately expressed cadherins, restoring normal cell adhesive properties and reducing or eliminating the tendency of the cells to metastasize.

Additionally, proteins of the present invention with cadherin activity, and polynucleotides of the present invention encoding such proteins, can used to generate antibodies recognizing and binding to cadherins. Such antibodies can be used to block the adhesion of inappropriately expressed tumor-cell cadherins, preventing the cells from forming a tumor elsewhere. Such an anti-cadherin antibody can also be used as a marker for the grade, pathological type, and prognosis of a cancer, i.e. the more progressed the cancer, the less cadherin expression there will be, and this decrease in cadherin expression can be detected by the use of a cadherin-binding antibody.

Fragments of proteins of the present invention with cadherin activity, preferably a polypeptide comprising a decapeptide of the cadherin recognition site, and polynucleotides of the present invention encoding such protein fragments, can also be used

to block cadherin function by binding to cadherins and preventing them from binding in ways that produce undesirable effects. Additionally, fragments of proteins of the present invention with cadherin activity, preferably truncated soluble cadherin fragments which have been found to be stable in the circulation of cancer patients, and polynucleotides encoding such protein fragments, can be used to disturb proper cell-cell adhesion.

Assays for cadherin adhesive and invasive suppressor activity include, without limitation, those described in: Hortsch et al. J Biol Chem 270 (32): 18809-18817, 1995; Miyaki et al. Oncogene 11: 2547-2552, 1995; Ozawa et al. Cell 63: 1033-1038, 1990.

Tumor Inhibition Activity

In addition to the activities described above for immunological treatment or prevention of tumors, a protein of the invention may exhibit other anti-tumor activities. A protein may inhibit tumor growth directly or indirectly (such as, for example, via ADCC). A protein may exhibit its tumor inhibitory activity by acting on tumor tissue or tumor precursor tissue, by inhibiting formation of tissues necessary to support tumor growth (such as, for example, by inhibiting angiogenesis), by causing production of other factors, agents or cell types which inhibit tumor growth, or by suppressing, eliminating or inhibiting factors, agents or cell types which promote tumor growth.

Other Activities

A protein of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth, infection or function of, or killing, infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting (suppressing or enhancing) bodily characteristics, including, without limitation, height, weight, hair color, eye color, skin, fat to lean ratio or other tissue pigmentation, or organ or body part size or shape (such as, for example, breast augmentation or diminution, change in bone form or shape); effecting biorhythms or caricadic cycles or rhythms; effecting the fertility of male or female subjects; effecting the metabolism, catabolism, anabolism, processing, utilization, storage or elimination of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional factors or component(s); effecting behavioral characteristics, including, without limitation, appetite, libido, stress, cognition (including cognitive disorders), depression (including depressive disorders) and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic

lineages; hormonal or endocrine activity; in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases; treatment of hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such as, for example, the ability to bind antigens or complement); and the ability to act as an antigen in a vaccine composition to raise an immune response against such protein or another material or entity which is cross-reactive with such protein.

ADMINISTRATION AND DOSING

A protein of the present invention (from whatever source derived, including without limitation from recombinant and non-recombinant sources) may be used in a pharmaceutical composition when combined with a pharmaceutically acceptable carrier. Such a composition may also contain (in addition to protein and a carrier) diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration. The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, TNF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IFN, TNF0, TNF1, TNF2, G-CSF, Meg-CSF, thrombopoietin, stem cell factor, and erythropoietin. The pharmaceutical composition may further contain other agents which either enhance the activity of the protein or compliment its activity or use in treatment. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with protein of the invention, or to minimize side effects. Conversely, protein of the present invention may be included in formulations of the particular cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent.

A protein of the present invention may be active in multimers (e.g., heterodimers or homodimers) or complexes with itself or other proteins. As a result, pharmaceutical compositions of the invention may comprise a protein of the invention in such multimeric or complexed form.

The pharmaceutical composition of the invention may be in the form of a complex of the protein(s) of present invention along with protein or peptide antigens. The protein

and/or peptide antigen will deliver a stimulatory signal to both B and T lymphocytes. B lymphocytes will respond to antigen through their surface immunoglobulin receptor. T lymphocytes will respond to antigen through the T cell receptor (TCR) following presentation of the antigen by MHC proteins. MHC and structurally related proteins including those encoded by class I and class II MHC genes on host cells will serve to present the peptide antigen(s) to T lymphocytes. The antigen components could also be supplied as purified MHC-peptide complexes alone or with co-stimulatory molecules that can directly signal T cells. Alternatively antibodies able to bind surface immunolgobulin and other molecules on B cells as well as antibodies able to bind the TCR and other molecules on T cells can be combined with the pharmaceutical composition of the invention.

The pharmaceutical composition of the invention may be in the form of a liposome in which protein of the present invention is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; and U.S. Patent No. 4,737,323, all of which are incorporated herein by reference.

