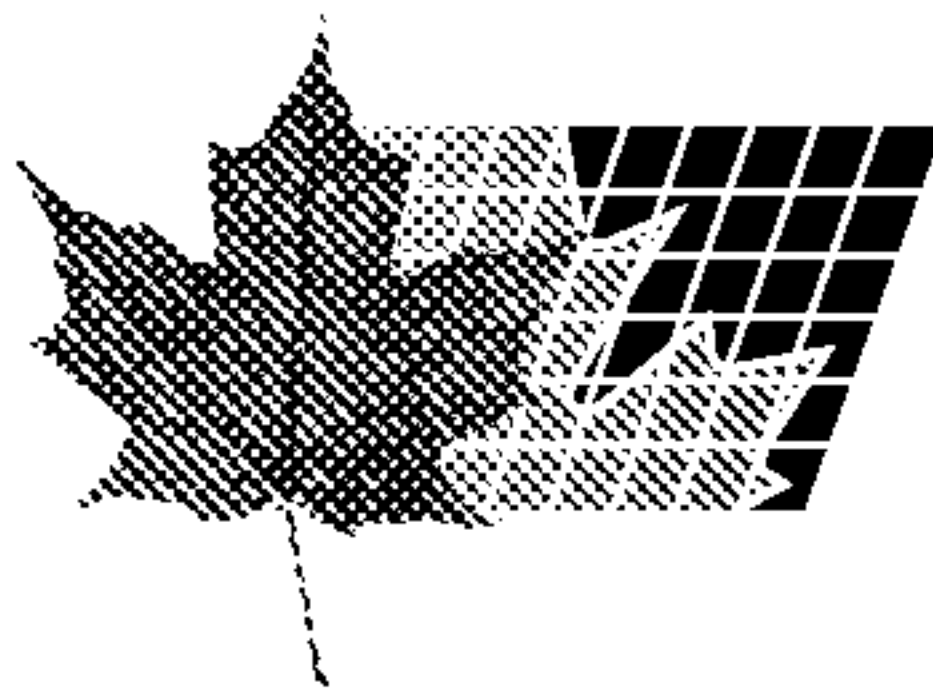




(72) HILLMAN, JENNIFER L., US

(72) YUE, HENRY, US

(72) TANG, Y. TOM, US

(72) CORLEY, NEIL C., US

(72) GUEGLER, KARL J., US

(72) PATTERSON, CHANDRA, US

(71) INCYTE PHARMACEUTICALS, INC., US

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(54) **MOLECULES ASSOCIEES A L'APOPTOSE**

(54) **MOLECULES ASSOCIATED WITH APOPTOSIS**

(57) La présente invention concerne des molécules humaines associées à l'apoptose ou "MAPOP" et des polynucléotides qui identifient et codent les MAPOP. L'invention concerne également des vecteurs d'expression, des cellules hôtes, des anticorps, des agonistes et des antagonistes. L'invention concerne enfin le diagnostic, le traitement et la prévention de troubles associés à l'expression des MAPOP.

(57) The invention provides human molecules associated with apoptosis (MAPOP) and polynucleotides which identify and encode MAPOP. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating or preventing disorders associated with expression of MAPOP.

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(21) International Application Number: PCT/US99/14188 (22) International Filing Date: 23 June 1999 (23.06.99) (30) Priority Data: 09/106,920 29 June 1998 (29.06.98) US (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 09/106,920 (CIP) Filed on 29 June 1998 (29.06.98) (71) Applicant (for all designated States except US): INCYTE PHARMACEUTICALS, INC. [US/US]; 3174 Porter Drive, Palo Alto, CA 94304 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): HILLMAN, Jennifer, L. [US/US]; 230 Monroe Drive #12, Mountain View, CA 94040 (US). YUE, Henry [US/US]; 826 Lois Avenue, Sunnyvale, CA 94087 (US). TANG, Y., Tom [CN/US]; 4230 Ranwick Court, San Jose, CA 95118 (US). CORLEY, Neil, C. [US/US]; 1240 Dale Avenue #30, Mountain View, CA 94040 (US). GUEGLER, Karl, J. [CH/US]; 1048 Oakland Avenue, Menlo Park, CA 94025 (US).		PATTERSON, Chandra [US/US]; 2189 Leland Avenue, Mountain View, CA 94040 (US). (74) Agents: BILLINGS, Lucy, J. et al.; Incyte Pharmaceuticals, Inc., 3174 Porter Drive, Palo Alto, CA 94304 (US). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>	

(54) Title: MOLECULES ASSOCIATED WITH APOPTOSIS

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1  MS - MAVETFGFFMATVGLL - - MLGVTLPNS 1456746
1  MANAGLQLLGFI LAF LGWIG AIVSTALPQ - 2057608
1  MS - MGLEITGTALAV LGWLG TIVCCALPM - GI 2570129

28 YWRVSTVHGN - VITNTNIFENLWFSCATDS 1456746
30 - WRIYSYAGDNIVTAQAMY EGLWMSCVS QS 2057608
29 - WRVSAFIGSNIITSQN IW EGLWMNCVV QS GI 2570129

57 LGVYN CWFPSMLALS GYIQACRALMITAI 1456746
59 TGQIQCKVFD SLLNLS STLQATRALMVVG I 2057608
58 TGQM QCKVYD SLLALPQDLQAARALIVVAI GI 2570129

87 LLGFLGLL LGIAGLRCTNIGGLELSRKAQA 1456746
89 LLGVIAIFVA TVGMKCMKCLEDDDEVQKMRM 2057608
88 LLAAFGLLVALVGAQCTNCVQDDTA - KAKI GI 2570129

117 GGHRRALHILAGICGMVAISWYAFNITRDF 1456746
119 AVIGGAIFFLAGLAILVA TAWYGNRIVQEF 2057608
117 TIVAGVLFLLAALLTLVPVSW SANTIIRDF GI 2570129

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(57) Abstract

The invention provides human molecules associated with apoptosis (MAPOP) and polynucleotides which identify and encode MAPOP. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating or preventing disorders associated with expression of MAPOP.

MOLECULES ASSOCIATED WITH APOPTOSIS

TECHNICAL FIELD

5 This invention relates to nucleic acid and amino acid sequences of molecules associated with apoptosis and to the use of these sequences in the diagnosis, treatment, and prevention of cell proliferative and autoimmune disorders.

BACKGROUND OF THE INVENTION

10 Apoptosis is the genetically controlled process by which unneeded or defective cells undergo programmed cell death. Apoptotic events are part of the normal developmental programs of many multicellular organisms. Selective elimination of cells is as important for morphogenesis and tissue remodeling as is cell proliferation and differentiation. Lack of apoptosis may result in hyperplasia and other disorders associated with increased cell proliferation. Apoptosis is also a
15 critical component of the immune response. Immune cells such as cytotoxic T-cells and natural killer cells prevent the spread of disease by inducing apoptosis in tumor cells and virus-infected cells. In addition, immune cells that fail to distinguish self molecules from foreign molecules must be eliminated by apoptosis to avoid an autoimmune response.

 Apoptotic cells undergo distinct morphological changes. Hallmarks of apoptosis include
20 cell shrinkage, nuclear and cytoplasmic condensation, and alterations in plasma membrane topology. Biochemically, apoptotic cells are characterized by increased intracellular calcium concentration, fragmentation of chromosomal DNA into nucleosomal-length units, and expression of novel cell surface components.

 The molecular mechanisms of apoptosis are highly conserved, and many of the key
25 protein regulators and effectors of apoptosis have been identified. Apoptosis generally proceeds in response to a signal which is transduced intracellularly and results in altered patterns of gene expression and protein activity. Signaling molecules such as hormones and cytokines are known to regulate apoptosis both positively and negatively through their interactions with cell surface receptors. Transcription factors also play an important role in the onset of apoptosis. A number
30 of downstream effector molecules, especially proteases, have been implicated in the degradation of cellular components and the proteolytic activation of other apoptotic effectors.

 The rat ventral prostate (RVP) is a model system for the study of hormone-regulated apoptosis. RVP epithelial cells undergo apoptosis in response to androgen deprivation. Messenger RNA (mRNA) transcripts that are up-regulated in the apoptotic RVP have been

identified. (Briehl, M. M. and Miesfeld, R. L. (1991) Mol. Endocrinol. 5:1381-1388.) One such transcript encodes RVP.1, the precise role of which in apoptosis has not been determined. The human homolog of RVP.1, hRVP1, is 89% identical to the rat protein. (Katahira, J. et al. (1997) J. Biol. Chem. 272:26652-26658.) hRVP1 is 220 amino acids in length and contains four
 5 transmembrane domains. hRVP1 is highly expressed in the lung, intestine, and liver. Interestingly, hRVP1 functions as a low affinity receptor for the Clostridium perfringens enterotoxin, a causative agent of diarrhea in humans and other animals.

Apoptosis also plays a critical role in the developing immune system and in the down-regulation of the immune response. Immature T-cells in the thymus are subjected to negative
 10 selection, a process that eliminates self-reactive T-cells which would otherwise mount an autoimmune response. Negative selection occurs through apoptotic mechanisms triggered by activation of the T-cell receptor (TCR) on the T-cell surface. A similar mechanism exists for the elimination of mature, antigen-activated T-cells when down-regulation of the immune response is required. This activation-induced apoptosis is mediated by another cell surface receptor, Fas,
 15 which is a member of the tumor necrosis factor receptor family. Fas expression is up-regulated in response to TCR stimulation and requires the activity of yet another gene, T cell death-associated gene 51 (TDAG51). (Park, C. G. et al. (1996) Immunity 4:583-591.) TDAG51 encodes a novel 261-amino acid protein.

The discovery of new human molecules associated with apoptosis and the polynucleotides
 20 encoding them satisfies a need in the art by providing new compositions which are useful in the diagnosis, treatment, and prevention of cell proliferative and autoimmune disorders.

SUMMARY OF THE INVENTION

The invention is based on the discovery of new molecules associated with apoptosis
 25 (MAPOP), the polynucleotides encoding MAPOP, and the use of these compositions for the diagnosis, treatment, or prevention of cell proliferative and autoimmune disorders.

The invention features substantially purified polypeptides, molecules associated with apoptosis, referred to collectively as "MAPOP" and individually as "MAPOP-1," "MAPOP-2," and "MAPOP-3." In one aspect, the invention provides a substantially purified polypeptide
 30 comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, a fragment of SEQ ID NO:1, a fragment of SEQ ID NO:2, and a fragment of SEQ ID NO:3.

The invention further provides a substantially purified variant having at least 90% amino acid identity to the amino acid sequence of SEQ ID NO:1-3, or to a fragment of any of these

sequences. The invention also provides an isolated and purified polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-3 and fragments thereof. The invention also includes an isolated and purified polynucleotide variant having at least 70% polynucleotide sequence identity to the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-3 and fragments thereof.

Additionally, the invention provides an isolated and purified polynucleotide which hybridizes under stringent conditions to the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-3 and fragments thereof, as well as an isolated and purified polynucleotide having a sequence which is complementary to the polynucleotide encoding the polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1-3 and fragments thereof.

The invention also provides an isolated and purified polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, a fragment of SEQ ID NO:4, a fragment of SEQ ID NO:5, and a fragment of SEQ ID NO:6. The invention further provides an isolated and purified polynucleotide variant having at least 70% polynucleotide sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:4-6 and fragments thereof, as well as an isolated and purified polynucleotide having a sequence which is complementary to a polynucleotide sequence selected from the group consisting of SEQ ID NO:4-6 and fragments thereof.

The invention further provides an expression vector containing at least a fragment of the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-3 and fragments thereof. In another aspect, the expression vector is contained within a host cell.

The invention also provides a method for producing a polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1-3 and fragments thereof, the method comprising the steps of: (a) culturing the host cell containing an expression vector containing at least a fragment of a polynucleotide encoding the polypeptide under conditions suitable for the expression of the polypeptide; and (b) recovering the polypeptide from the host cell culture.

The invention also provides a pharmaceutical composition comprising a substantially purified polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO:1-3 and fragments thereof, in conjunction with a suitable pharmaceutical carrier.

The invention further includes a purified antibody which binds to a polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1-3 and

fragments thereof, as well as a purified agonist and a purified antagonist to the polypeptide.

The invention also provides a method for treating or preventing a cell proliferative disorder, the method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising a substantially purified polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-3 and fragments thereof.

The invention also provides a method for treating or preventing an autoimmune disorder, the method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising a substantially purified polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-3 and fragments thereof.

The invention also provides a method for detecting a polynucleotide encoding the polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1-3 and fragments thereof in a biological sample containing nucleic acids, the method comprising the steps of: (a) hybridizing the complement of the polynucleotide sequence encoding the polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1-3 and fragments thereof to at least one of the nucleic acids of the biological sample, thereby forming a hybridization complex; and (b) detecting the hybridization complex, wherein the presence of the hybridization complex correlates with the presence of a polynucleotide encoding the polypeptide in the biological sample. In one aspect, the method further comprises amplifying the polynucleotide prior to hybridization.

BRIEF DESCRIPTION OF THE FIGURES AND TABLES

Figures 1A and 1B show the amino acid sequence alignments among MAPOP-1 (1456746; SEQ ID NO:1), MAPOP-2 (2057608; SEQ ID NO:2), and human hRVP1 (GI 2570129; SEQ ID NO:29). The alignments were produced using the multisequence alignment program of LASERGENE™ software (DNASTAR Inc, Madison WI).

Figure 2 shows the amino acid sequence alignment between MAPOP-3 (2840978; SEQ ID NO:3), and mouse TDAG51 (GI 1469400; SEQ ID NO:30).

Table 1 shows the programs, their descriptions, references, and threshold parameters (where appropriate) used to identify and characterize MAPOP.

DESCRIPTION OF THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular methodology, protocols, cell lines, vectors, and reagents described, as these may vary. It is also to be understood that the terminology

used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, vectors, and methodologies which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

DEFINITIONS

"MAPOP," as used herein, refers to the amino acid sequences, or variant thereof, of substantially purified MAPOP obtained from any species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and preferably the human species, from any source, whether natural, synthetic, semi-synthetic, or recombinant.

The term "agonist," as used herein, refers to a molecule which, when bound to MAPOP, increases or prolongs the duration of the effect of MAPOP. Agonists may include proteins, nucleic acids, carbohydrates, or any other molecules which bind to and modulate the effect of MAPOP.

An "allelic variant," as this term is used herein, is an alternative form of the gene encoding MAPOP. Allelic variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. Any given natural or recombinant gene may have none, one, or many allelic forms. Common mutational changes which give rise to allelic variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

"Altered" nucleic acid sequences encoding MAPOP, as described herein, include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a

polynucleotide the same as MAPOP or a polypeptide with at least one functional characteristic of MAPOP. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding MAPOP, and improper or unexpected hybridization to allelic variants, with a locus other than the normal
5 chromosomal locus for the polynucleotide sequence encoding MAPOP. The encoded protein may also be "altered," and may contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent MAPOP. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the
10 biological or immunological activity of MAPOP is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid, positively charged amino acids may include lysine and arginine, and amino acids with uncharged polar head groups having similar hydrophilicity values may include leucine, isoleucine, and valine; glycine and alanine; asparagine and glutamine; serine and threonine; and phenylalanine and tyrosine.

