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(21) International Application Number: PCT/US99/08165 (22) International Filing Date: 14 April 1999 (14.04.99) (30) Priority Data: 09/060,410 14 April 1998 (14.04.98) US (71) Applicant: BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM [US/US]; 201 West 7th Street, Austin, TX 78701-2981 (US). (72) Inventors: COBB, Melanie; 11724 Valley Dale, Dallas, TX 75230 (US). HUTCHISON, Michele; 1019 North Montclair, Dallas, TX 75208 (US). CHEN, Zhu; 2508A Rampart Street, Dallas, TX 75235 (US). BERMAN, Kevin; Apartment 2208, 4144 Office Parkway, Dallas, TX 75204 (US). (74) Agents: MAKI, David, J. et al.; Seed and Berry LLP, 6300 Columbia Center, 701 Fifth Avenue, Seattle, WA 98104-7092 (US).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, 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, UZ, VN, YU, ZA, 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>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: TAO PROTEIN KINASES AND METHODS OF USE THEREFOR (57) Abstract Compositions and methods are provided for potentiating the activity of the mitogen-activated protein kinase p38. In particular the mitogen-activated protein kinase MEK3, and variants thereof that stimulate phosphorylation of p38 are provided. Such compounds may be used, for example, for therapy of diseases associated with the p38 cascade and to identify antibodies and other agents that inhibit or activate signal transduction via p38.		

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TAO PROTEIN KINASES AND METHODS OF USE THEREFOR

STATEMENT OF GOVERNMENT INTEREST

5 The Government owns certain rights in the present invention pursuant to NIH Grant GM53032.

TECHNICAL FIELD

 The present invention relates generally to compositions and methods for
10 modulating the activity of the MAP/ERK kinase MEK3 and/or other MEK family members. The invention is more particularly related to TAO proteins, and variants thereof that stimulate phosphorylation and activation of MEK substrates, such as MEK3. The invention is further related to the use of such proteins, for example, to activate a stress-responsive MAP kinase pathway in an organism and to identify
15 antibodies and other agents that inhibit or activate signal transduction via such a pathway.

BACKGROUND OF THE INVENTION

 MAP kinase pathways are conserved signal transduction pathways that
20 activate transcription factors, translation factors and other target molecules in response to a variety of extracellular signals. Each pathway contains a MAP kinase module, consisting of a MAP kinase or ERK, a MAP/ERK kinase (MEK), and a MEK kinase (MEKK). In higher eucaryotes, activation of MAP kinase pathways has been correlated with cellular events such as proliferation, oncogenesis, development and differentiation.
25 Accordingly, the ability to regulate signal transduction via these pathways could lead to the development of treatments and preventive therapies for human diseases associated with MAP kinase pathways, such as inflammatory diseases, autoimmune diseases and cancer.

 Several MAP kinase pathways have been found in *S. cerevisiae* (Hunter
30 and Plowman, *Trends in Biochem. Sci.* 22:18-22, 1997), and parallel mammalian pathways have been identified based upon sequences of mammalian ERKs and yeast MAP kinases, KSS1 and FUS3 (Boulton et al., *Science* 249:64-67, 1990; Courchesne et

al., *Cell* 58: 1107-1119, 1989; Elion et al., *Cell* 60:649-664, 1990). The best delineated yeast MAP kinase pathway, activated by mating pheromones, is controlled by a receptor-G protein system, includes a Cdc42 small G protein, and requires at least three protein kinases, Ste20p (Leberer et al., *EMBO J.* 11:4815-4828, 1992; Ramer et al.,
5 *Proc. Natl. Acad. Sci. USA* 90:452-456, 1993), Ste11p (Rhodes et al., *Genes Dev.* 4:1862-1874, 1990), and Ste7p (Teague et al., *Proc. Natl. Acad. Sci. USA* 83:7371-7375, 1986), upstream of the MAP kinase Fus3p (Elion et al., *Cell* 60:649-664, 1990).