As used herein, the term "therapeutically effective amount" means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, i.e., treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of protein of the present invention is administered to a mammal having a condition to be treated. Protein of the present invention may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines, lymphokines

or other hematopoietic factors. When co-administered with one or more cytokines, lymphokines or other hematopoietic factors, protein of the present invention may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering protein of the present invention in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

Administration of protein of the present invention used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, topical application or cutaneous, subcutaneous, intraperitoneal, parenteral or intravenous injection. Intravenous administration to the patient is preferred.

When a therapeutically effective amount of protein of the present invention is administered orally, protein of the present invention will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% protein of the present invention, and preferably from about 25 to 90% protein of the present invention. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of protein of the present invention, and preferably from about 1 to 50% protein of the present invention.

When a therapeutically effective amount of protein of the present invention is administered by intravenous, cutaneous or subcutaneous injection, protein of the present invention will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to protein of the present invention, an isotonic vehicle such as Sodium

Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art.

The amount of protein of the present invention in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of protein of the present invention with which to treat each individual patient. Initially, the attending physician will administer low doses of protein of the present invention and observe the patient's response. Larger doses of protein of the present invention may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.01 µg to about 100 mg (preferably about 0.1ng to about 10 mg, more preferably about 0.1 µg to about 1 mg) of protein of the present invention per kg body weight.

The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It is contemplated that the duration of each application of the protein of the present invention will be in the range of 12 to 24 hours of continuous intravenous administration. Ultimately the attending physician will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

Protein of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the protein. Such antibodies may be obtained using either the entire protein or fragments thereof as an immunogen. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Methods for synthesizing such peptides are known in the art, for example, as in R.P. Merrifield, J. Amer.Chem.Soc. 85, 2149-2154 (1963); J.L. Krstenansky, *et al.*, FEBS Lett. 211, 10 (1987). Monoclonal antibodies binding to the protein of the invention may be useful diagnostic agents for the immunodetection of the protein. Neutralizing monoclonal antibodies binding to the protein may also be useful therapeutics for both conditions associated with the protein and also in the treatment of some forms of cancer where

abnormal expression of the protein is involved. In the case of cancerous cells or leukemic cells, neutralizing monoclonal antibodies against the protein may be useful in detecting and preventing the metastatic spread of the cancerous cells, which may be mediated by the protein.

For compositions of the present invention which are useful for bone, cartilage, tendon or ligament regeneration, the therapeutic method includes administering the composition topically, systematically, or locally as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Therapeutically useful agents other than a protein of the invention which may also optionally be included in the composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the composition in the methods of the invention. Preferably for bone and/or cartilage formation, the composition would include a matrix capable of delivering the protein-containing composition to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid, polyglycolic acid and polyanhydrides. Other potential materials are biodegradable and biologically well-defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability.

Presently preferred is a 50:50 (mole weight) copolymer of lactic acid and glycolic acid in the form of porous particles having diameters ranging from 150 to 800 microns. In some applications, it will be useful to utilize a sequestering agent, such as carboxymethyl cellulose or autologous blood clot, to prevent the protein compositions from disassociating from the matrix.

A preferred family of sequestering agents is cellulosic materials such as alkylcelluloses (including hydroxyalkylcelluloses), including methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropyl-methylcellulose, and carboxymethylcellulose, the most preferred being cationic salts of carboxymethylcellulose (CMC). Other preferred sequestering agents include hyaluronic acid, sodium alginate, poly(ethylene glycol), polyoxyethylene oxide, carboxyvinyl polymer and poly(vinyl alcohol). The amount of sequestering agent useful herein is 0.5-20 wt%, preferably 1-10 wt% based on total formulation weight, which represents the amount necessary to prevent desorbtion of the protein from the polymer matrix and to provide appropriate handling of the composition, yet not so much that the progenitor cells are prevented from infiltrating the matrix, thereby providing the protein the opportunity to assist the osteogenic activity of the progenitor cells.

In further compositions, proteins of the invention may be combined with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents include various growth factors such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth factors (TGF-α and TGF-β), and insulin-like growth factor (IGF).

The therapeutic compositions are also presently valuable for veterinary applications. Particularly domestic animals and thoroughbred horses, in addition to humans, are desired patients for such treatment with proteins of the present invention.

The dosage regimen of a protein-containing pharmaceutical composition to be used in tissue regeneration will be determined by the attending physician considering various factors which modify the action of the proteins, e.g., amount of tissue weight desired to be formed, the site of damage, the condition of the damaged tissue, the size of a wound, type of damaged tissue (e.g., bone), the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and with inclusion of other proteins in the pharmaceutical composition. For example, the addition of other known growth factors, such as IGF I (insulin like growth factor I), to the final composition, may also effect

the dosage. Progress can be monitored by periodic assessment of tissue/bone growth and/or repair, for example, X-rays, histomorphometric determinations and tetracycline labeling.