15 The terms "amino acid" or "amino acid sequence," as used herein, refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. In this context, "fragments," "immunogenic fragments," or "antigenic fragments" refer to fragments of MAPOP which are preferably at least 5 to about 15 amino acids in length, most preferably at least 14 amino acids, and which retain some
20 biological activity or immunological activity of MAPOP. Where "amino acid sequence" is recited herein to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

"Amplification," as used herein, relates to the production of additional copies of a nucleic
25 acid sequence. Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art. (See, e.g., Dieffenbach, C.W. and G.S. Dveksler (1995) PCR Primer, a Laboratory Manual, Cold Spring Harbor Press, Plainview, NY, pp.1-5.)

The term "antagonist," as it is used herein, refers to a molecule which, when bound to MAPOP, decreases the amount or the duration of the effect of the biological or immunological
30 activity of MAPOP. Antagonists may include proteins, nucleic acids, carbohydrates, antibodies, or any other molecules which decrease the effect of MAPOP.

As used herein, the term "antibody" refers to intact molecules as well as to fragments thereof, such as Fab, F(ab')₂, and Fv fragments, which are capable of binding the epitopic determinant. Antibodies that bind MAPOP polypeptides can be prepared using intact polypeptides
35 or using fragments containing small peptides of interest as the immunizing antigen. The

polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is
5 then used to immunize the animal.

The term "antigenic determinant," as used herein, refers to that fragment of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to antigenic determinants (given regions or three-
10 dimensional structures on the protein). An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The term "antisense," as used herein, refers to any composition containing a nucleic acid sequence which is complementary to the "sense" strand of a specific nucleic acid sequence. Antisense molecules may be produced by any method including synthesis or transcription. Once
15 introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form duplexes and to block either transcription or translation. The designation "negative" can refer to the antisense strand, and the designation "positive" can refer to the sense strand.

As used herein, the term "biologically active," refers to a protein having structural,
20 regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic MAPOP, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The terms "complementary" or "complementarity," as used herein, refer to the natural
25 binding of polynucleotides by base pairing. For example, the sequence "5' A-G-T 3'" binds to the complementary sequence "3' T-C-A 5'." Complementarity between two single-stranded molecules may be "partial," such that only some of the nucleic acids bind, or it may be "complete," such that total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength
30 of the hybridization between the nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acids strands, and in the design and use of peptide nucleic acid (PNA) molecules.

A "composition comprising a given polynucleotide sequence" or a "composition comprising a given amino acid sequence," as these terms are used herein, refer broadly to any
35 composition containing the given polynucleotide or amino acid sequence. The composition may

comprise a dry formulation or an aqueous solution. Compositions comprising polynucleotide sequences encoding MAPOP or fragments of MAPOP may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing
5 salts, e.g., NaCl, detergents, e.g., sodium dodecyl sulfate (SDS), and other components, e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.

"Consensus sequence," as used herein, refers to a nucleic acid sequence which has been resequenced to resolve uncalled bases, extended using XL-PCR™ kit (The Perkin-Elmer Corp., Norwalk, CT) in the 5' and/or the 3' direction, and resequenced, or which has been assembled from
10 the overlapping sequences of more than one Incyte Clone using a computer program for fragment assembly, such as the GELVIEW™ Fragment Assembly system (GCG, Madison, WI). Some sequences have been both extended and assembled to produce the consensus sequence.

As used herein, the term "correlates with expression of a polynucleotide" indicates that the detection of the presence of nucleic acids, the same or related to a nucleic acid sequence encoding
15 MAPOP, by Northern analysis is indicative of the presence of nucleic acids encoding MAPOP in a sample, and thereby correlates with expression of the transcript from the polynucleotide encoding MAPOP.

A "deletion," as the term is used herein, refers to a change in the amino acid or nucleotide sequence that results in the absence of one or more amino acid residues or nucleotides.

20 The term "derivative," as used herein, refers to the chemical modification of a polypeptide sequence, or a polynucleotide sequence. Chemical modifications of a polynucleotide sequence can include, for example, replacement of hydrogen by an alkyl, acyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains at least one biological or immunological function of the natural molecule. A derivative polypeptide is one modified by
25 glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

The term "similarity," as used herein, refers to a degree of complementarity. There may be partial similarity or complete similarity. The word "identity" may substitute for the word "similarity." A partially complementary sequence that at least partially inhibits an identical
30 sequence from hybridizing to a target nucleic acid is referred to as "substantially similar." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or Northern blot, solution hybridization, and the like) under conditions of reduced stringency. A substantially similar sequence or hybridization probe will compete for and inhibit the binding of a completely similar (identical) sequence to the
35 target sequence under conditions of reduced stringency. This is not to say that conditions of

reduced stringency are such that non-specific binding is permitted, as reduced stringency conditions require that the binding of two sequences to one another be a specific (i.e., a selective) interaction. The absence of non-specific binding may be tested by the use of a second target sequence which lacks even a partial degree of complementarity (e.g., less than about 30% similarity or identity). In the absence of non-specific binding, the substantially similar sequence or probe will not hybridize to the second non-complementary target sequence.

The phrases "percent identity" or "% identity" refer to the percentage of sequence similarity found in a comparison of two or more amino acid or nucleic acid sequences. Percent identity can be determined electronically, e.g., by using the MegAlign™ program (DNASTAR, Inc., Madison WI). The MegAlign™ program can create alignments between two or more sequences according to different methods, e.g., the clustal method. (See, e.g., Higgins, D.G. and P.M. Sharp (1988) Gene 73:237-244.) The clustal algorithm groups sequences into clusters by examining the distances between all pairs. The clusters are aligned pairwise and then in groups. The percentage similarity between two amino acid sequences, e.g., sequence A and sequence B, is calculated by dividing the length of sequence A, minus the number of gap residues in sequence A, minus the number of gap residues in sequence B, into the sum of the residue matches between sequence A and sequence B, times one hundred. Gaps of low or of no similarity between the two amino acid sequences are not included in determining percentage similarity. Percent identity between nucleic acid sequences can also be counted or calculated by other methods known in the art, e.g., the Jotun Hein method. (See, e.g., Hein, J. (1990) Methods Enzymol. 183:626-645.) Identity between sequences can also be determined by other methods known in the art, e.g., by varying hybridization conditions.

"Human artificial chromosomes" (HACs), as described herein, are linear microchromosomes which may contain DNA sequences of about 6 kb to 10 Mb in size, and which contain all of the elements required for stable mitotic chromosome segregation and maintenance. (See, e.g., Harrington, J.J. et al. (1997) Nat Genet. 15:345-355.)

The term "humanized antibody," as used herein, refers to antibody molecules in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

"Hybridization," as the term is used herein, refers to any process by which a strand of nucleic acid binds with a complementary strand through base pairing.

As used herein, the term "hybridization complex" refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed in solution (e.g., C₀t or R₀t analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence

immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

The words "insertion" or "addition," as used herein, refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively, to the sequence found in the naturally occurring molecule.

"Immune response" can refer to conditions associated with inflammation, trauma, immune disorders, or infectious or genetic disease, etc. These conditions can be characterized by expression of various factors, e.g., cytokines, chemokines, and other signaling molecules, which may affect cellular and systemic defense systems.

The term "microarray," as used herein, refers to an arrangement of distinct polynucleotides arrayed on a substrate, e.g., paper, nylon or any other type of membrane, filter, chip, glass slide, or any other suitable solid support.

The terms "element" or "array element" as used herein in a microarray context, refer to hybridizable polynucleotides arranged on the surface of a substrate.

The term "modulate," as it appears herein, refers to a change in the activity of MAPOP. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of MAPOP.

The phrases "nucleic acid" or "nucleic acid sequence," as used herein, refer to a nucleotide, oligonucleotide, polynucleotide, or any fragment thereof. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material. In this context, "fragments" refers to those nucleic acid sequences which, when translated, would produce polypeptides retaining some functional characteristic, e.g., antigenicity, or structural domain characteristic, e.g., ATP-binding site, of the full-length polypeptide.

The terms "operably associated" or "operably linked," as used herein, refer to functionally related nucleic acid sequences. A promoter is operably associated or operably linked with a coding sequence if the promoter controls the translation of the encoded polypeptide. While operably associated or operably linked nucleic acid sequences can be contiguous and in the same reading frame, certain genetic elements, e.g., repressor genes, are not contiguously linked to the sequence encoding the polypeptide but still bind to operator sequences that control expression of the polypeptide.

The term "oligonucleotide," as used herein, refers to a nucleic acid sequence of at least about 6 nucleotides to 60 nucleotides, preferably about 15 to 30 nucleotides, and most preferably about 20 to 25 nucleotides, which can be used in PCR amplification or in a hybridization assay or

microarray. As used herein, the term "oligonucleotide" is substantially equivalent to the terms "amplimer," "primer," "oligomer," and "probe," as these terms are commonly defined in the art.

"Peptide nucleic acid" (PNA), as used herein, refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers solubility to the composition. PNAs preferentially bind complementary single stranded DNA or RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell. (See, e.g., Nielsen, P.E. et al. (1993) Anticancer Drug Des. 8:53-63.)

The term "sample," as used herein, is used in its broadest sense. A biological sample suspected of containing nucleic acids encoding MAPOP, or fragments thereof, or MAPOP itself, may comprise a bodily fluid; an extract from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or cDNA, in solution or bound to a solid support; a tissue; a tissue print; etc.

As used herein, the terms "specific binding" or "specifically binding" refer to that interaction between a protein or peptide and an agonist, an antibody, or an antagonist. The interaction is dependent upon the presence of a particular structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding molecule. For example, if an antibody is specific for epitope "A," the presence of a polypeptide containing the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

As used herein, the term "stringent conditions" refers to conditions which permit hybridization between polynucleotides and the claimed polynucleotides. Stringent conditions can be defined by salt concentration, the concentration of organic solvent, e.g., formamide, temperature, and other conditions well known in the art. In particular, stringency can be increased by reducing the concentration of salt, increasing the concentration of formamide, or raising the hybridization temperature.

The term "substantially purified," as used herein, refers to nucleic acid or amino acid sequences that are removed from their natural environment and are isolated or separated, and are at least about 60% free, preferably about 75% free, and most preferably about 90% free from other components with which they are naturally associated.

A "substitution," as used herein, refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

"Transformation," as defined herein, describes a process by which exogenous DNA enters and changes a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the

insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed" cells includes stably transformed cells in which the
5 inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome, as well as transiently transformed cells which express the inserted DNA or RNA for limited periods of time.

A "variant" of MAPOP polypeptides, as used herein, refers to an amino acid sequence that is altered by one or more amino acid residues. The variant may have "conservative" changes,
10 wherein a substituted amino acid has similar structural or chemical properties (e.g., replacement of leucine with isoleucine). More rarely, a variant may have "nonconservative" changes (e.g., replacement of glycine with tryptophan). Analogous minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing biological or immunological activity may be
15 found using computer programs well known in the art, for example, LASERGENE™ software (DNASTAR Inc).

The term "variant," when used in the context of a polynucleotide sequence, may encompass a polynucleotide sequence related to MAPOP. This definition may also include, for example, "allelic" (as defined above), "splice," "species," or "polymorphic" variants. A splice
20 variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternate splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or an absence of domains. Species variants are polynucleotide sequences that vary from one species to another. The resulting polypeptides generally will have significant amino acid identity relative to each other. A
25 polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs) in which the polynucleotide sequence varies by one base. The presence of SNPs may be indicative of, for example, a certain population, a disease state, or a propensity for a disease state.

30

THE INVENTION

The invention is based on the discovery of new human molecules associated with apoptosis (MAPOP), the polynucleotides encoding MAPOP, and the use of these compositions for the diagnosis, treatment, or prevention of cell proliferative and autoimmune disorders.

Nucleic acids encoding the MAPOP-1 of the present invention were first identified in Incyte Clone 1456746 from the fetal colon cDNA library (COLNFET02) using a computer search, e.g., BLAST, for amino acid sequence alignments. A consensus sequence, SEQ ID NO:4, was derived from the following overlapping and/or extended nucleic acid sequences (SEQ ID NO:7-
 5 11): Incyte Clones 1456746H1 (COLNFET02), 1512130H1 (LUNGNOT14), 1456746T6 (COLNFET02), 2922036H1 (SININOT04), and 1430349F1 (SINTBST01).

In one embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:1. MAPOP-1 is 228 amino acids in length and has one potential N-glycosylation site at N141; four potential casein kinase II phosphorylation sites at T43, T154,
 10 S187, and S211; two potential protein kinase C phosphorylation sites at S111 and S194; and one potential tyrosine kinase phosphorylation site at Y151. A signal peptide sequence is predicted from M1 to P25. BLOCKS analysis indicates that the regions of MAPOP-1 from S2 to V31, from I44 to L57, from M68 to E109, and from L158 to C185 are similar to PMP-22/EMP/MP20 family motifs. Such motifs are characteristic of small integral membrane glycoproteins found in neural,
 15 tumor, and epithelial cells. In addition, PRINTS analysis indicates that the regions of MAPOP-1 from T7 to S32, from Y74 to I105, from H124 to I142, and from L162 to C180 are similar to transmembrane domains in the rhesus blood group proteins. As shown in Figures 1A and 1B, MAPOP-1 has chemical and structural similarity with hRVP1 (GI 2570129; SEQ ID NO:29). In particular, MAPOP-1 and hRVP1 share 33% identity. Hydrophobicity plots and Hidden Markov
 20 Model analysis demonstrate that the four transmembrane domains of hRVP1 are well conserved in MAPOP-1 from about amino acid 1 to about amino acid 20; from about amino acid 80 to about amino acid 100; from about amino acid 125 to about amino acid 140; and from amino acid 160 to about amino acid 185. Northern analysis shows the expression of this sequence in various libraries, at least 65% of which are associated with cancer or proliferating cells and at least 29% of
 25 which are associated with the immune response or trauma. In particular, 35% of the libraries expressing MAPOP-1 are derived from gastrointestinal tissue and 29% are derived from reproductive tissue.