Ste20p was isolated from *S. cerevisiae* as a gene whose product
10 functions downstream of the $\beta\gamma$ subunits of a heterotrimeric G protein but upstream of enzymes in the MAP kinase module (MEKK, MEK, ERK) of the pheromone response pathway (Leberer et al., *EMBO J.* 11:4815-4828, 1992; Ramer et al., *Proc. Natl. Acad. Sci. USA* 90:452-456, 1993). Ste11p, the MEKK, may be one of the Ste20p substrates (Wu et al., *J. Biol. Chem.* 270:15984-15992, 1990); thus, Ste20p-like enzymes may
15 activate MEKKs in mammalian MAP kinase pathways. Ste20p, like its best studied mammalian counterparts, the p21-activated protein kinases (PAKs), is thought to be regulated by binding to Cdc42 through a conserved Cdc42/Rac interactive binding region, or CRIB domain (Burbelo et al., *J. Biol. Chem.* 270:29071-29074, 1995).

Mammalian relatives of Ste20p are diverse and include the PAK
20 subfamily (PAK1,2,3) and the mixed lineage kinase (MLK) subfamily, including the dual leucine zipper kinase (DLK), germinal center kinase (GCK), and the Nck-interacting kinase, NIK. In the past year, newly identified Ste20p-related kinases include members of the MLK subfamily, SOK-1, Krs-1 and -2, and MUK. MUK was isolated in a screen for MEKK isoforms, but in fact shows more identity to MLK. In
25 transfected cells several of these enzymes, as first shown with GCK, increase the activity of the stress-responsive kinases, particularly SAPK/JNK. In the case of NIK and GCK, they may work by binding to MEKK (Su et al., *EMBO J.* 16:1279-1290, 1997). However, several of these Ste20p-related enzymes also have MEKK activity. For example, DLK phosphorylates and potently activates MEKs that lie in the
30 stress-responsive cascades.

Further characterization of members of these pathways, and the identification of additional members, is critical for understanding the signal transduction pathways involved and for developing methods for activating or inactivating MEKs and MAP kinase pathways *in vivo*. Accordingly, there is a need in the art for improved methods for modulating the activity of members of MAP kinase pathways, and for treating diseases associated with such pathways. The present invention fulfills these needs and further provides other related advantages.

SUMMARY OF THE INVENTION

Briefly stated, the present invention provides compositions and methods for modulating the activity of MAP/ERK kinases such as MEK3, and stress-responsive MAP kinase pathways. Within certain aspects, the present invention provides TAO polypeptides. Within one such aspect, the polypeptide may comprise an amino acid sequence provided in SEQ ID NO:2 or SEQ ID NO:4, or a variant thereof in which the ability to phosphorylate MEK3 is not substantially diminished. In certain embodiments, such a polypeptide may comprise a sequence that differs from a sequence recited in SEQ ID NO:2 or SEQ ID NO:4 only in conservative substitutions and/or modifications at no more than 10% of the amino acid residues. In certain other embodiments, the polypeptide may be a constitutively active variant.

Within other aspects, the present invention provides polypeptides comprising an amino acid sequence provided in SEQ ID NO:2 or SEQ ID NO:4 modified at no more than 10% of the amino acid residues, such that the polypeptide is rendered constitutively inactive.

Within further aspects, the present invention provides polypeptides capable of phosphorylating MEK3, wherein the polypeptide does not detectably phosphorylate MEK1 or MEK2.

The present invention further provides, within other aspects, isolated polynucleotides encoding polypeptides as described above. Isolated polynucleotides comprising one or more sequences recited in any one of SEQ ID NOs:5-16, or a variant thereof, wherein the polynucleotide encodes a polypeptide capable of phosphorylating MEK3, are also provided. Polypeptides encoded by such polynucleotides are further

provided. Recombinant expression vectors comprising any of the above polynucleotides, and host cells transformed or transfected with such expression vectors, are provided within related aspects.