Polynucleotides of the present invention can also be used for gene therapy. Such polynucleotides can be introduced either *in vivo* or *ex vivo* into cells for expression in a mammalian subject. Polynucleotides of the invention may also be administered by other known methods for introduction of nucleic acid into a cell or organism (including, without limitation, in the form of viral vectors or naked DNA).

Cells may also be cultured *ex vivo* in the presence of proteins of the present invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced *in vivo* for therapeutic purposes.

Patent and literature references cited herein are incorporated by reference as if fully set forth.

SEQUENCE LISTING

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Gly Glu Asn Tyr Gly Lys Glu Tyr Tyr Gln Ala Leu Leu Gln Glu Gln
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Glu Glu His Tyr Gln Thr Arg Ala Thr Ser Leu Lys Arg Gln Ile Ala
65                  70                  75                  80

Gln Leu Lys Gln Glu Leu Gln Glu Met Ser Glu Lys Met Arg Ser Leu
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Lys Glu Gln Ala Pro Ser Asp Leu Leu Glu Phe Leu His Ser Gln Ile
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Asp Lys Ala Glu Val Ser Ile Gly Ala Lys Leu Pro Ser Glu Tyr Gly
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Met Gly Leu Thr Arg His Pro Glu Glu Lys Pro Val Arg Lys Asp Lys
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Arg Asp Glu Leu Val Glu Val Ile Glu Ala Gly Leu Glu Val Ile Asn
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Asn Pro Asp Glu Asp Asp Glu Gln Glu Asp Glu Glu Gly Pro Leu Gly
        195                 200                 205

Glu Lys Leu Ile Phe Asn Glu Asn Asp Phe Val Glu Gly Tyr Tyr Arg
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Thr Glu Arg Asp Lys Gly Thr Gln Tyr Glu Leu Phe Phe Lys Lys Ala
225                 230                 235                 240

Asp Leu Thr Glu Tyr Arg Leu Val Thr Leu Phe Arg Pro Phe Gly Pro
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Leu Met Lys Val Lys Ser Glu Met Ile Asp Ile Thr Arg Ser Ile Ile
            260                 265                 270

Asn Ile Ile Val Pro Leu Ala Glu Arg Thr Glu Ala Phe Val Gln Phe
        275                 280                 285

Met Gln Asn Phe Arg Asp Val Cys Ile His Gln Asp Lys Lys Ile His
    290                 295                 300

Leu Thr Val Val Tyr Phe Gly Lys Glu Gly Leu Ser Lys Val Asn Ser
305                 310                 315                 320

Ile Leu Glu Ser Val Thr Ser Glu Ser Asn Phe His Asn Tyr Thr Leu
                325                 330                 335

Val Ser Leu Asn Glu Glu Phe Asn Arg Gly Arg Gly Leu Ile Val Gly
            340                 345                 350

Ala Arg Ala Trp Asp Lys Gly Glu Val Leu Met Phe Phe Cys Asp Val
        355                 360                 365

Asp Ile Tyr Phe Ser Ala Glu Phe Leu Asn Ser Cys Arg Leu Asn Ala
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Glu Pro Gly Lys Lys Val Phe Tyr Pro Val Val Phe Ser Leu Tyr Asn
385                 390                 395                 400

Pro Ala Ile Val Tyr Ala Asn Gln Lys Leu Pro Pro Pro Val Glu Gln
                405                 410                 415

Gln Leu Val His Lys Lys Asp Ser Gly Phe Trp Gln Asn Phe Gly Phe
            420                 425                 430

Gly Met Thr Cys Gln Tyr Arg Pro Asn Phe Leu Thr Ile Gly Gly Phe
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Asp Met Glu Leu Lys Gly Trp Gly Gly Lys Asn Phe His Leu Tyr Gln
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Lys Tyr Leu His Gly Asp Leu Ile Val Ile Arg Thr Pro Val Pro Gly
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Leu Phe His Leu Trp His Glu Lys Arg Cys Ala Asp Glu Leu Thr Pro
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Glu Gln Tyr Arg Met Cys Ile Gln Ser Lys Ala Met Asn Glu Ala Ser
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<213> Homo sapiens

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Ala Ser Pro Val Leu Cys Cys Ser Gly Gln Pro Thr Glu Val His Glu
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Ser Asn Ala Arg Ser Ser Arg His Leu Ser Phe Gln Lys Ala Arg Gly
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Asp Glu Met Ser Glu Phe Gln Asn
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<213> Homo sapiens

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Phe Asp Gly Phe Arg Ala Asp Tyr Leu Lys Asn Tyr Glu Phe Pro His
35 40 45