Nucleic acids encoding the MAPOP-2 of the present invention were first identified in Incyte Clone 2057608 from the bronchial epithelium cDNA library (BEPINOT01) using a
 30 computer search, e.g., BLAST, for amino acid sequence alignments. A consensus sequence, SEQ ID NO:5, was derived from the following overlapping and/or extended nucleic acid sequences (SEQ ID NO:12-22): Incyte Clones 2057608H1 (BEPINOT01), 821149H1 (KERANOT02), 2662526F6 (ADRENOT08), shotgun sequence SAEB01552R1, 2734032F6 (OVARTUT04), 2795896T6 (NPOLNOT01), 2696284F6 (UTRSNOT12), 2254004R6 (OVARTUT01),
 35 1433735R1 (BEPINON01), 1554306F6 (BLADTUT04), and 1554306T6 (BLADTUT04).

In one embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:2. MAPOP-2 is 211 amino acids in length and has one potential N-glycosylation site at N72; one potential cAMP- and cGMP-dependent protein kinase phosphorylation site at T191; one potential casein kinase II phosphorylation site at S206; and two potential protein kinase C phosphorylation sites at T195 and S206. Analysis of the MAPOP-2 sequence reveals similarities to motifs and sequence signatures also identified in MAPOP-1. A signal peptide sequence is predicted in MAPOP-2 from M1 to A21. BLOCKS analysis indicates that the regions of MAPOP-2 from N3 to I32, from M46 to T59, from L70 to D111, and from F161 to R188 are similar to PMP-22/EMP/MP20 family motifs as described above. In addition, PRINTS analysis indicates that the regions of MAPOP-2 from F11 to L27, from M84 to L108, from G123 to W139, and from S173 to C186 are similar to transmembrane domains in the rhesus blood group proteins. As shown in Figures 1A and 1B, MAPOP-2 has chemical and structural similarity with hRVP1 (GI 2570129; SEQ ID NO:29). In particular, MAPOP-2 and hRVP1 share 47% identity. Hydrophobicity plots and Hidden Markov Model analysis demonstrate that the four transmembrane domains of hRVP1 are well conserved in MAPOP-2 from about amino acid 1 to about amino acid 30; from about amino acid 80 to about amino acid 105; from about amino acid 115 to about amino acid 140; and from amino acid 160 to about amino acid 185. Northern analysis shows the expression of this sequence in various libraries, at least 75% of which are associated with cancer or proliferating cells and at least 29% of which are associated with the immune response or trauma. In particular, 29% of the libraries expressing MAPOP-2 are derived from reproductive tissue.

Nucleic acids encoding the MAPOP-3 of the present invention were first identified in Incyte Clone 2840978 from the dorsal root ganglion cDNA library (DRGLNOT01) using a computer search, e.g., BLAST, for amino acid sequence alignments. A consensus sequence, SEQ ID NO:6, was derived from the following overlapping and/or extended nucleic acid sequences (SEQ ID NO:23-28): Incyte Clones 2840978H1 (DRGLNOT01), 3415476H1 (PTHYNOT04), 2099593R6 (BRAITUT02), 1441568F1 (THYRNOT03), 893117R6 (STOMTUT01), and 1441568R1 (THYRNOT03).

In one embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:3. MAPOP-3 is 127 amino acids in length and has four potential protein kinase C phosphorylation sites at T19, T34, T67, and T113. Pfam analysis indicates that the region of MAPOP-3 from T8 to T67 is similar to a pleckstrin homology domain signature. This domain is found in a wide range of proteins involved in intracellular signal transduction, including membrane- and receptor-associated proteins. As shown in Figure 2, MAPOP-3 has chemical and structural similarity with TDAG51 (GI 1469400; SEQ ID NO:30). In particular, MAPOP-3 and

TDAG51 share 44% identity. Note that in Figure 2, only the first 155 amino acids of TDAG51 are shown in the alignment for reasons of clarity. However, the complete amino acid sequence of TDAG51 as shown in the Sequence Listing was included in the calculation of amino acid identity with MAPOP-3. Northern analysis shows the expression of this sequence in various libraries, at least 69% of which are associated with cancer or proliferating cells and at least 29% of which are associated with the immune response or trauma. In particular, 25% of the libraries expressing MAPOP-3 are derived from reproductive tissue and 23% from neural tissue.

The invention also encompasses MAPOP variants. A preferred MAPOP variant is one which has at least about 80%, more preferably at least about 90%, and most preferably at least about 95% amino acid sequence identity to the MAPOP amino acid sequence, and which contains at least one functional or structural characteristic of MAPOP.

The invention also encompasses polynucleotides which encode MAPOP. In a particular embodiment, the invention encompasses a polynucleotide sequence comprising the sequence of SEQ ID NO:4, which encodes MAPOP-1. In a further embodiment, the invention encompasses the polynucleotide sequence comprising the sequence of SEQ ID NO:5, which encodes MAPOP-2. In still another embodiment, the invention encompasses the polynucleotide sequence comprising the sequence of SEQ ID NO:6, which encodes MAPOP-3.

The invention also encompasses a variant of a polynucleotide sequence encoding MAPOP. In particular, such a variant polynucleotide sequence will have at least about 70%, more preferably at least about 85%, and most preferably at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding MAPOP, selected from the group consisting of SEQ ID NO:4-6. Any one of the polynucleotide variants described above can encode an amino acid sequence which contains at least one functional or structural characteristic of MAPOP.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding MAPOP, some bearing minimal similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequence of naturally occurring MAPOP, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode MAPOP and its variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring MAPOP under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding MAPOP possessing a substantially different codon usage, e.g., inclusion of

non-naturally occurring codons. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding MAPOP and its derivatives without altering the
5 encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode MAPOP and MAPOP derivatives, or fragments thereof, entirely by synthetic chemistry. After production,
10 the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding MAPOP or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID
15 NO:4-6 and fragments thereof, under various conditions of stringency. (See, e.g., Wahl, G.M. and S.L. Berger (1987) *Methods Enzymol.* 152:399-407; Kimmel, A.R. (1987) *Methods Enzymol.* 152:507-511.) For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and most preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low
20 stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and most preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30°C, more preferably of at least about 37°C, and most preferably of at least about 42°C. Varying additional parameters, such as hybridization time,
25 the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred embodiment, hybridization will occur at 30°C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37°C in 500 mM NaCl, 50 mM trisodium
30 citrate, 1% SDS, 35% formamide, and 100 µg/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42°C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50 % formamide, and 200 µg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

The washing steps which follow hybridization can also vary in stringency. Wash
35 stringency conditions can be defined by salt concentration and by temperature. As above, wash

- stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include
- 5 temperature of at least about 25°C, more preferably of at least about 42°C, and most preferably of at least about 68°C. In a preferred embodiment, wash steps will occur at 25°C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a most preferred embodiment, wash steps will occur at 68°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS.
- 10 Additional variations on these conditions will be readily apparent to those skilled in the art.

Methods for DNA sequencing and analysis are well known in the art. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE® (Amersham Pharmacia Biotech Ltd., Uppsala, Sweden), Taq polymerase (Perkin-Elmer), thermostable T7 polymerase (Amersham Pharmacia Biotech), or combinations of polymerases and proofreading

15 exonucleases, such as those found in the ELONGASE™ amplification system (Life Technologies, Inc., Rockville, MD). Preferably, sequence preparation is automated with machines, e.g., the ABI CATALYST™ 800 (Perkin-Elmer) or MICROLAB® 2200 (Hamilton Co., Reno, NV) systems, in combination with thermal cyclers. Sequencing can also be automated, such as by ABI PRISM™ 373 or 377 systems (Perkin-Elmer) or the MEGABACE™ 1000 capillary electrophoresis system

20 (Molecular Dynamics, Inc., Sunnyvale, CA). Sequences can be analyzed using computer programs and algorithms well known in the art. (See, e.g., Ausubel (1997), supra, unit 7.7; and Meyers, R.A. (1995) Molecular Biology and Biotechnology, Wiley VCH, Inc, New York, NY.)

The nucleic acid sequences encoding MAPOP may be extended utilizing a partial nucleotide sequence and employing various PCR-based methods known in the art to detect

25 upstream sequences, such as promoters and regulatory elements. For example, one method which may be employed, restriction-site PCR, uses universal and nested primers to amplify unknown sequence from genomic DNA within a cloning vector. (See, e.g., Sarkar, G. (1993) PCR Methods Applic. 2:318-322.) Another method, inverse PCR, uses primers that extend in divergent directions to amplify unknown sequence from a circularized template. The template is derived

30 from restriction fragments comprising a known genomic locus and surrounding sequences. (See, e.g., Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186.) A third method, capture PCR, involves PCR amplification of DNA fragments adjacent to known sequences in human and yeast artificial chromosome DNA. (See, e.g., Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119.) In this method, multiple restriction enzyme digestions and ligations may be used to insert an

35 engineered double-stranded sequence into a region of unknown sequence before performing PCR.

Other methods which may be used to retrieve unknown sequences are known in the art. (See, e.g., Parker, J.D. et al. (1991) Nucleic Acids Res. 19:3055-306). Additionally, one may use PCR, nested primers, and PromoterFinder™ libraries (Clontech, Palo Alto, CA) to walk genomic DNA. This procedure avoids the need to screen libraries and is useful in finding intron/exon junctions.

- 5 For all PCR-based methods, primers may be designed using commercially available software, such as OLIGO™ 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, MN) or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the template at temperatures of about 68°C to 72°C.

When screening for full-length cDNAs, it is preferable to use libraries that have been
10 size-selected to include larger cDNAs. In addition, random-primed libraries, which often include sequences containing the 5' regions of genes, are preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to
15 analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different nucleotide-specific, laser-stimulated fluorescent dyes, and a charge coupled device camera for detection of the emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate software (e.g., Genotyper™ and Sequence Navigator™, (Perkin-Elmer)), and
20 the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for sequencing small DNA fragments which may be present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode MAPOP may be cloned in recombinant DNA molecules that direct expression of
25 MAPOP, or fragments or functional equivalents thereof, in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and used to express MAPOP.

The nucleotide sequences of the present invention can be engineered using methods
30 generally known in the art in order to alter MAPOP-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create
35 new restriction sites, alter glycosylation patterns, change codon preference, produce splice

variants, and so forth.

In another embodiment, sequences encoding MAPOP may be synthesized, in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) Nucl. Acids Res. Symp. Ser. 215-223, and Horn, T. et al. (1980) Nucl. Acids Res. Symp. Ser. 225-232.)

5 Alternatively, MAPOP itself or a fragment thereof may be synthesized using chemical methods. For example, peptide synthesis can be performed using various solid-phase techniques. (See, e.g., Roberge, J.Y. et al. (1995) Science 269:202-204.) Automated synthesis may be achieved using the ABI 431A Peptide Synthesizer (Perkin-Elmer). Additionally, the amino acid sequence of MAPOP, or any part thereof, may be altered during direct synthesis and/or combined with
10 sequences from other proteins, or any part thereof, to produce a variant polypeptide.

The peptide may be substantially purified by preparative high performance liquid chromatography. (See, e.g., Chiez, R.M. and F.Z. Regnier (1990) Methods Enzymol. 182:392-421.) The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing. (See, e.g., Creighton, T. (1984) Proteins, Structures and Molecular Properties, WH
15 Freeman and Co., New York, NY.)

In order to express a biologically active MAPOP, the nucleotide sequences encoding MAPOP or derivatives thereof may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. These elements include regulatory sequences, such as
20 enhancers, constitutive and inducible promoters, and 5' and 3' untranslated regions in the vector and in polynucleotide sequences encoding MAPOP. Such elements may vary in their strength and specificity. Specific initiation signals may also be used to achieve more efficient translation of sequences encoding MAPOP. Such signals include the ATG initiation codon and adjacent sequences, e.g. the Kozak sequence. In cases where sequences encoding MAPOP and its initiation
25 codon and upstream regulatory sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including an in-frame ATG initiation codon should be provided by the vector. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic.
30 The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the particular host cell system used. (See, e.g., Scharf, D. et al. (1994) Results Probl. Cell Differ. 20:125-162.)

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding MAPOP and appropriate transcriptional and
35 translational control elements. These methods include in vitro recombinant DNA techniques,

synthetic techniques, and in vivo genetic recombination. (See, e.g., Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview, NY, ch. 4, 8, and 16-17; and Ausubel, F.M. et al. (1995, and periodic supplements) Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY, ch. 9, 13, and 16.)

5 A variety of expression vector/host systems may be utilized to contain and express sequences encoding MAPOP. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral expression vectors
10 (e.g., cauliflower mosaic virus (CaMV) or tobacco mosaic virus (TMV)) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems. The invention is not limited by the host cell employed.

In bacterial systems, a number of cloning and expression vectors may be selected depending upon the use intended for polynucleotide sequences encoding MAPOP. For example,
15 routine cloning, subcloning, and propagation of polynucleotide sequences encoding MAPOP can be achieved using a multifunctional E. coli vector such as pBluescript® plasmid (Stratagene) or pSport1™ plasmid (Life Technologies). Ligation of sequences encoding MAPOP into the vector's multiple cloning site disrupts the *lacZ* gene, allowing a colorimetric screening procedure for identification of transformed bacteria containing recombinant molecules. In addition, these
20 vectors may be useful for in vitro transcription, dideoxy sequencing, single strand rescue with helper phage, and creation of nested deletions in the cloned sequence. (See, e.g., Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509.) When large quantities of MAPOP are needed, e.g. for the production of antibodies, vectors which direct high level expression of MAPOP may be used. For example, vectors containing the strong, inducible T5 or T7
25 bacteriophage promoter may be used.

Yeast expression systems may be used for production of MAPOP. A number of vectors containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH, may be used in the yeast Saccharomyces cerevisiae or Pichia pastoris. In addition, such vectors direct either the secretion or intracellular retention of expressed proteins and enable integration of
30 foreign sequences into the host genome for stable propagation. (See, e.g., Ausubel, supra; and Grant et al. (1987) Methods Enzymol. 153:516-54; Scorer, C. A. et al. (1994) Bio/Technology 12:181-184.)

Plant systems may also be used for expression of MAPOP. Transcription of sequences encoding MAPOP may be driven viral promoters, e.g., the 35S and 19S promoters of CaMV used
35 alone or in combination with the omega leader sequence from TMV. (Takamatsu, N. (1987)

EMBO J. 6:307-311.) Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used. (See, e.g., Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105.) These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. (See, e.g., Hobbs, S. or Murry, L.E. in McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York, NY; pp. 191-196.)

In mammalian cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding MAPOP may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain infective virus which expresses MAPOP in host cells. (See, e.g., Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells. SV40 or EBV-based vectors may also be used for high-level protein expression.

Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained in and expressed from a plasmid. HACs of about 6 kb to 10 Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes.