Leu Gln Asn Phe Ile Lys Glu Gly Val Leu Val Glu His Val Lys Asn
50 55 60

Val Phe Ile Thr Lys Thr Phe Pro Asn His Tyr Ser Ile Val Thr Gly
65 70 75 80

Leu Tyr Glu Glu Ser His Gly Ile Val Ala Asn Ser Met Tyr Asp Ala

Val Thr Lys Lys His Phe Ser Asp Ser Asn Asp Lys Asp Pro Phe Trp
100 105 110

Trp Asn Glu Ala Val Pro Ile Trp Val Thr Asn Gln Leu Gln Glu Asn
115 120 125

Arg Ser Ser Ala Ala Ala Met Trp Pro Gly Thr Asp Val Pro Ile His
130 135 140

Asp Thr Ile Ser Ser Tyr Phe Met Asn Tyr Asn Ser Ser Val Ser Phe
145 150 155 160

Glu Glu Arg Leu Asn Asn Ile Thr Met Trp Leu Asn Asn Ser Asn Pro
165 170 175

Pro Val Thr Phe Ala Thr Leu Tyr Trp Glu Glu Pro Asp Ala Ser Gly
180 185 190

His Lys Tyr Gly Pro Glu Asp Lys Glu Asn Met Ser Arg Val Leu Lys
195 200 205

Lys Ile Asp Asp Leu Ile Gly Asp Leu Val Gln Arg Leu Lys Met Leu
210 215 220

Gly Leu Trp Glu Asn Leu Asn Val Ile Ile Thr Ser Asp His Gly Met
225 230 235 240

Thr Gln Cys Ser Gln Asp Arg Leu Ile Asn Leu Asp Ser Cys Ile Asp
245 250 255

His Ser Tyr Tyr Thr Leu Ile Asp Leu Ser Pro Val Ala Ala Ile Leu
260 265 270

Pro Lys Ile Asn Arg Thr Glu Val Tyr Asn Lys Leu Lys Asn Cys Ser
275 280 285

Pro His Met Asn Val Tyr Leu Lys Glu Asp Ile Pro Asn Arg Phe Tyr
290 295 300

Tyr Gln His Asn Asp Arg Ile Gln Pro Ile Ile Leu Val Ala Asp Glu
305 310 315 320

Gly Trp Thr Ile Val Leu Asn Glu Ser Ser Gln Lys Leu Gly Asp His
325 330 335

Gly Tyr Asp Asn Ser Leu Pro Ser Met His Pro Phe Leu Ala Ala His
340 345 350

Gly Pro Ala Phe His Lys Gly Tyr Lys His Ser Thr Ile Asn Ile Val
355 360 365

Asp Ile Tyr Pro Met Met Cys His Ile Leu Gly Leu Lys Pro His Pro
370 375 380

Asn Asn Gly Thr Phe Gly His Thr Lys Cys Leu Leu Val Asp Gln Trp
385 390 395 400

Cys Ile Asn Leu Pro Glu Ala Ile Ala Ile Val Ile Gly Ser Leu Leu
405 410 415

Val Leu Thr Met Leu Thr Cys Leu Ile Ile Ile Met Gln Asn Arg Leu
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Ser Val Pro Arg Pro Phe Ser Arg Leu Gln Leu Gln Glu Asp Asp Asp
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Asp Pro Leu Ile Gly
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<213> Homo sapiens

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<211> 155
<212> PRT
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Leu Cys Phe Arg Val Cys Glu Asn Ala Gly Val Asp His Cys Ala Gln
35 40 45

Gln Asp His Gly Cys Glu Gln Leu Cys Leu Asn Thr Glu Asp Ser Phe
50 55 60

Val Cys Gln Cys Ser Glu Gly Phe Leu Ile Asn Glu Asp Leu Lys Thr
65 70 75 80

Cys Ser Arg Val Asp Tyr Cys Leu Leu Ser Asp His Gly Cys Glu Tyr
85 90 95

Ser Cys Val Asn Met Asp Arg Ser Phe Ala Cys Gln Cys Pro Glu Gly
100 105 110

His Val Leu Arg Ser Asp Gly Lys Thr Cys Ala Lys Leu Asp Ser Cys
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<212> DNA
<213> Homo sapiens

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What is claimed is:

1. A composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 27 to nucleotide 1652;

(c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 129 to nucleotide 1652;

(d) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 1 to nucleotide 413;

(e) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone AM349_2 deposited under accession number ATCC 98155;

(f) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone AM349_2 deposited under accession number ATCC 98155;

(g) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AM349_2 deposited under accession number ATCC 98155;

(h) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AM349_2 deposited under accession number ATCC 98155;

(i) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:2;

(j) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:2 having biological activity;

(k) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(h) above;

(l) a polynucleotide which encodes a species homologue of the protein of (i) or (j) above ; and

(m) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(j).

2. A composition of claim 1 wherein said polynucleotide is operably linked to at least one expression control sequence.

3. A host cell transformed with a composition of claim 2.

4. The host cell of claim 3, wherein said cell is a mammalian cell.

5. A process for producing a protein encoded by a composition of claim 2, which process comprises:

(a) growing a culture of the host cell of claim 3 in a suitable culture medium; and

(b) purifying said protein from the culture.