For long term production of recombinant proteins in mammalian systems, stable expression of MAPOP in cell lines is preferred. For example, sequences encoding MAPOP can be transformed into cell lines using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media before being switched to selective media. The purpose of the selectable marker is to confer resistance to a selective agent, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase and adenine phosphoribosyltransferase genes, for use in *tk* or *apr* cells, respectively. (See, e.g., Wigler, M. et al. (1977) Cell 11:223-232; and Lowy, I. et al. (1980) Cell 22:817-823.) Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate; *neo* confers resistance to the aminoglycosides neomycin and G-418; and *als* or *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase,

respectively. (See, e.g., Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. 77:3567-3570; Colbere-Garapin, F. et al (1981) J. Mol. Biol. 150:1-14; and Murry, supra.) Additional selectable genes have been described, e.g., *trpB* and *hisD*, which alter cellular requirements for metabolites. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. 85:8047-8051.) Visible
 5 markers, e.g., anthocyanins, green fluorescent proteins (GFP) (Clontech), β glucuronidase and its substrate β -D-glucuronoside, or luciferase and its substrate luciferin may be used. These markers can be used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, C.A. et al. (1995) Methods Mol. Biol. 55:121-131.)

10 Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding MAPOP is inserted within a marker gene sequence, transformed cells containing sequences encoding MAPOP can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence
 15 encoding MAPOP under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

In general, host cells that contain the nucleic acid sequence encoding MAPOP and that express MAPOP may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations, PCR
 20 amplification, and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

Immunological methods for detecting and measuring the expression of MAPOP using either specific polyclonal or monoclonal antibodies are known in the art. Examples of such
 25 techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on MAPOP is preferred, but a competitive binding assay may be employed. These and other assays are well known in the art. (See, e.g., Hampton, R. et al. (1990) Serological Methods, a Laboratory Manual, APS Press,
 30 St Paul, MN, Section IV; Coligan, J. E. et al. (1997 and periodic supplements) Current Protocols in Immunology, Greene Pub. Associates and Wiley-Interscience, New York, NY; and Maddox, D.E. et al. (1983) J. Exp. Med. 158:1211-1216).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled
 35 hybridization or PCR probes for detecting sequences related to polynucleotides encoding MAPOP

include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding MAPOP, or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits, such as those provided by Amersham Pharmacia Biotech, Promega (Madison, WI), and U.S. Biochemical Corp. (Cleveland, OH). Suitable reporter molecules or labels which may be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding MAPOP may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or retained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode MAPOP may be designed to contain signal sequences which direct secretion of MAPOP through a prokaryotic or eukaryotic cell membrane.

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to specify protein targeting, folding, and/or activity. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC, Bethesda, MD) and may be chosen to ensure the correct modification and processing of the foreign protein.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding MAPOP may be ligated to a heterologous sequence resulting in translation of a fusion protein in any of the aforementioned host systems. For example, a chimeric MAPOP protein containing a heterologous moiety that can be recognized by a commercially available antibody may facilitate the screening of peptide libraries for inhibitors of MAPOP activity. Heterologous protein and peptide moieties may also facilitate purification of fusion proteins using commercially available affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose binding protein (MBP), thioredoxin (Trx), calmodulin binding peptide (CBP), 6-His, FLAG, *c-myc*, and hemagglutinin (HA). GST, MBP, Trx, CBP, and 6-His enable purification of their cognate fusion proteins on immobilized glutathione, maltose,

phenylarsine oxide, calmodulin, and metal-chelate resins, respectively. FLAG, *c-myc*, and hemagglutinin (HA) enable immunoaffinity purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize these epitope tags. A fusion protein may also be engineered to contain a proteolytic cleavage site located between the
 5 MAPOP encoding sequence and the heterologous protein sequence, so that MAPOP may be cleaved away from the heterologous moiety following purification. Methods for fusion protein expression and purification are discussed in Ausubel, F. M. et al. (1995 and periodic supplements) Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY, ch 10. A variety of commercially available kits may also be used to facilitate expression and purification of fusion
 10 proteins.

In a further embodiment of the invention, synthesis of radiolabeled MAPOP may be achieved in vitro using the TNT™ rabbit reticulocyte lysate or wheat germ extract systems (Promega). These systems couple transcription and translation of protein-coding sequences operably associated with the T7, T3, or SP6 promoters. Translation takes place in the presence of
 15 a radiolabeled amino acid precursor, preferably ³⁵S-methionine.

Fragments of MAPOP may be produced not only by recombinant production, but also by direct peptide synthesis using solid-phase techniques. (See, e.g., Creighton, supra pp. 55-60.) Protein synthesis may be performed by manual techniques or by automation. Automated synthesis may be achieved, for example, using the Applied Biosystems 431A Peptide Synthesizer (Perkin-
 20 Elmer). Various fragments of MAPOP may be synthesized separately and then combined to produce the full length molecule.

THERAPEUTICS

Partial chemical and structural similarity, e.g., in the context of sequences and motifs,
 25 exists among regions of MAPOP-1, MAPOP-2, hRVP1, and transmembrane proteins. In addition, partial chemical and structural similarity exists among regions of MAPOP-3, TDAG51, and pleckstrin homology domains. Furthermore, MAPOP is expressed in tissues associated with cell proliferation and the immune system. Therefore, MAPOP appears to play a role in cell proliferative and autoimmune disorders.

30 Therefore, in one embodiment, MAPOP or a fragment or derivative thereof may be administered to a subject to treat or prevent a cell proliferative disorder. Such disorders can include, but are not limited to, actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including

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adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus.

5 In another embodiment, a vector capable of expressing MAPOP or a fragment or derivative thereof may be administered to a subject to treat or prevent a cell proliferative disorder including, but not limited to, those described above.

In a further embodiment, a pharmaceutical composition comprising a substantially purified MAPOP in conjunction with a suitable pharmaceutical carrier may be administered to a
10 subject to treat or prevent a cell proliferative disorder including, but not limited to, those provided above.

In still another embodiment, an agonist which modulates the activity of MAPOP may be administered to a subject to treat or prevent a cell proliferative disorder including, but not limited to, those listed above.

15 In another embodiment, MAPOP or a fragment or derivative thereof may be administered to a subject to treat or prevent an autoimmune disorder. Such disorders can include, but are not limited to, acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, bronchitis, cholecystitis, contact
20 dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis,
25 pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma.

30 In another embodiment, a vector capable of expressing MAPOP or a fragment or derivative thereof may be administered to a subject to treat or prevent an autoimmune disorder including, but not limited to, those described above.

In a further embodiment, a pharmaceutical composition comprising a substantially purified MAPOP in conjunction with a suitable pharmaceutical carrier may be administered to a
35 subject to treat or prevent an autoimmune disorder including, but not limited to, those provided

above.

In still another embodiment, an agonist which modulates the activity of MAPOP may be administered to a subject to treat or prevent an autoimmune disorder including, but not limited to, those listed above.

5 In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment
10 or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

An antagonist of MAPOP may be produced using methods which are generally known in the art. In particular, purified MAPOP may be used to produce antibodies or to screen libraries of
15 pharmaceutical agents to identify those which specifically bind MAPOP. Antibodies to MAPOP may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit dimer formation) are especially preferred for therapeutic use.

20 For the production of polyclonal antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with MAPOP or with any fragment or oligopeptide thereof which has immunogenic properties. Rats and mice are preferred hosts for downstream applications involving monoclonal antibody production. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants
25 include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable. (For review of methods for antibody production and analysis, see, e.g., Harlow, E. and Lane, D. (1988) Antibodies: A Laboratory
30 Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.)

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to MAPOP have an amino acid sequence consisting of at least about 5 amino acids, and, more preferably, of at least about 14 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein and
35 contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of

MAPOP amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to MAPOP may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but
5 are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (See, e.g., Kohler, G. et al. (1975) *Nature* 256:495-497; Kozbor, D. et al. (1985) *J. Immunol. Methods* 81:31-42; Cote, R.J. et al. (1983) *Proc. Natl. Acad. Sci.* 80:2026-2030; and Cole, S.P. et al. (1984) *Mol. Cell Biol.* 62:109-120.)

In addition, techniques developed for the production of "chimeric antibodies," such as the
10 splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. (See, e.g., Morrison, S.L. et al. (1984) *Proc. Natl. Acad. Sci.* 81:6851-6855; Neuberger, M.S. et al. (1984) *Nature* 312:604-608; and Takeda, S. et al. (1985) *Nature* 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce
15 MAPOP-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (See, e.g., Burton D.R. (1991) *Proc. Natl. Acad. Sci.* 88:10134-10137.)

Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents
20 as disclosed in the literature. (See, e.g., Orlandi, R. et al. (1989) *Proc. Natl. Acad. Sci.* 86: 3833-3837; and Winter, G. et al. (1991) *Nature* 349:293-299.)

Antibody fragments which contain specific binding sites for MAPOP may also be generated. For example, such fragments include, but are not limited to, F(ab')₂ fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing
25 the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse, W.D. et al. (1989) *Science* 246:1275-1281.)

Various immunoassays may be used for screening to identify antibodies having the desired specificity and minimal cross-reactivity. Numerous protocols for competitive binding or
30 immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between MAPOP and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering MAPOP epitopes is preferred, but a competitive binding assay may also be employed. (Maddox, supra.)

35 Various methods such as Scatchard analysis in conjunction with radioimmunoassay

techniques may be used to assess the affinity of antibodies for MAPOP. Affinity is expressed as an association constant, K_a , which is defined as the molar concentration of MAPOP-antibody complex divided by the molar concentrations of free antigen and free antibody under equilibrium conditions. The K_a determined for a preparation of polyclonal antibodies, which are
5 heterogeneous in their affinities for multiple MAPOP epitopes, represents the average affinity, or avidity, of the antibodies for MAPOP. The K_a determined for a preparation of monoclonal antibodies, which are monospecific for a particular MAPOP epitope, represents a true measure of affinity. High-affinity antibody preparations with K_a ranging from about 10^9 to 10^{12} L/mole are preferred for use in immunoassays in which the MAPOP-antibody complex must withstand
10 rigorous manipulations. Low-affinity antibody preparations with K_a ranging from about 10^6 to 10^7 L/mole are preferred for use in immunopurification and similar procedures which ultimately require dissociation of MAPOP, preferably in active form, from the antibody. (Catty, D. (1988) Antibodies. Volume I: A Practical Approach, IRL Press, Washington, D. C.; and Liddell, J. E. and Cryer, A. (1991) A Practical Guide to Monoclonal Antibodies, John Wiley & Sons, New York,
15 NY.)

The titre and avidity of polyclonal antibody preparations may be further evaluated to determine the quality and suitability of such preparations for certain downstream applications. For example, a polyclonal antibody preparation containing at least 1-2 mg specific antibody/ml, preferably 5-10 mg specific antibody/ml, is preferred for use in procedures requiring precipitation
20 of MAPOP-antibody complexes. Procedures for evaluating antibody specificity, titer, and avidity, and guidelines for antibody quality and usage in various applications, are generally available. (See, e.g., Catty, supra, and Coligan et al. supra.)

In another embodiment of the invention, the polynucleotides encoding MAPOP, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, the
25 complement of the polynucleotide encoding MAPOP may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding MAPOP. Thus, complementary molecules or fragments may be used to modulate MAPOP activity, or to achieve regulation of gene function. Such technology is now well known in the art, and sense or antisense oligonucleotides or larger
30 fragments can be designed from various locations along the coding or control regions of sequences encoding MAPOP.

Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. Methods which are well known to those skilled in the art
35 can be used to construct vectors to express nucleic acid sequences complementary to the

polynucleotides encoding MAPOP. (See, e.g., Sambrook, supra; and Ausubel, supra.)

Genes encoding MAPOP can be turned off by transforming a cell or tissue with expression vectors which express high levels of a polynucleotide, or fragment thereof, encoding MAPOP. Such constructs may be used to introduce untranslatable sense or antisense sequences
5 into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector, and may last even longer if appropriate replication elements are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by designing
10 complementary sequences or antisense molecules (DNA, RNA, or PNA) to the control, 5', or regulatory regions of the gene encoding MAPOP. Oligonucleotides derived from the transcription initiation site, e.g., between about positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using triple helix base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently
15 for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature. (See, e.g., Gee, J.E. et al. (1994) in Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing Co., Mt. Kisco, NY, pp. 163-177.) A complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

20 Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. For example, engineered hammerhead motif ribozyme molecules may specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding MAPOP.

25 Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, including the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides, corresponding to the region of the target gene containing the cleavage site, may be evaluated for secondary structural features which may render the oligonucleotide inoperable.
30 The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Complementary ribonucleic acid molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase
35 phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro

and in vivo transcription of DNA sequences encoding MAPOP. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize complementary RNA, constitutively or inducibly, can be introduced into cell lines, cells, or tissues.

5 RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as
10 inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into
15 stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved using methods which are well known in the art. (See, e.g., Goldman, C.K. et al. (1997) Nature Biotechnology 15:462-466.)

Any of the therapeutic methods described above may be applied to any subject in need of
20 such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

An additional embodiment of the invention relates to the administration of a pharmaceutical or sterile composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist
25 of MAPOP, antibodies to MAPOP, and mimetics, agonists, antagonists, or inhibitors of MAPOP. The compositions may be administered alone or in combination with at least one other agent, such as a stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents,
30 drugs, or hormones.

The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

35 In addition to the active ingredients, these pharmaceutical compositions may contain

suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, PA).

5 Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

10 Pharmaceutical preparations for oral use can be obtained through combining active compounds with solid excipient and processing the resultant mixture of granules (optionally, after grinding) to obtain tablets or dragee cores. Suitable auxiliaries can be added, if desired. Suitable excipients include carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, and sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as
15 methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums, including arabic and tragacanth; and proteins, such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, and alginic acid or a salt thereof, such as sodium alginate.

 Dragee cores may be used in conjunction with suitable coatings, such as concentrated
20 sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

 Pharmaceutical preparations which can be used orally include push-fit capsules made of
25 gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with fillers or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

30 Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be
35 prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include

fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate, triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents to increase the solubility of the compounds and allow for the preparation of highly concentrated solutions.

5 For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

10 The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, and succinic acid. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder which may contain any or all of the following: 1 mM to 50 mM histidine, 0.1% to 2%
15 sucrose, and 2% to 7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of MAPOP, such labeling would include amount, frequency, and method of administration.

20 Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

For any compound, the therapeutically effective dose can be estimated initially either in
25 cell culture assays, e.g., of neoplastic cells or in animal models such as mice, rats, rabbits, dogs, or pigs. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example
30 MAPOP or fragments thereof, antibodies of MAPOP, and agonists, antagonists or inhibitors of MAPOP, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating the ED₅₀ (the dose therapeutically effective in 50% of the population) or LD₅₀ (the dose lethal to 50% of the population) statistics. The dose ratio of therapeutic to toxic
35 effects is the therapeutic index, and it can be expressed as the ED₅₀/LD₅₀ ratio. Pharmaceutical

compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used to formulate a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that includes the ED₅₀ with little or no toxicity. The dosage varies within this range depending
5 upon the dosage form employed, the sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and
10 gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from about 0.1 μ g to 100,000 μ g, up to a total dose of
15 about 1 gram, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

20

DIAGNOSTICS

In another embodiment, antibodies which specifically bind MAPOP may be used for the diagnosis of disorders characterized by expression of MAPOP, or in assays to monitor patients being treated with MAPOP or agonists, antagonists, or inhibitors of MAPOP. Antibodies useful
25 for diagnostic purposes may be prepared in the same manner as described above for therapeutics. Diagnostic assays for MAPOP include methods which utilize the antibody and a label to detect MAPOP in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by covalent or non-covalent attachment of a reporter molecule. A wide variety of reporter molecules, several of which are described above, are known
30 in the art and may be used.