6. A protein produced according to the process of claim 5.

7. The protein of claim 6 comprising a mature protein.

8. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:2;

(b) the amino acid sequence of SEQ ID NO:2 from amino acid 1 to amino acid 129;

(c) fragments of the amino acid sequence of SEQ ID NO:2; and

(d) the amino acid sequence encoded by the cDNA insert of clone AM349_2 deposited under accession number ATCC 98155;

the protein being substantially free from other mammalian proteins.

9. The composition of claim 8, wherein said protein comprises the amino acid sequence of SEQ ID NO:2.

10. The composition of claim 8, wherein said protein comprises the amino acid sequence of SEQ ID NO:2 from amino acid 1 to amino acid 129.

11. The composition of claim 8, further comprising a pharmaceutically acceptable carrier.

12. A method for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition of claim 11.

13. An isolated gene corresponding to the cDNA sequence of SEQ ID NO:1.

14. A composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 788 to nucleotide 1153;

(c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 1759 to nucleotide 2146;

(d) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone AR310_3 deposited under accession number ATCC 98155;

(e) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone AR310_3 deposited under accession number ATCC 98155;

(f) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AR310_3 deposited under accession number ATCC 98155;

(g) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AR310_3 deposited under accession number ATCC 98155;

(h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:4;

(i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:4 having biological activity;

(j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;

(k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above ; and

(l) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i).

15. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:4;

(b) fragments of the amino acid sequence of SEQ ID NO:4; and

(c) the amino acid sequence encoded by the cDNA insert of clone AR310_3 deposited under accession number ATCC 98155;

the protein being substantially free from other mammalian proteins.

16. An isolated gene corresponding to the cDNA sequence of SEQ ID NO:3.

17. A composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 448 to nucleotide 663;

(c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 547 to nucleotide 663;

(d) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 321 to nucleotide 603;

(e) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone AS186_3 deposited under accession number ATCC 98155;

(f) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone AS186_3 deposited under accession number ATCC 98155;

(g) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AS186_3 deposited under accession number ATCC 98155;

(h) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AS186_3 deposited under accession number ATCC 98155;

(i) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:6;

(j) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 having biological activity;

(k) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(h) above;

(l) a polynucleotide which encodes a species homologue of the protein of (i) or (j) above ; and

(m) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(j).

18. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:6;

(b) the amino acid sequence of SEQ ID NO:6 from amino acid 1 to amino acid 52;

(c) fragments of the amino acid sequence of SEQ ID NO:6; and

(d) the amino acid sequence encoded by the cDNA insert of clone AS186_3 deposited under accession number ATCC 98155;

the protein being substantially free from other mammalian proteins.

19. An isolated gene corresponding to the cDNA sequence of SEQ ID NO:5.

20. A composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 from nucleotide 140 to nucleotide 1498;

(c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 from nucleotide 185 to nucleotide 1498;

(d) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 from nucleotide 132 to nucleotide 457;

(e) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone AY160_2 deposited under accession number ATCC 98155;

(f) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone AY160_2 deposited under accession number ATCC 98155;

(g) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AY160_2 deposited under accession number ATCC 98155;

(h) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AY160_2 deposited under accession number ATCC 98155;

(i) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:8;

(j) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:8 having biological activity;

(k) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(h) above;

(l) a polynucleotide which encodes a species homologue of the protein of (i) or (j) above ; and

(m) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(j).

21. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:8;

(b) the amino acid sequence of SEQ ID NO:8 from amino acid 1 to amino acid 106;

(c) fragments of the amino acid sequence of SEQ ID NO:8; and

(d) the amino acid sequence encoded by the cDNA insert of clone AY160_2 deposited under accession number ATCC 98155;

the protein being substantially free from other mammalian proteins.

22. An isolated gene corresponding to the cDNA sequence of SEQ ID NO:7.

23. A composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 84 to nucleotide 548;

(c) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone BD127_16 deposited under accession number ATCC 98155;

(d) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone BD127_16 deposited under accession number ATCC 98155;

(e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone BD127_16 deposited under accession number ATCC 98155;

(f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone BD127_16 deposited under accession number ATCC 98155;

(g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:10;

(h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:10 having biological activity;

(i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(f) above;

(j) a polynucleotide which encodes a species homologue of the protein of (g) or (h) above ; and

(k) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(h).

24. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:10;

(b) fragments of the amino acid sequence of SEQ ID NO:10; and

(c) the amino acid sequence encoded by the cDNA insert of clone BD127_16 deposited under accession number ATCC 98155;

the protein being substantially free from other mammalian proteins.

25. An isolated gene corresponding to the cDNA sequences of SEQ ID NO:9 and SEQ ID NO:11.

26. A composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:12;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:12 from nucleotide 236 to nucleotide 1480;

(c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:12 from nucleotide 450 to nucleotide 800;

(d) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone BL205_14 deposited under accession number ATCC 98155;

(e) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone BL205_14 deposited under accession number ATCC 98155;

(f) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone BL205_14 deposited under accession number ATCC 98155;

(g) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone BL205_14 deposited under accession number ATCC 98155;

(h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:13;

(i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:13 having biological activity;

(j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;

(k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above ; and

(l) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i).

27. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:13;

(b) the amino acid sequence of SEQ ID NO:13 from amino acid 89 to amino acid 188;

(c) fragments of the amino acid sequence of SEQ ID NO:13; and

(d) the amino acid sequence encoded by the cDNA insert of clone BL205_14 deposited under accession number ATCC 98155;

the protein being substantially free from other mammalian proteins.

28. An isolated gene corresponding to the cDNA sequence of SEQ ID NO:12.

29. A composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:14;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:14 from nucleotide 69 to nucleotide 371;

(c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:14 from nucleotide 109 to nucleotide 350;

(d) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone H438_1 deposited under accession number ATCC 98140;

(e) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone H438_1 deposited under accession number ATCC 98140;

(f) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone H438_1 deposited under accession number ATCC 98140;

(g) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone H438_1 deposited under accession number ATCC 98140;

(h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:15;

(i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:15 having biological activity;

(j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;

(k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above ; and

(l) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i).

30. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:15;

(b) the amino acid sequence of SEQ ID NO:15 from amino acid 17 to amino acid 94;

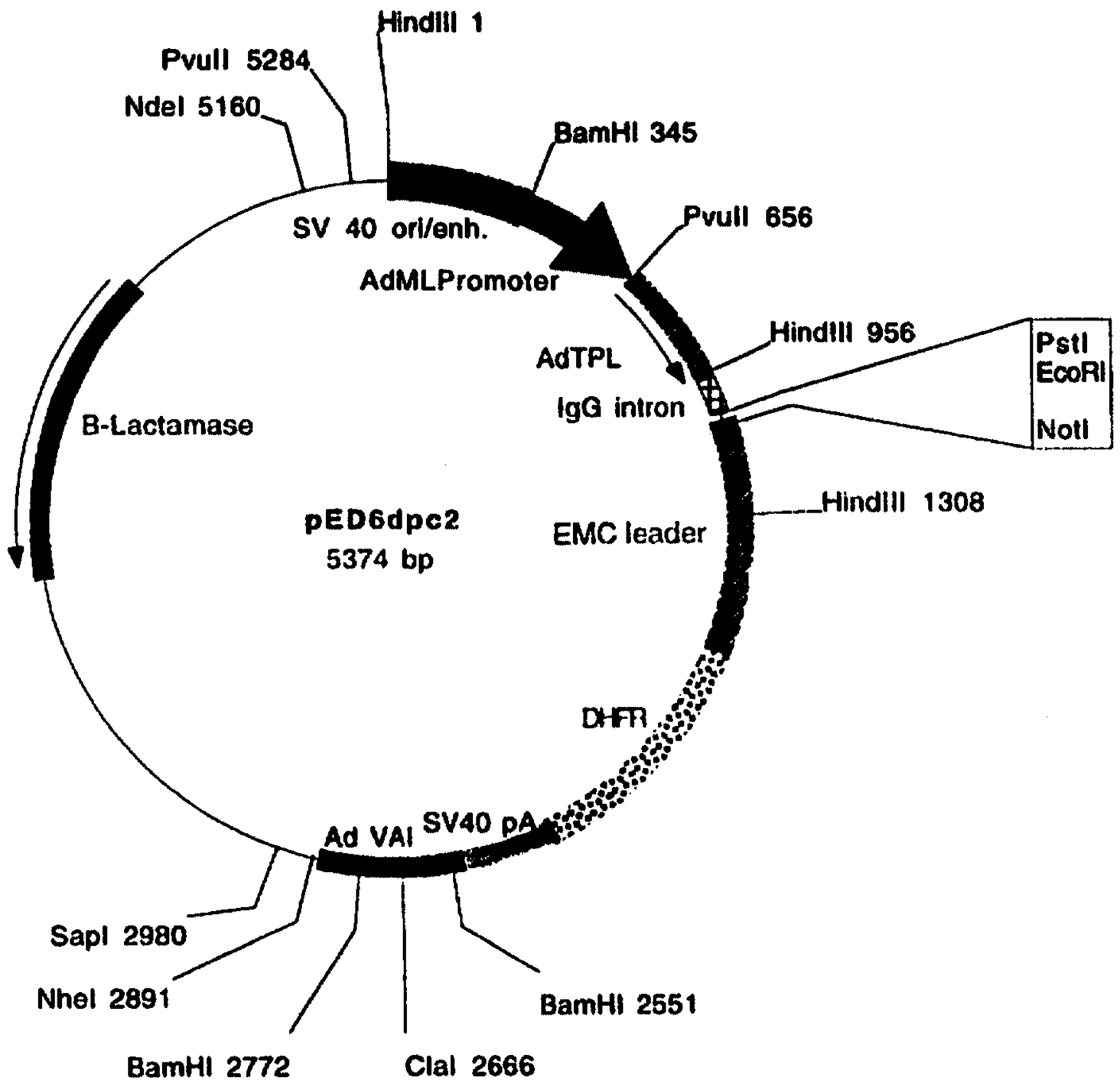
(c) fragments of the amino acid sequence of SEQ ID NO:15; and