A variety of protocols for measuring MAPOP, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of MAPOP expression. Normal or standard values for MAPOP expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, preferably human, with antibody to

MAPOP under conditions suitable for complex formation. The amount of standard complex formation may be quantitated by various methods, preferably by photometric means. Quantities of MAPOP expressed in subject, control, and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the
5 parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding MAPOP may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantitate gene expression in biopsied tissues in which expression of MAPOP
10 may be correlated with disease. The diagnostic assay may be used to determine absence, presence, and excess expression of MAPOP, and to monitor regulation of MAPOP levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding MAPOP or closely related
15 molecules may be used to identify nucleic acid sequences which encode MAPOP. The specificity of the probe, whether it is made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification (maximal, high, intermediate, or low), will determine whether the probe identifies only naturally occurring sequences encoding MAPOP, allelic variants, or related sequences.

20 Probes may also be used for the detection of related sequences, and should preferably have at least 50% sequence identity to any of the MAPOP encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from the sequences of SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, or from genomic sequences including promoters, enhancers, and introns of the MAPOP gene.

25 Means for producing specific hybridization probes for DNAs encoding MAPOP include the cloning of polynucleotide sequences encoding MAPOP or MAPOP derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled
30 by a variety of reporter groups, for example, by radionuclides such as ^{32}P or ^{35}S , or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding MAPOP may be used for the diagnosis of a disorder associated with expression of MAPOP. Examples of such a disorder include, but are not limited
35 to, a cell proliferative disorder such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis,

cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall
 5 bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; and an autoimmune disorder such as acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis,
 10 bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis,
 15 pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma. The polynucleotide sequences encoding MAPOP may be used
 20 in Southern or Northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and ELISA assays; and in microarrays utilizing fluids or tissues from patients to detect altered MAPOP expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding MAPOP may be useful in assays
 25 that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding MAPOP may be labeled by standard methods and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantitated and compared with a standard value. If the amount of signal in the patient sample is significantly
 30 altered in comparison to a control sample then the presence of altered levels of nucleotide sequences encoding MAPOP in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor the treatment of an individual patient.

In order to provide a basis for the diagnosis of a disorder associated with expression of
 35 MAPOP, a normal or standard profile for expression is established. This may be accomplished by

combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding MAPOP, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with values from an experiment in which a known amount of a substantially
5 purified polynucleotide is used. Standard values obtained in this manner may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

Once the presence of a disorder is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to determine if the level of expression in
10 the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may
15 provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding
20 MAPOP may involve the use of PCR. These oligomers may be chemically synthesized, generated enzymatically, or produced in vitro. Oligomers will preferably contain a fragment of a polynucleotide encoding MAPOP, or a fragment of a polynucleotide complementary to the polynucleotide encoding MAPOP, and will be employed under optimized conditions for identification of a specific gene or condition. Oligomers may also be employed under less
25 stringent conditions for detection or quantitation of closely related DNA or RNA sequences.

Methods which may also be used to quantitate the expression of MAPOP include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (See, e.g., Melby, P.C. et al. (1993) J. Immunol. Methods 159:235-244; and Duplaa, C. et al. (1993) Anal. Biochem. 229-236.) The speed of
30 quantitation of multiple samples may be accelerated by running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

In further embodiments, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used as targets in a microarray. The
35 microarray can be used to monitor the expression level of large numbers of genes simultaneously

and to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, and to develop and monitor the activities of therapeutic agents.

Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g., Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) Proc. Natl. Acad. Sci. 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.)

In another embodiment of the invention, nucleic acid sequences encoding MAPOP may be used to generate hybridization probes useful in mapping the naturally occurring genomic sequence. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries. (See, e.g., Price, C.M. (1993) Blood Rev. 7:127-134; and Trask, B.J. (1991) Trends Genet. 7:149-154.)

Fluorescent in situ hybridization (FISH) may be correlated with other physical chromosome mapping techniques and genetic map data. (See, e.g., Heinz-Ulrich, et al. (1995) in Meyers, R.A. (ed.) Molecular Biology and Biotechnology, VCH Publishers New York, NY, pp. 965-968.) Examples of genetic map data can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) site. Correlation between the location of the gene encoding MAPOP on a physical chromosomal map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA associated with that disorder. The nucleotide sequences of the invention may be used to detect differences in gene sequences among normal, carrier, and affected individuals.

In situ hybridization of chromosomal preparations and physical mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localized by genetic linkage to a particular genomic region, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R.A. et al. (1988) Nature 336:577-580.) The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal

location due to translocation, inversion, etc., among normal, carrier, or affected individuals.

In another embodiment of the invention, MAPOP, its catalytic or immunogenic fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, 5 affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between MAPOP and the agent being tested may be measured.

Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the protein of interest. (See, e.g., Geysen, et al. (1984) PCT application WO84/03564.) In this method, large numbers of different small test 10 compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The test compounds are reacted with MAPOP, or fragments thereof, and washed. Bound MAPOP is then detected by methods well known in the art. Purified MAPOP can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

15 In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding MAPOP specifically compete with a test compound for binding MAPOP. In this manner, antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with MAPOP.

In additional embodiments, the nucleotide sequences which encode MAPOP may be used 20 in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

The examples below are provided to illustrate the subject invention and are not included for the purpose of limiting the invention.

25

EXAMPLES

I. cDNA Library Construction

The COLNFET02 cDNA library was constructed using RNA isolated from the colon tissue of a Caucasian female fetus, who died at 20 weeks' gestation.

30 The BEPINOT01 cDNA library was constructed using RNA isolated from a bronchial epithelium primary cell line derived from a 54-year-old Caucasian male.

The DRGLNOT01 cDNA library was constructed using RNA isolated from dorsal root ganglion tissue removed from the low thoracic/high lumbar region of a 32-year-old Caucasian male who died from acute pulmonary edema and bronchopneumonia, bilateral pleural and

pericardial effusions, and malignant lymphoma (natural killer cell type). Patient history included probable cytomegalovirus infection, hepatic congestion and steatosis, splenomegaly, hemorrhagic cystitis, thyroid hemorrhage, and Bell's palsy. Surgeries included colonoscopy, large intestine biopsy, adenotonsillectomy, and nasopharyngeal endoscopy and biopsy; treatment included
5 radiation therapy.

For the construction of the COLNFET02 and BEPINOT01 cDNA libraries, frozen tissue was homogenized and lysed in guanidinium isothiocyanate solution using a Brinkmann Homogenizer Polytron PT-3000 (Brinkmann Instruments, Westbury, NY). The lysate was centrifuged over a CsCl cushion to isolate RNA. The RNA was extracted with phenol and
10 precipitated with sodium acetate and ethanol. For the construction of the DRGLNOT01 cDNA library, frozen tissue was homogenized and lysed in TRIzol reagent (1 gm tissue/10 ml TRIzol, Life Technologies), a monophasic solution of phenol and guanidine isothiocyanate. After brief incubation on ice, chloroform was added (1:5 v/v), and the mixture was centrifuged to separate the phases. The upper aqueous phase was removed to a fresh tube, and isopropanol was added to
15 precipitate RNA. All RNA preparations were resuspended in RNase-free water, treated with DNase, re-extracted with acid phenol, and reprecipitated with sodium acetate and ethanol.

From each RNA preparation, poly(A⁺) RNA was isolated using the Qiagen OLIGOTEX kit (QIAGEN Inc, Chatsworth, CA). Poly(A⁺) RNA was used for cDNA synthesis and construction of each cDNA library according to the recommended protocols in the
20 SUPERScript plasmid system (Life Technologies). The cDNAs were fractionated on a SEPHAROSE CL4B column (Amersham Pharmacia Biotech), and those cDNAs exceeding 400 bp were ligated into either pINCY 1 plasmid (Incyte Pharmaceuticals, Palo Alto, CA) (COLNFET02 and DRGLNOT01) or pSPORT1 plasmid (Life Technologies) (BEPINOT01). Recombinant plasmids were transformed into DH5 α TM competent cells (Life Technologies).

25

II. Isolation of cDNA Clones

Plasmid DNA was released from the cells and purified using the REAL Prep 96 plasmid kit (QIAGEN Inc). The recommended protocol was employed except for the following changes:
1) the bacteria were cultured in 1 ml of sterile Terrific Broth (Life Technologies) with carbenicillin
30 at 25 mg/l and glycerol at 0.4%; 2) after the cultures were incubated for 19 hours, the cells were lysed with 0.3 ml of lysis buffer; and 3) following isopropanol precipitation, the plasmid DNA pellets were each resuspended in 0.1 ml of distilled water. The DNA samples were stored at 4°C.

III. Sequencing and Analysis

35 The cDNAs were prepared for sequencing using either an ABI CATALYSTTM 800

(Perkin-Elmer Applied Biosystems, Foster City, CA) or a MICROLAB® 2200 (Hamilton Co., Reno, NV) sequencing preparation system in combination with Peltier PTC-200 thermal cyclers (MJ Research, Inc., Watertown, MA). The cDNAs were sequenced using the ABI PRISM™ 373 or 377 sequencing systems and ABI protocols, base calling software, and kits (Perkin-Elmer Applied Biosystems). Alternatively, solutions and dyes from Amersham Pharmacia Biotech, Ltd. were used. Reading frames were determined using standard methods (Ausubel, supra). Some of the cDNA sequences were selected for extension using the techniques disclosed in Example V.

The polynucleotide sequences derived from cDNA, extension, and shotgun sequencing were assembled and analyzed using a combination of software programs which utilize algorithms well known to those skilled in the art. Table 1 summarizes the software programs, their descriptions, references, and threshold parameters where applicable. The references cited in the third column of Table 1 are incorporated by reference herein. Sequences were also analyzed using MACDNASIS PRO software (Hitachi Software Engineering Co., Ltd. San Bruno, CA) and LASERGENE software (DNASTAR Inc).

The polynucleotide sequences were validated by removing vector, linker, and polyA tail sequences and by masking ambiguous bases, using algorithms and programs based on BLAST, dynamic programming, and dinucleotide nearest neighbor analysis. The sequences were then queried against a selection of public databases such as GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases, and BLOCKS to acquire annotation, using programs based on BLAST, FASTA, and BLIMPS. The sequences were assembled using Phred, Phrap, and Consed, and were analyzed for open reading frames using programs based on GeneMark, BLAST, and FASTA. This was followed by translation of the full length polynucleotide sequences to derive the corresponding full length amino acid sequences. These full length polynucleotide and amino acid sequences were subsequently analyzed by querying against databases such as the GenBank databases described above and SwissProt, BLOCKS, PRINTS, PFAM, and Prosite.

IV. Northern Analysis

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, supra, ch. 7; and Ausubel, supra, ch. 4 and 16.)

Electronic northern analysis was produced using analogous computer techniques. These techniques apply BLAST to search for identical or related molecules in nucleotide databases such as GenBank or LIFESEQ® database (Incyte Pharmaceuticals). The sensitivity of the computer search was modified to determine the specificity of the match. The basis of the search is the

product score, which is defined as:

$$\frac{\% \text{ sequence identity} \times \% \text{ maximum BLAST score}}{100}$$

100

5

The product score encompasses both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match may have a possibility of a 1% to 2% error, in contrast, a product score of 70 indicates that the match will be exact. Similar molecules were identified by product scores between 15 and 40, although lower
10 scores may identify related molecules.

Electronic northern analysis further involved the categorization of cDNA libraries by organ/tissue and disease. The organ/tissue categories included cardiovascular, dermatologic, developmental, endocrine, gastrointestinal, hematopoietic/immune, musculoskeletal, nervous, reproductive, and urologic. The disease categories included cancer, inflammation/trauma, fetal,
15 neurological, and pooled. For each category, the number of libraries expressing the sequence of interest was divided by the total number of libraries across all categories. The results above were reported as a percentage distribution.

V. Extension of MAPOP Encoding Polynucleotides

20 The full length nucleic acid sequences were produced by extension of the component fragments as disclosed in the invention description. Oligonucleotide primer pairs designed from at least one of the component fragments were used to initiate extension of sense and antisense polynucleotide strands. Primers were used to facilitate the extension of the known sequence "outward" generating amplicons containing new unknown nucleotide sequence for the region of
25 interest. The initial primers were designed from the cDNA using OLIGO™ 4.06 (National Biosciences, Plymouth, MN), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

30 Selected human cDNA libraries (Life Technologies) were used to extend the sequence. If more than one extension is necessary or desired, additional sets of primers are designed to further extend the known region.

High fidelity amplification was obtained by following the instructions for the XL-PCR™ kit (Perkin-Elmer) and thoroughly mixing the enzyme and reaction mix. PCR was performed
35 using the PTC-200 thermal cycler (MJ Research, Inc., Watertown, MA), beginning with 40 pmol

of each primer and the recommended concentrations of all other components of the kit, with the following parameters:

	Step 1	94° C for 1 min (initial denaturation)
	Step 2	65° C for 1 min
5	Step 3	68° C for 6 min
	Step 4	94° C for 15 sec
	Step 5	65° C for 1 min
	Step 6	68° C for 7 min
	Step 7	Repeat steps 4 through 6 for an additional 15 cycles
10	Step 8	94° C for 15 sec
	Step 9	65° C for 1 min
	Step 10	68° C for 7:15 min
	Step 11	Repeat steps 8 through 10 for an additional 12 cycles
	Step 12	72° C for 8 min
15	Step 13	4° C (and holding)

A 5 μ l to 10 μ l aliquot of the reaction mixture was analyzed by electrophoresis on a low concentration (about 0.6% to 0.8%) agarose mini-gel to determine which reactions were successful in extending the sequence. Bands thought to contain the largest products were excised from the gel, purified using QIAQUICK™ (QIAGEN Inc.), and trimmed of overhangs using Klenow enzyme to facilitate religation and cloning.