(d) the amino acid sequence encoded by the cDNA insert of clone H438_1 deposited under accession number ATCC 98140;

the protein being substantially free from other mammalian proteins.

31. An isolated gene corresponding to the cDNA sequence of SEQ ID NO:14.

FIGURE 1A

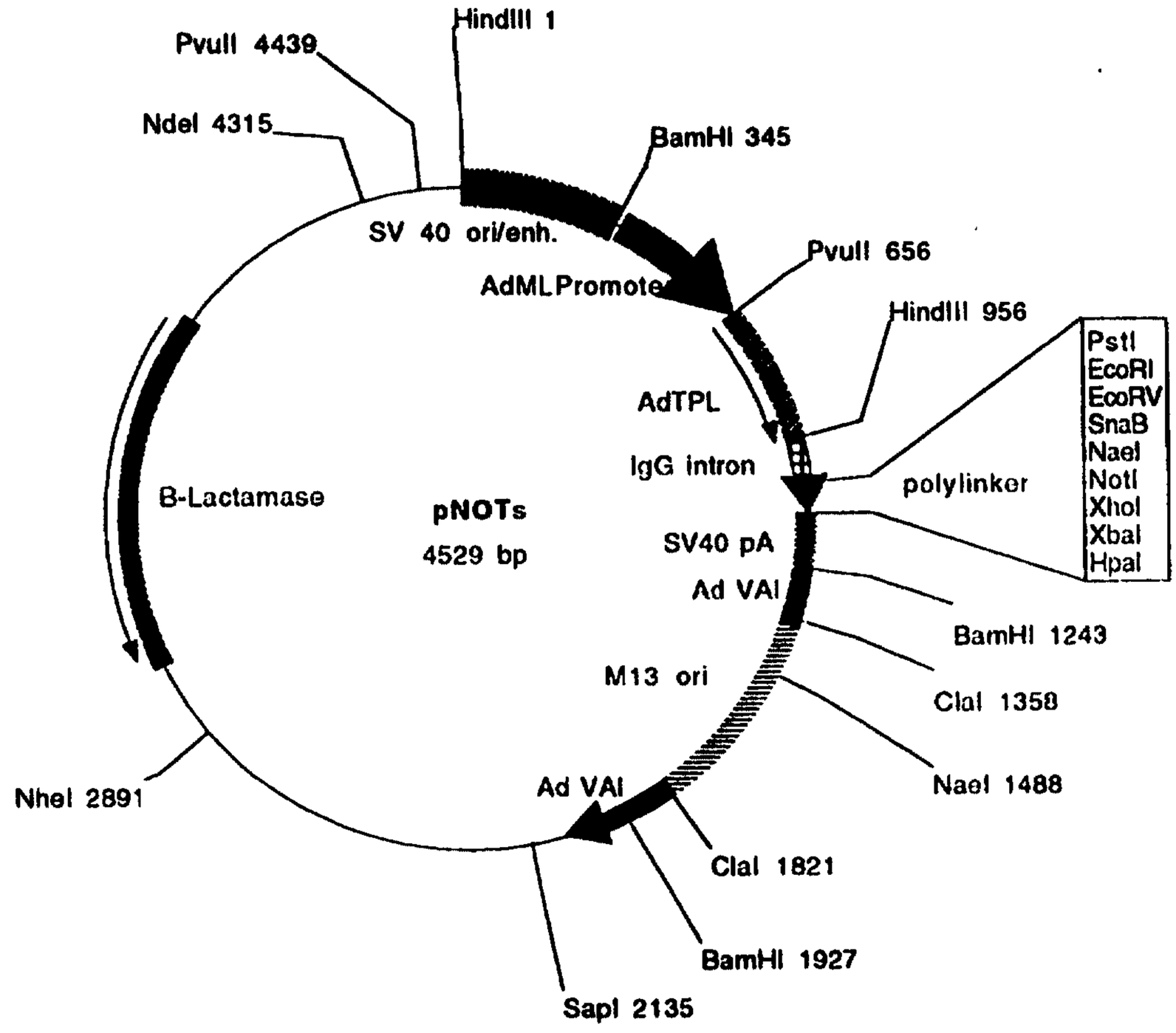


Plasmid name: pED6dpc2

Plasmid size: 5374 bp

Comments/References: pED6dpc2 is derived from pED6dpc1 by insertion of a new polylinker to facilitate cDNA cloning. SST cDNAs are cloned between EcoRI and NotI. pED vectors are described in Kaufman et al.(1991), NAR 19: 4485-4490.