After ethanol precipitation, the products were redissolved in 13 μ l of ligation buffer, 1 μ l T4-DNA ligase (15 units) and 1 μ l T4 polynucleotide kinase were added, and the mixture was incubated at room temperature for 2 to 3 hours, or overnight at 16° C. Competent *E. coli* cells (in 40 μ l of appropriate media) were transformed with 3 μ l of ligation mixture and cultured in 80 μ l of SOC medium. (See, e.g., Sambrook, *supra*, Appendix A, p. 2.) After incubation for one hour at 37° C, the *E. coli* mixture was plated on Luria Bertani (LB) agar (See, e.g., Sambrook, *supra*, Appendix A, p. 1) containing carbenicillin (2x carb). The following day, several colonies were randomly picked from each plate and cultured in 150 μ l of liquid LB/2x carb medium placed in an individual well of an appropriate commercially-available sterile 96-well microtiter plate. The following day, 5 μ l of each overnight culture was transferred into a non-sterile 96-well plate and, after dilution 1:10 with water, 5 μ l from each sample was transferred into a PCR array.

For PCR amplification, 18 μ l of concentrated PCR reaction mix (3.3x) containing 4 units of rTth DNA polymerase, a vector primer, and one or both of the gene specific primers used for the extension reaction were added to each well. Amplification was performed using the following conditions:

	Step 1	94° C for 60 sec
	Step 2	94° C for 20 sec
	Step 3	55° C for 30 sec
40	Step 4	72° C for 90 sec
	Step 5	Repeat steps 2 through 4 for an additional 29 cycles

Step 6	72° C for 180 sec
Step 7	4° C (and holding)

Aliquots of the PCR reactions were run on agarose gels together with molecular weight
5 markers. The sizes of the PCR products were compared to the original partial cDNAs, and
appropriate clones were selected, ligated into plasmid, and sequenced.

In like manner, the nucleotide sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID
NO:6 are used to obtain 5' regulatory sequences using the procedure above, oligonucleotides
designed for 5' extension, and an appropriate genomic library.

10

VI. Labeling and Use of Individual Hybridization Probes

Hybridization probes derived from SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6 are
employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of
oligonucleotides, consisting of about 20 base pairs, is specifically described, essentially the same
15 procedure is used with larger nucleotide fragments. Oligonucleotides are designed using state-of-
the-art software such as OLIGO™ 4.06 software (National Biosciences) and labeled by combining
50 pmol of each oligomer, 250 μ Ci of [γ -³²P] adenosine triphosphate (Amersham, Chicago, IL),
and T4 polynucleotide kinase (DuPont NEN®, Boston, MA). The labeled oligonucleotides are
substantially purified using a Sephadex™ G-25 superfine size exclusion dextran bead column
20 (Pharmacia & Upjohn, Kalamazoo, MI). An aliquot containing 10⁷ counts per minute of the
labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA
digested with one of the following endonucleases: Ase I, Bgl II, Eco RI, Pst I, Xba I, or Pvu II
(DuPont NEN, Boston, MA).

The DNA from each digest is fractionated on a 0.7% agarose gel and transferred to nylon
25 membranes (Nytran Plus, Schleicher & Schuell, Durham, NH). Hybridization is carried out for 16
hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature
under increasingly stringent conditions up to 0.1 x saline sodium citrate and 0.5% sodium dodecyl
sulfate. After XOMAT AR™ film (Kodak, Rochester, NY) is exposed to the blots to film for
several hours, hybridization patterns are compared visually.

30

VII. Microarrays

A chemical coupling procedure and an ink jet device can be used to synthesize array
elements on the surface of a substrate. (See, e.g., Baldeschweiler, supra.) An array analogous to a
dot or slot blot may also be used to arrange and link elements to the surface of a substrate using
35 thermal, UV, chemical, or mechanical bonding procedures. A typical array may be produced by
hand or using available methods and machines and contain any appropriate number of elements.

After hybridization, nonhybridized probes are removed and a scanner used to determine the levels and patterns of fluorescence. The degree of complementarity and the relative abundance of each probe which hybridizes to an element on the microarray may be assessed through analysis of the scanned images.

5 Full-length cDNAs, Expressed Sequence Tags (ESTs), or fragments thereof may comprise the elements of the microarray. Fragments suitable for hybridization can be selected using software well known in the art such as LASERGENE™. Full-length cDNAs, ESTs, or fragments thereof corresponding to one of the nucleotide sequences of the present invention, or selected at random from a cDNA library relevant to the present invention, are arranged on an
10 appropriate substrate, e.g., a glass slide. The cDNA is fixed to the slide using, e.g., UV cross-linking followed by thermal and chemical treatments and subsequent drying. (See, e.g., Schena, M. et al. (1995) Science 270:467-470; and Shalon, D. et al. (1996) Genome Res. 6:639-645.) Fluorescent probes are prepared and used for hybridization to the elements on the substrate. The substrate is analyzed by procedures described above.

15

VIII. Complementary Polynucleotides

Sequences complementary to the MAPOP-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring MAPOP. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same
20 procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using OLIGO™ 4.06 software and the coding sequence of MAPOP. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the MAPOP-encoding transcript.

25

IX. Expression of MAPOP

Expression and purification of MAPOP is achieved using bacterial or virus-based expression systems. For expression of MAPOP in bacteria, cDNA is subcloned into an appropriate vector containing an antibiotic resistance gene and an inducible promoter that directs
30 high levels of cDNA transcription. Examples of such promoters include, but are not limited to, the *trp-lac* (*tac*) hybrid promoter and the T5 or T7 bacteriophage promoter in conjunction with the *lac* operator regulatory element. Recombinant vectors are transformed into suitable bacterial hosts, e.g., BL21(DE3). Antibiotic resistant bacteria express MAPOP upon induction with isopropyl beta-D-thiogalactopyranoside (IPTG). Expression of MAPOP in eukaryotic cells is achieved by
35 infecting insect or mammalian cell lines with recombinant Autographica californica nuclear

polyhedrosis virus (AcMNPV), commonly known as baculovirus. The nonessential polyhedrin gene of baculovirus is replaced with cDNA encoding MAPOP by either homologous recombination or bacterial-mediated transposition involving transfer plasmid intermediates. Viral infectivity is maintained and the strong polyhedrin promoter drives high levels of cDNA transcription. Recombinant baculovirus is used to infect Spodoptera frugiperda (Sf9) insect cells in most cases, or human hepatocytes, in some cases. Infection of the latter requires additional genetic modifications to baculovirus. (See Engelhard, E. K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945.)

In most expression systems, MAPOP is synthesized as a fusion protein with, e.g., glutathione S-transferase (GST) or a peptide epitope tag, such as FLAG or 6-His, permitting rapid, single-step, affinity-based purification of recombinant fusion protein from crude cell lysates. GST, a 26-kilodalton enzyme from Schistosoma japonicum, enables the purification of fusion proteins on immobilized glutathione under conditions that maintain protein activity and antigenicity (Pharmacia, Piscataway, NJ). Following purification, the GST moiety can be proteolytically cleaved from MAPOP at specifically engineered sites. FLAG, an 8-amino acid peptide, enables immunoaffinity purification using commercially available monoclonal and polyclonal anti-FLAG antibodies (Eastman Kodak, Rochester, NY). 6-His, a stretch of six consecutive histidine residues, enables purification on metal-chelate resins (QIAGEN Inc, Chatsworth, CA). Methods for protein expression and purification are discussed in Ausubel, F. M. et al. (1995 and periodic supplements) Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY, ch 10, 16. Purified MAPOP obtained by these methods can be used directly in the following activity assay.

X. Demonstration of MAPOP Activity

An assay for MAPOP activity measures the induction of apoptosis when MAPOP is expressed at physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a mammalian expression vector containing a strong promoter that drives high levels of cDNA expression. Vectors of choice include pCMV SPORT™ (Life Technologies) and pCR™ 3.1 (Invitrogen, Carlsbad, CA), both of which contain the cytomegalovirus promoter. 5-10 µg of recombinant vector are transiently transfected into a human cell line, preferably of endothelial or hematopoietic origin, using either liposome formulations or electroporation. 1-2 µg of an additional plasmid containing sequences encoding a marker protein are co-transfected. Expression of a marker protein provides a means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., Green Fluorescent Protein (GFP) (Clontech), CD64, or a

CD64-GFP fusion protein. Flow cytometry (FCM), an automated, laser optics-based technique, is used to identify transfected cells expressing GFP or CD64-GFP and to evaluate their apoptotic state. FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell death. These events include changes in nuclear DNA content as measured by staining of DNA with propidium iodide; changes in cell size and granularity as measured by forward light scatter and 90 degree side light scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in expression of cell surface and intracellular proteins as measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow cytometry are discussed in Ormerod, M. G. (1994) Flow Cytometry, Oxford, New York, NY.

XI. Production of MAPOP Specific Antibodies

MAPOP substantially purified using polyacrylamide gel electrophoresis (PAGE)(see, e.g., Harrington, M.G. (1990) *Methods Enzymol.* 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols.

Alternatively, the MAPOP amino acid sequence is analyzed using LASERGENE™ software (DNASTAR Inc.) to determine regions of high immunogenicity, and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Methods for selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well described in the art. (See, e.g., Ausubel supra, ch. 11.)

Typically, oligopeptides 15 residues in length are synthesized using an Applied Biosystems Peptide Synthesizer Model 431A using fmoc-chemistry and coupled to KLH (Sigma, St. Louis, MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase immunogenicity. (See, e.g., Ausubel supra.) Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for antipeptide activity by, for example, binding the peptide to plastic, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG.

XII. Purification of Naturally Occurring MAPOP Using Specific Antibodies

Naturally occurring or recombinant MAPOP is substantially purified by immunoaffinity chromatography using antibodies specific for MAPOP. An immunoaffinity column is constructed by covalently coupling anti-MAPOP antibody to an activated chromatographic resin, such as CNBr-activated SEPHAROSE resin (Amersham Pharmacia Biotech). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing MAPOP are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of MAPOP (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/MAPOP binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and MAPOP is collected.

XIII. Identification of Molecules Which Interact with MAPOP

MAPOP, or biologically active fragments thereof, are labeled with ^{125}I Bolton-Hunter reagent. (See, e.g., Bolton et al. (1973) Biochem. J. 133:529.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled MAPOP, washed, and any wells with labeled MAPOP complex are assayed. Data obtained using different concentrations of MAPOP are used to calculate values for the number, affinity, and association of MAPOP with the candidate molecules.

Various modifications and variations of the described methods and systems of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

TABLE 1

Program	Description	Reference	Parameter Threshold
ABI FACTURA	A program that removes vector sequences and masks ambiguous bases in nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA.	
ABI/PARACEL FDF	A Fast Data Finder useful in comparing and annotating amino acid or nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA; Paracel Inc., Pasadena, CA.	Mismatch <50%
ABI AutoAssembler	A program that assembles nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA.	
BLAST	A Basic Local Alignment Search Tool useful in sequence similarity search for amino acid and nucleic acid sequences. BLAST includes five functions: blastp, blastn, blastx, tblastn, and tblastx.	Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410; Altschul, S.F. et al. (1997) Nucleic Acids Res. 25: 3389-3402.	ESTs: Probability value= 1.0E-8 or less Full Length sequences: Probability value= 1.0E-10 or less
FASTA	A Pearson and Lipman algorithm that searches for similarity between a query sequence and a group of sequences of the same type. FASTA comprises at least five functions: fasta, tfasta, fastx, tfastx, and ssearch.	Pearson, W.R. and D.J. Lipman (1988) Proc. Natl. Acad. Sci. 85:2444-2448; Pearson, W.R. (1990) Methods Enzymol. 183: 63-98; and Smith, T.F. and M. S. Waterman (1981) Adv. Appl. Math. 2:482-489.	ESTs: fasta E value=1.06E-6 Assembled ESTs: fasta Identity= 95% or greater and Match length=200 bases or greater; fastx E value=1.0E-8 or less Full Length sequences: fastx score=100 or greater
BLIMPS	A BLocks IMProved Searcher that matches a sequence against those in BLOCKS and PRINTS databases to search for gene families, sequence homology, and structural fingerprint regions.	Henikoff, S and J.G. Henikoff, Nucl. Acid Res., 19:6565-72, 1991. J.G. Henikoff and S. Henikoff (1996) Methods Enzymol. 266:88-105; and Attwood, T.K. et al. (1997) J. Chem. Inf. Comput. Sci. 37: 417-424.	Score=1000 or greater; Ratio of Score/Strength = 0.75 or larger; and Probability value= 1.0E-3 or less
PFAM	A Hidden Markov Models-based application useful for protein family search.	Krogh, A. et al. (1994) J. Mol. Biol., 235:1501-1531; Sonnhammer, E.L.L. et al. (1988) Nucleic Acids Res. 26:320-322.	Score=10-50 bits, depending on individual protein families

TABLE 1 (con)

GeneMark	A gene prediction algorithm that is based on inhomogeneous Markov chain models and is useful for DNA sequence analysis and particularly for gene prediction.	Borodovsky, M. and J. McIninch (1993) Computers Chem. 17: 123-133; Blattner, F.R. et al. (1993) Nucleic Acids Res. 21: 5408-5417.	Score= 0.4 or greater
ProfileScan	An algorithm that searches for structural and sequence motifs in protein sequences that match sequence patterns defined in Prosite.	Gribkov, M. et al. (1988) CABIOS 4:61-66; Gribkov, et al. (1989) Methods Enzymol. 183:146-159; Bairoch, A. et al. (1997) Nucleic Acids Res. 25: 217-221.	Score= 4.0 or greater
Phred	A base-calling algorithm that examines automated sequencer traces with high sensitivity and probability.	Ewing, B. et al. (1998) Genome Res. 8:175-185; Ewing, B. and P. Green (1998) Genome Res. 8:186-194.	
Phrap	A Phils Revised Assembly Program including SWAT and CrossMatch, programs based on efficient implementation of the Smith-Waterman algorithm, useful in searching sequence homology and assembling DNA sequences.	Smith, T.F. and M. S. Waterman (1981) Adv. Appl. Math. 2:482-489; Smith, T.F. and M. S. Waterman (1981) J. Mol. Biol. 147:195-197; and Green, P., University of Washington, Seattle, WA.	Score= 120 or greater; Match length= 56 or greater
Consed	A graphical tool for viewing and editing Phrap assemblies	Gordon, D. et al. (1998) Genome Res. 8:195-202.	
SPScan	A weight matrix analysis program that scans protein sequences for the presence of secretory signal peptides.	Nielson, H. et al. (1997) Protein Engineering 10:1-6; Claverie, J.M. and S. Audic (1997) CABIOS 12: 431-439.	Score=5 or greater
Motifs	A program that searches amino acid sequences for patterns that matched those defined in Prosite.	Bairoch et al. <u>supra</u> ; Wisconsin Package Program Manual, version 9, page M51-59, Genetics Computer Group, Madison, WI.	

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TANG, Y. Tom

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GUEGLER, Karl J.

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 gctccagtgt ccgtgatgcc cgtcgccacc tcggaccaag aaggcgacan agctttggca 360
 aatacggcag aaacgcctac gtgtagcagt ctggcccgtg ggcccgtgt ttccactgcc 420
 caaaggagag ggacttgcc gggccattcc ctatagtaac tcaagggcgg cangcccgn 480
 tccgtagccc cgcccggcag ggcccgtgt tgaatccatg gcctcaaggc naaaaatgtc 540
 t 541

<210> 12
 <211> 258
 <212> DNA
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> Incyte clone 2057608H1

<400> 12
 cccctgagcc agcgcgggcg cccgagcgag tcatggccaa cgcggggctg cagctgttgg 60
 gcttcattct cgcttctctg ggatggatcg gcgccatcgt cagcactgcc ctgccccagt 120

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```

ggaggatttta ctccatatgcc ggcgacaaca tcgtgaccgc ccaggccatg tacgaggggc 180
tgtggatgtc ctgcgtgtcg cagagcaccg ggcagatcca gtgcaaagtc tttgactcct 240
tgctgaatct gagcagca                                     258

```

<210> 13
 <211> 245
 <212> DNA
 <213> Homo sapiens

<220>
 <221> unsure
 <222> 8, 143
 <223> a or g or c or t, unknown, or other

<220>
 <221> misc_feature
 <223> Incyte clone 821149H1

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<400> 13
ccagactnca gcgccgcccc gggcgcgagc cccaaccccg acccagagct tctccagcgg 60
cggcgagcga gcagggtccc ccgccttaac ttcttcgcgc gggcccagcc accttcggga 120
gtccgggttg cccacctgca aantctccgc cttctgcacc tgccaccctt gagccagcgc 180
gggcgccccga gcgagtcacg gccaacgcgc ggctgcagct gttgggctta ttctcgcctt 240
cctgg                                     245

```

<210> 14
 <211> 573
 <212> DNA
 <213> Homo sapiens

<220>
 <221> unsure
 <222> 87, 93, 149, 222, 224, 232, 257, 268, 303, 327, 344, 350, 389,
 403, 409, 473, 485, 506, 529
 <223> a or g or c or t, unknown, or other

<220>
 <221> misc_feature
 <223> Incyte clone 2662526F6

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<400> 14
caaccgcagc ttctagtatc cagactccag cgccgccccg ggcgcggacc ccaacccccga 60
cccagagctt ctccagcggc ggcgcangag canggtccc cgccttaact tcttcgcgcg 120
ggcccagcca ccttcgggag tccgggttnc ccacctgcaa actctccgcc ttctgcacct 180
gccacccttg agccagcgcg ggcgcccagc cgagtcacgg cnancgcggg gntgcagctg 240
ttgggcttca ttctcgnctt cctgggangg atcggcgcca tcgtcagcac tgccctgccc 300
cantggagga tttactccta tgccgngac aacatcgtga ccgnccaggn catgtacgag 360
gggctgtgga tgtcctgcgt gtcgagatna ccgggcagat ccnagtgcna agtctttgac 420
tccttgctga atctgagcag cacattgcaa acaaccctg ccttgatggg ggntgcaacc 480
tcctnngagt gatagcaatc tttgtngcca ccgttgggat gaagtgtang aagtgttgga 540
agacgatgag gtgcacaaga tgagatggct gtc                                     573

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<210> 15
 <211> 486
 <212> DNA
 <213> Homo sapiens

<220>
 <221> unsure
 <222> 36
 <223> a or g or c or t, unknown, or other

<220>
 <221> misc_feature
 <223> Incyte clone SAEB01552R1

<400> 15
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 aagtgccttg aagacgatga ggtgcagaag atgaggatgg ctgtcattgg gggcgcgata 120
 tttcttcttg caggtctggc tatttttagtt gccacagcat ggtatggcaa tagaatcggt 180
 caagaattct atgaccctat gacccagtc aatgccaggt acgaatttgg tcaggctctc 240
 ttcactggct gggctgctgc ttctctctgc cttctgggag gtgccctact ttgctgttcc 300
 tgtccccgaa aaacaacctc ttaccaaca ccaaggcct atccaaaacc tgcaccttcc 360
 agcgggaaag actacgtgtg acacagcag 389

<210> 16
 <211> 389
 <212> DNA
 <213> Homo sapiens

<220>
 <221> unsure
 <222> 106, 135, 211, 407, 421, 423, 439, 440
 <223> a or g or c or t, unknown, or other

<220>
 <221> misc_feature
 <223> Incyte clone 2734032F6

<400> 16
 catgtgttaa aatactcagt gctaaacatg gcttaatctt attttatctt ctttctctcaa 60
 tataggaggg aagatttttc catttgtatt actgcttccc attgantaat cataactcaaa 120
 tgggggaagg ggtgntcctt aaatatatat agatatgtat atatacatgt ttttctatta 180
 aaaatagaca gtaaaatact attctcatta ngttgatact agcatactta aaatatctct 240
 aaaataggta aatgtattta attccatatt gatgaagatg tttattggta tattttcttt 300
 ttcgtcctta tatacatatg taacagtcaa atatcattta ctcttcttca ttagcttttg 360
 gtgcctttgc cacaagacct agcctaattt accaaggatg aattctntca attcttcatg 420
 ngngcccttt tcatatacnn attttatctt ttaccataat ctatagcact tgcacgttta 480
 ttaagc 486

<210> 17
 <211> 645
 <212> DNA
 <213> Homo sapiens

<220>
 <221> misc_feature

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<223> Incyte clone 2795896T6