FIGURE 1B



Plasmid name: pNOTs
Plasmid size: 4529 bp

Comments/References: pNOTs is a derivative of pMT2 (Kaufman et al,1989. Mol.Cell.Biol.9:1741-1750). DHFR was deleted and a new polylinker was inserted between EcoRI and HpaI. M13 origin of replication was inserted in the ClaI site. SST cDNAs are cloned between EcoRI and NotI

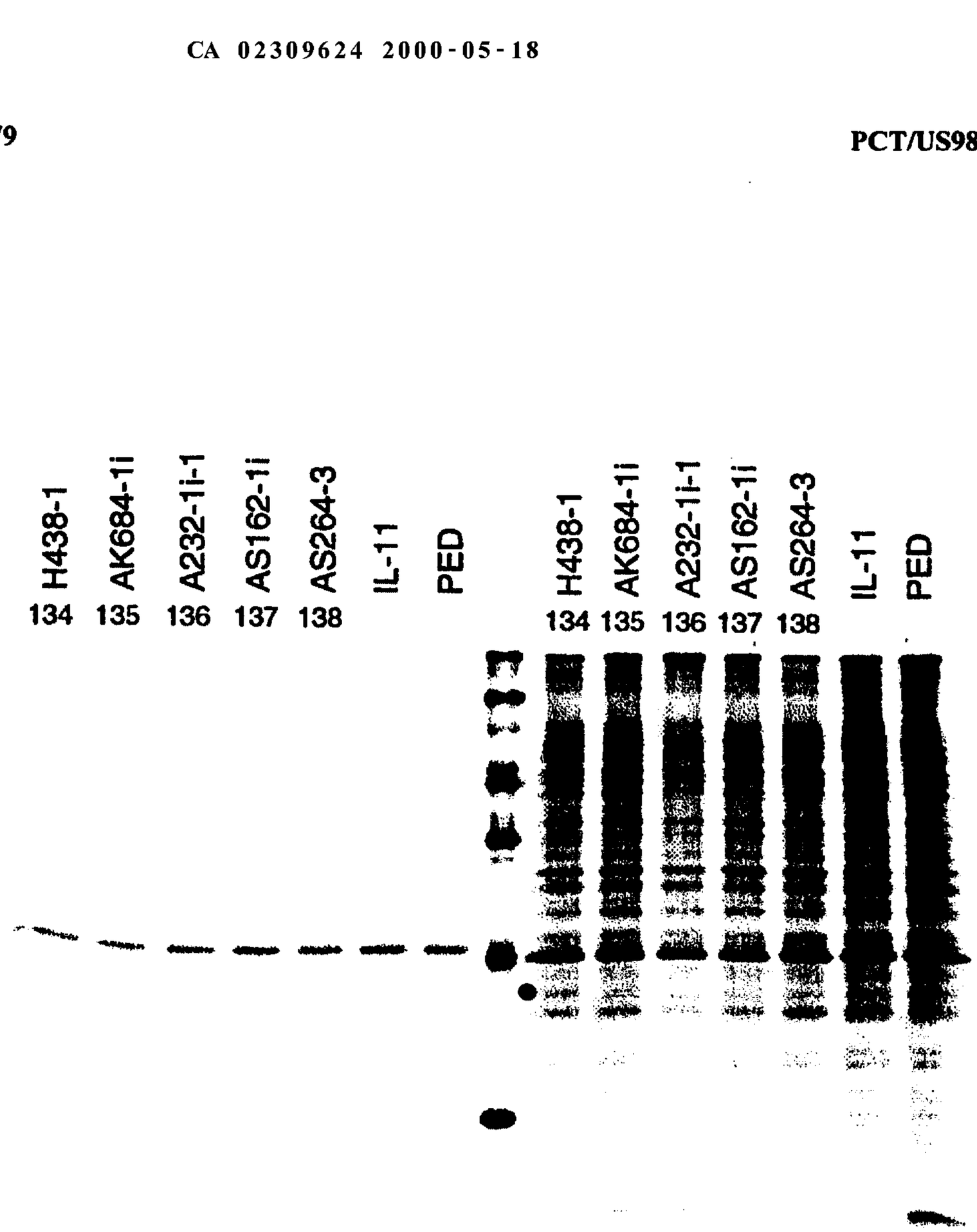

H438-1
AK684-1i
A232-1i-1
AS162-1i
AS264-3
IL-11
PED
134 135 136 137 138
H438-1
AK684-1i
A232-1i-1
AS162-1i
AS264-3
IL-11
PED
134 135 136 137 138

Fig. 2