<400> 17

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tataaaacgc atgataatta cacttgggaa aacaatacaa ttaaggaatg gggaacaaaa 60
aggtagttta gggggatata aaactatgaa taagtaactt aaaaattcaa tcaataaagt 120
tgtgttacaa cacctggggg aacagcatgc agctactcaa ttggacaaat attttcaaag 180
caaacaagag tgctatgggt cagagtacag ggattgtgtg ggaagggtcaa caggaattta 240
tcactttggg catgcagaaa gatttgcctc caaagttctg atttgtaata aattcttctt 300
ggcttttagaa actaaaatgt aggctatgaa atgtgttaga ttcaggagat agagaccaat 360
gaaacacaaa acaaataagg gcttaataac gatgcaagtg ctataagatt atggtaaaaa 420
ataaaataag tatatgaaaa gggcacgcat gaagaattga aagaattcat ccttggtaaa 480
ttaggctagg tcttgtggca aaggcaccca aagctaataga agaagagtaa atgatatttg 540
actgttacat atgtatatat agacgaaaaa gaaaatatac caataaacat cttcatcaat 600
atggaattaa atacatttac ctattttaga gatattttta gtagt 645

```

<210> 18

<211> 506

<212> DNA

<213> Homo sapiens

<220>

<221> misc_feature

<223> Incyte clone 2696284F6

<400> 18

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cttattcata gttttatata cccctaaact acctttttgt tccccattcc ttaattgtat 60
tgttttccca agtgtaatta tcatgcgttt tatatcttcc taataagggt tggtctgttt 120
gtctgaacaa agtgctagac tttctggagt gataatctgg tgacaaatat tctctctgta 180
gctgtaagca agtcacttaa tctttctacc tcttttttct atctgccaaa ttgagataat 240
gatacttaac cagttagaag aggtagtgtg aatattaatt agtttatatt actctcattc 300
tttgaacatg aactatgcct atgtagtgtc tttatttgct cagctggctg agacactgaa 360
gaagtcactg aacaaaacct acacacgtac cttcatgtga ttcactgcct tcctctctct 420
accagtctat ttccactgga acaaaacctt cacacatacc ttcattgtgg ttagtggtct 480
cctctctcta ccagtctatt tccact 506

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<210> 19

<211> 477

<212> DNA

<213> Homo sapiens

<220>

<221> unsure

<222> 450, 462

<223> a or g or c or t, unknown, or other

<220>

<221> misc_feature

<223> Incyte clone 2254004R6

<400> 19

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tgtctttatt tgctcagctg gctgagacac tgaagaagtc actgaacaaa acctacacac 120
gtaccttcac gtgattcact gccttcctct ctctaccagt ctatttccac tgaacaaaac 180
ctacacacat accttcattg gggtcagtcg cttcctctct ctaccagtct atttccactg 240
aacaaaacct acgcacatac cttcatgtgg ctcagtgcct tcctctctct accagtctat 300

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ttccattctt tcagctgtgt ctgacatggt tgtgctctgt tccattttta caactgctct 360
 tacttttcca gtctgtacag aatgctatct cacttgagca agatgatgta atggaaaggg 420
 tggtggcatt ggtgtctgga gaacctgga ttgagtcttg gngcatcaat cacggtc 477

<210> 20
 <211> 540
 <212> DNA
 <213> Homo sapiens

<220>
 <221> unsure
 <221> 485
 <223> a or g or c or t, unkown, or other

<220>
 <221> misc_feature
 <223> Incyte clone 1433735R1

<400> 20
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 acatgtaagt gtggttttgt aattttaaaaa gtgctatact aagggaaga attgaggaat 120
 taactgcata cgttttggtg ttgcttttca aatgtttgaa aacaaaaaaa atgttaagaa 180
 atgggtttct tgccttaacc agtctctcaa gtgatgagac agtgaagtaa aattgagtgc 240
 actaaacaaa taagattctg aggaagtctt atcttctgca gtgagtatgg cccgatgctt 300
 tctgtggcta aacagatgta atgggaagaa ataaaagcct acgtgttggt aaatccaaca 360
 gcaagggaga tttttgaatc ataataactc ataagggtgt atctgttcag tgatgccctc 420
 agagctcttg ctgttagctg gcagctgacg ctgctaggat agttagtttg gaaatggtac 480
 ttcanaataa acttaccag ggaaagtcag ccactgtggc ctatgaggaa ttggacctaa 540

<210> 21
 <211> 570
 <212> DNA
 <213> Homo sapiens

<220>
 <221> unsure
 <222> 366
 <223> a or g or c or t, unknown, or other

<220>
 <221> misc_feature
 <223> Incyte clone 1554306F6

<400> 21
 gtaatttaaa aagtgctata ctaagggaaa gaattgagga attaactgca tacgttttgg 60
 tggtgctttt caaatgtttg aaaacaaaaa aaatgttaag aaatgggttt cttgccttaa 120
 ccagtctctc aagtgatgag acagtgaagt aaaattgagt gcactaaaca aataagattc 180
 tgaggaagtc ttatcttctg cagtgagtat ggcccgatgc tttctgtggc taaacagatg 240
 taatgggaag aaataaaaagc ctacgtgttg gtaaatccaa cagcaaggga gatttttgaa 300
 tcataataac tcataagggtg ctatctgttc agtgatgcc tcagagctct tgctgttagc 360
 tggcantgac gctgctagga tagttagttt ggaaatggta cttcataata aactacacaa 420
 ggaaagtcag ccactgtgtc ttatgaggaa ttggacctaa taaatttttag tgtgccttcc 480
 aaacctgaga atatatgctt ttggaagtta aatttaaatg gctttgcaca tactagatct 540
 catgatgtgt gagtgttaatt cctgggggta 570

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<210> 22
 <211> 677
 <212> DNA
 <213> Homo sapiens

<220>
 <221> unsure
 <222> 407, 665, 669
 <223> a or g or c or t, unknown, or other

<220>
 <221> misc_feature
 <223> Incyte clone 1554306T6

<400> 22
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 aacccccaaa tgtctattgg tctgtttcca ggtgtggttag aagaatataa aaagatcaaa 120
 attggataaa ttctattgta acaatttcgt tggtcatttt gggccataaa atttttttgt 180
 aatgtttggt aactgatatc cacatggaat tacactcaca catcatgaag atctatgtat 240
 gtggcaaaaag ccattttaaatt tttaacttcc aaaagcatat attctcaggt ttggaaggca 300
 cactaaaatt tattaggtcc aattcctcat aagacacagt ggctgacttt ccttgtgtag 360
 tttattatga agtaccattt ccaaactaac tatectagca gcgtcantgc cagctaacag 420
 caagagctct gagggcatca ctgaacagat agcaccttat gagttattat gattcaaaaa 480
 tctcccttgc tgttggattt accaacacgt agggctttta tttcttccca ttacatctgt 540
 ttagccacag aaagcatcgg ggccatactc actggcagaa gataagactt cctcagaatc 600
 ttatttggtt agtgcctcaa ttttacttca ctgtctcatc acttgagaga tgggtaaggc 660
 agaancctt tctttat 677

<210> 23
 <211> 260
 <212> DNA
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> Incyte clone 2840978H1

<400> 23
 accgaaggga gcggcgagat cgacttccgc tgccccctgg aagatcccgg ctggaacgcc 60
 cagatcaccc taggcctggt caagttcaag aaccagcagg ccatccagac agtgcggggc 120
 cggcagagcc tcgggaccgg gaccctcgtg tcctaaacca ccgggcgcac catctttcct 180
 tcatgctacc caccacctca gtgctgaggt caaggcagct tcgttggtcc ctctggcttg 240
 tgggggcagg ctgtgtccat 260

<210> 24
 <211> 243
 <212> DNA
 <213> Homo sapiens

<220>
 <221> unsure
 <222> 24, 27, 46, 121, 136, 138, 182, 188, 190, 192, 224
 <223> a or g or c or t, unknown, or other

<220>

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<221> misc_feature
 <223> Incyte clone 3415476H1

<400> 24
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 caccactgcc tcaccggcag ccgggtgctg agggccgcgc tgtgggtgcg cggacagtca 120
 nggcgcaggt gggcancncg cacggcctgc cagccccggg cgccagaatc ctgcgctgcg 180
 gngccganan gngcgccgcg cccgccgcag cctggagctt tccncgaacc tcggggcgcc 240
 cat 243

<210> 25
 <211> 499
 <212> DNA
 <213> Homo sapiens

<220>
 <221> unsure
 <222> 243, 350, 409, 449, 468, 471
 <223> a or g or c or t, unknown, or other

<220>
 <221> misc_feature
 <223> Incyte clone 2099593R6

<400> 25
 agcgcaggac ggggctgctg cagctgtgga agcgggaaggc tgcgtcctca ccgaacgcgg 60
 gctgcagctc ttcgaggcca agggcacggg cggccggccc aaggagctca gcttcgcccc 120
 catcaaggcc gtggagtgcg tggagagcac cgggcgccac atctacttca cgctggtgac 180
 cgaagggggcg gcgagatcga cttccgctgc cccctggaag atcccggctg gaacgcccag 240
 atnaccctag gcctggtcaa gttcaagaac cagcaggcca tccagacagt gcgggcccgg 300
 cagagcctcg ggaccgggac cctcgtgtcc taaaccaccg ggcgcaccan ctttccttca 360
 tgctacccac cacctcagtg ctgaggtcaa ggcagcttcg ttgttccnc tggettggtg 420
 gggcacggct gtgtccatgt ggggaaggtng aaggcatgac gtgtgganga nggcgtggaa 480
 gtgaaggatg gacgagcct 499

<210> 26
 <211> 583
 <212> DNA
 <213> Homo sapiens

<220>
 <221> unsure
 <222> 224, 351, 379, 397, 428, 451, 502, 513, 523, 527, 529, 535, 539,
 545, 572
 <223> a or g or c or t, unknown, or other

<220>
 <221> misc_feature
 <223> Incyte clone 1441568F1

<400> 26
 ggcacggggcg gccggcccaa ggagctcagc ttcgcccgc tcaaggccgt ggagtgcgtg 60
 gagagcaccg ggcgccacat ctacttcacg ctggtgaccg aagggtgcgg cgagatcgac 120
 ttccgctgcc ccctggaaga tcccggctgg aacgccca taccctagg cctggtcaag 180

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ttcaagaacc agcaggccat ccagacagtg cgggccccggc agancctcgg gaccggggacc 240
ctcgtgtcct aaaccaccgg gcgcaccatc ttctcttcat gctaccacc acctcagtgc 300
tgagggtcaag gcagcttcgt tgttccctct ggcttgtggg ggcaggctgt ntccatgtgg 360
caagtggaag gcatggacnt gtggaagagg cctgganctg aaagaatgga cgaaccctgg 420
gaaggaangg cagaaggcta acgcagggct ngaaggatga agttgcaagc cccctggatg 480
gtccccagaa ctcttcatga anatggccca agnttcaggg ggnttcnang ccacnaggnc 540
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<210> 27

<211> 509

<212> DNA

<213> Homo sapiens

<220>

<221> unsure

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<222> 310, 349, 354, 367, 392, 404, 406, 434, 437, 438, 445, 447, 451,
      452, 454, 455, 457, 458, 460, 461, 462, 463, 464, 465, 466, 467,
      469, 470, 473, 474, 475, 476, 477, 478, 479, 480, 481, 483, 486,
      488, 489, 491, 492, 494, 495, 497, 498, 500, 501, 502, 503, 504,
      505, 506, 507, 508

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<223> a or g or c or t, unknown, or other

<220>

<221> misc_feature

<223> Incyte clone 893117R6

<400> 27

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agctggcata ggagccccct ccctgctgtg gtcctgccct ctgtcctgca gactgctctt 60
agccccctgg ctttgtgccca ggcctggagg agggcagtc cccatggggg gccgagccaa 120
cgcctcagga atcaggaggc cagcctggta ccaaaaggag taccagggc ctggtaccca 180
ggcccactcc agaatggcct ctggactcac cttgagaagg gggagcttct gggcctaaag 240
cccactcctg ggggtctcct gctgcttagg tccttttggg acccccaccc atccaggccc 300
tttctttgan aattttcccc cacctctagg atttccccca atgggtttng gctnaagggtg 360
ttggggnaag gggggttgca ttagattaat tnttccccaa aagnanatcc ccttaaagggtg 420
ggtaaccacg aaanaanna aaaananttg nngnngnnan nnnnnnnncnn tttnnnnnnnn 480
nanaananna nnanntnncn nnnnnnnna 509

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<210> 28

<211> 401

<212> DNA

<213> Homo sapiens

<220>

<221> unsure

<222> 35, 58, 68, 284, 384

<223> a or g or c or t, unknown, or other

<220>

<221> misc_feature

<223> Incyte clone 1441568R1

<400> 28

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actgcccttt gcagggcata ttcatttggg gaaanacatg aaatgctaag ggccaaancc 60
cccctcancc ccaccacctt caggccgaac accgcagtgg ggggaagatg catagagggtg 120

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ggggaagaag tgtgcaaaga aagggcctgg atgggtgggg gtcccaaaag gacctaagca 180
gcaggagacc cccaggagtg ggctttaggc ccagcagctc ccccttctca aggtgagtcc 240
agaggccatt ctggagtggg cctgggtacc aggccctggg tacncctttt ggtaccaggc 300
tggcctcctg attcctgagg cgttggctcg gcaccccatg ggggaatgcc ctcctccagg 360
gcctggcaca aagccaaggg ggcnaaaaag cagtttgcaa g 401

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<210> 29
 <211> 220
 <212> PRT
 <213> Homo sapiens

<300>
 <308> 2570129, GenBank

<400> 29
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 5 10 15
 Gly Trp Leu Gly Thr Ile Val Cys Cys Ala Leu Pro Met Trp Arg
 20 25 30
 Val Ser Ala Phe Ile Gly Ser Asn Ile Ile Thr Ser Gln Asn Ile
 35 40 45
 Trp Glu Gly Leu Trp Met Asn Cys Val Val Gln Ser Thr Gly Gln
 50 55 60
 Met Gln Cys Lys Val Tyr Asp Ser Leu Leu Ala Leu Pro Gln Asp
 65 70 75
 Leu Gln Ala Ala Arg Ala Leu Ile Val Val Ala Ile Leu Leu Ala
 80 85 90
 Ala Phe Gly Leu Leu Val Ala Leu Val Gly Ala Gln Cys Thr Asn
 95 100 105
 Cys Val Gln Asp Asp Thr Ala Lys Ala Lys Ile Thr Ile Val Ala
 110 115 120
 Gly Val Leu Phe Leu Leu Ala Ala Leu Leu Thr Leu Val Pro Val
 125 130 135
 Ser Trp Ser Ala Asn Thr Ile Ile Arg Asp Phe Tyr Asn Pro Val
 140 145 150
 Val Pro Glu Ala Gln Lys Arg Glu Met Gly Ala Gly Leu Tyr Val
 155 160 165
 Gly Trp Ala Ala Ala Ala Leu Gln Leu Leu Gly Gly Ala Leu Leu
 170 175 180
 Cys Cys Ser Cys Pro Pro Arg Glu Lys Lys Tyr Thr Ala Thr Lys
 185 190 195
 Val Val Tyr Ser Ala Pro Arg Ser Thr Gly Pro Gly Ala Ser Leu
 200 205 210
 Gly Thr Gly Tyr Asp Arg Lys Asp Tyr Val
 215 220

<210> 30
 <211> 261
 <212> PRT
 <213> Homo sapiens

<300>
 <308> 1469400, GenBank

<400> 30

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Met	Leu	Glu	Asn	Ser	Gly	Cys	Lys	Ala	Leu	Lys	Glu	Gly	Val	Leu	5	10	15
Glu	Lys	Arg	Ser	Asp	Gly	Leu	Leu	Gln	Leu	Trp	Lys	Lys	Lys	Cys	20	25	30
Cys	Ile	Leu	Thr	Glu	Glu	Gly	Leu	Leu	Leu	Ile	Pro	Pro	Lys	Gln	35	40	45
Leu	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Pro	Gly	Gln	Gly	Thr	Ala	50	55	60
Glu	Pro	Ser	Gln	Pro	Ser	Gly	Pro	Thr	Val	Ala	Ser	Leu	Glu	Pro	65	70	75
Pro	Val	Lys	Leu	Lys	Glu	Leu	His	Phe	Ser	Asn	Met	Lys	Thr	Val	80	85	90
Asp	Cys	Val	Glu	Arg	Lys	Gly	Lys	Tyr	Met	Tyr	Phe	Thr	Val	Val	95	100	105
Met	Thr	Glu	Gly	Lys	Glu	Ile	Asp	Phe	Arg	Cys	Pro	Gln	Asp	Gln	110	115	120
Gly	Trp	Asn	Ala	Glu	Ile	Thr	Leu	Gln	Met	Val	Gln	Tyr	Lys	Asn	125	130	135
Arg	Gln	Ala	Ile	Leu	Ala	Val	Lys	Ser	Thr	Arg	Gln	Lys	Gln	Gln	140	145	150
His	Leu	Val	Gln	Gln	Gln	Pro	Pro	Gln	Thr	Gln	Gln	Ile	Gln	Pro	155	160	165
Gln	Pro	Gln	Pro	Gln	Ile	Gln	Pro	Gln	Pro	Gln	Pro	Gln	Ile	Gln	170	175	180
Pro	Gln	Pro	Gln	Pro	Gln	Pro	Gln	Pro	Gln	Pro	Gln	Pro	Gln	Pro	185	190	195
Gln	Pro	Gln	Pro	Gln	Gln	Leu	His	Ser	Tyr	Pro	His	Pro	His	Pro	200	205	210
His	Pro	Tyr	Ser	His	Pro	His	Gln	His	Pro	His	Pro	His	Pro	His	215	220	225
Pro	His	Pro	His	Pro	His	Pro	His	Pro	Tyr	Gln	Leu	Gln	His	Ala	230	235	240
His	Gln	Pro	Leu	His	Ser	Gln	Pro	Gln	Gly	His	Arg	Leu	Leu	Arg	245	250	255
Ser	Thr	Ser	Asn	Ser	Ala										260		

What is claimed is:

1. A substantially purified polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, a fragment of SEQ ID NO:1, a fragment of SEQ ID NO:2, and a fragment of SEQ ID NO:3.
2. A substantially purified variant having at least 90% amino acid identity to the amino acid sequence of claim 1.
3. An isolated and purified polynucleotide encoding the polypeptide of claim 1.
4. An isolated and purified polynucleotide variant having at least 70% polynucleotide sequence identity to the polynucleotide of claim 3.
5. An isolated and purified polynucleotide which hybridizes under stringent conditions to the polynucleotide of claim 3.
6. An isolated and purified polynucleotide having a sequence which is complementary to the polynucleotide sequence of claim 3.
7. An isolated and purified polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, a fragment of SEQ ID NO:4, a fragment of SEQ ID NO:5, and a fragment of SEQ ID NO:6.
8. An isolated and purified polynucleotide variant having at least 70% polynucleotide sequence identity to the polynucleotide of claim 7.
9. An isolated and purified polynucleotide having a sequence which is complementary to the polynucleotide of claim 7.
10. An expression vector comprising at least a fragment of the polynucleotide of claim 3.
11. A host cell comprising the expression vector of claim 10.

12. A method for producing a polypeptide, the method comprising the steps of:
- a) culturing the host cell of claim 11 under conditions suitable for the expression of the polypeptide; and
 - b) recovering the polypeptide from the host cell culture.
- 5
13. A pharmaceutical composition comprising the polypeptide of claim 1 in conjunction with a suitable pharmaceutical carrier.
14. A purified antibody which specifically binds to the polypeptide of claim 1.
- 10
15. A purified agonist of the polypeptide of claim 1.
16. A purified antagonist of the polypeptide of claim 1.
- 15
17. A method for treating or preventing a cell proliferative disorder, the method comprising administering to a subject in need of such treatment an effective amount of the pharmaceutical composition of claim 13.
18. A method for treating or preventing an autoimmune disorder, the method
- 20 comprising administering to a subject in need of such treatment an effective amount of the antagonist of claim 16.
19. A method for detecting a polynucleotide in a biological sample, the method comprising the steps of:
- 25
- (a) hybridizing the polynucleotide of claim 6 to at least one of the nucleic acids in the biological sample, thereby forming a hybridization complex; and
 - (b) detecting the hybridization complex, wherein the presence of the hybridization complex correlates with the presence of the polynucleotide encoding the polypeptide in the biological sample.
- 30
20. The method of claim 19 further comprising amplifying the polynucleotide prior to hybridization.

1	MS	-	MA	VE	TF	GF	FM	AT	VG	LL	-	ML	GV	TL	PN	S	1456746													
1	MA	NA	AG	LQ	LL	GF	IL	AF	LG	WI	GA	IV	ST	AL	PQ	-	2057608													
1	MS	-	MG	LE	IT	GT	ALA	AV	LG	WL	GT	IV	CC	AL	PM	-	GI 2570129													
28	Y	WR	VS	TV	HG	GN	-	VI	TN	TI	FE	NL	WF	SC	AT	DS	1456746													
30	-	WR	IY	SY	AG	DN	NI	VT	AQ	AM	YE	GL	WM	SC	VS	QS	2057608													
29	-	WR	VS	AF	IG	SN	II	IT	SQ	NI	WE	GL	WM	NC	VV	QS	GI 2570129													
57	L	GV	YN	CW	EF	PS	M	L	ALS	GY	I	QA	C	R	AL	M	I	T	A	I	1456746									
59	T	G	Q	I	QC	KV	FD	SL	LN	LS	ST	LQ	AT	R	AL	M	V	V	G	I	2057608									
58	T	G	Q	M	QC	KV	YD	SL	L	AL	P	Q	D	L	Q	A	A	R	A	L	I	V	V	A	I	GI 2570129				
87	L	L	G	F	L	G	L	L	LG	I	AG	LR	CT	N	I	GG	LE	LS	R	K	A	Q	A	1456746						
89	L	L	G	V	I	A	I	F	VA	T	VG	M	K	CM	KL	ED	DE	V	Q	K	M	R	M	2057608						
88	L	L	A	A	F	G	L	L	VA	L	V	G	A	Q	C	T	N	C	V	Q	D	D	T	A	-	K	A	K	I	GI 2570129
117	G	G	H	R	A	L	H	I	L	AG	I	C	G	M	V	A	I	SW	Y	A	F	N	I	T	R	D	F	1456746		
119	A	V	I	G	A	I	F	L	L	AG	L	A	I	L	V	A	T	AW	Y	G	N	R	I	V	Q	E	F	2057608		
117	T	I	V	A	G	V	L	F	L	L	AA	L	L	T	L	V	P	SW	S	A	N	T	I	I	R	D	F	GI 2570129		

FIGURE 1A

147	F	D	P	L	Y	P	G	T	-	K	Y	E	L	G	P	A	L	Y	L	G	S	A	S	L	I	S	I	L	G	1456746		
149	Y	D	P	M	T	P	V	N	A	R	Y	E	F	G	Q	A	L	F	T	G	W	A	A	S	L	C	L	L	G	2057608		
147	Y	N	P	V	V	P	E	A	Q	K	R	E	M	G	A	G	L	Y	V	G	W	A	A	A	L	Q	L	L	G	GI 2570129		
176	G	L	C	L	C	S	A	C	C	C	G	S	D	E	D	P	A	A	S	A	R	R	P	Y	Q	A	P	V	S	V	1456746	
179	G	A	L	L	C	-	-	C	S	C	P	R	K	T	T	S	Y	P	T	P	R	P	-	Y	P	K	P	A	P	S	2057608	
177	G	A	L	L	C	-	-	C	S	C	P	P	R	E	K	K	Y	T	A	T	K	V	V	Y	S	A	P	R	S	T	GI 2570129	
206	M	P	V	A	T	S	D	Q	E	G	D	S	S	F	G	K	-	Y	G	R	N	A	Y	V								1456746
206	-	-	-	-	-	-	-	-	S	G	-	-	-	-	-	-	-	-	-	-	-	-	-	K	D	Y	V				2057608	
205	-	-	-	-	-	-	-	-	G	P	G	A	S	L	G	T	G	Y	D	R	K	D	Y	V								GI 2570129

FIGURE 1B