Within other aspects, the present invention provides antisense
5 polynucleotides comprising at least 10 nucleotides complementary to a polynucleotide as described above.

Within further aspects, pharmaceutical compositions are provided, comprising: (a) a polypeptide or polynucleotide as described above; and (b) a physiologically acceptable carrier.

10 The present invention further provides, within other aspects, methods for phosphorylating a MEK3 polypeptide, comprising contacting a MEK3 polypeptide with a polypeptide according to claim as described above, thereby phosphorylating the MEK3 polypeptide.

Within further aspects, the present invention provides methods for
15 activating a member of a stress-responsive MAP kinase pathway in an organism, comprising administering to an organism a polypeptide as described above, and thereby activating a member of a stress-responsive MAP kinase pathway.

Within other aspects, methods are provided for phosphorylating a MEK3 polypeptide comprising contacting a MEK3 polypeptide with a polypeptide as
20 described above, and thereby phosphorylating the MEK3 polypeptide.

The present invention further provides methods for activating a member of a stress-responsive MAP kinase pathway in an organism, comprising administering to an organism a polypeptide as described above, and thereby activating a member of the stress-responsive MAP kinase pathway.

25 Within further aspects, the present invention provides methods for screening for an agent that modulates signal transduction via a stress-responsive MAP kinase pathway, comprising: (a) contacting a candidate agent with a polypeptide as described above; and (b) subsequently measuring the ability of the polypeptide to modulate the activity of a MEK3 polypeptide, and thereby evaluating the ability of the
30 compound to modulate signal transduction via a stress-responsive MAP kinase pathway.

The present invention further provides, within other aspects, monoclonal antibodies and antigen-binding fragments thereof that specifically bind to a polypeptide as described above. Such monoclonal antibodies or fragments thereof may inhibit the phosphorylation of MEK3 by the polypeptide. Pharmaceutical compositions comprising: (a) an antibody or antigen-binding fragment thereof as described above; and (b) a physiologically acceptable carrier are also provided.

Within other aspects, the present invention provides methods for treating a patient afflicted with a disease associated with a stress-responsive MAP kinase pathway, comprising administering to a patient a compound that modulates the phosphorylation of MEK3. Within certain embodiments, the compound comprises a monoclonal antibody or antigen-binding fragment thereof or a nucleotide sequence. Within such methods, the compound may inhibit phosphorylation of MEK3 and the disease may be inflammation, an autoimmune disease, cancer or a degenerative disease. Alternatively, the compound may enhance the phosphorylation of MEK3 and the disease may be insulin-dependent diabetes or a neurodegenerative disease.

Within other aspects, methods are provided for determining the presence or absence of TAO kinase activity in a sample, comprising evaluating the ability of the sample to phosphorylate a MEK3 polypeptide, and thereby determining the presence or absence of TAO kinase activity in the sample.

Within related aspects, kits are provided for detecting TAO kinase activity in a sample, comprising a MEK3 polypeptide in combination with a suitable buffer.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 presents the nucleotide and predicted amino acid sequence of a representative TAO1 kinase (SEQ ID NOs: 1 and 2).

Figure 2 presents a comparison of the catalytic domains of TAO1 (residues 1-273 of SEQ ID NO:2), TAO2 (residues 1-273 of SEQ ID NO:4), STE20 (SEQ ID NO:17) and the *C. elegans* homolog (ceTAO) (SEQ ID NO:18). The catalytic domains were aligned by eye and the conserved amino acids bolded. The domains are indicated with roman numerals.

Figures 3A and 3B are Northern blots, showing TAO1 (Figure 3A) and TAO2 (Figure 3B) expression in various tissues. Various rat poly-A⁺ RNAs were probed, as indicated. Equal loading of RNA was verified by hybridizing the blot to an actin probe (not shown).

Figures 4A and 4B are Northern blots in which RNAs made from various human brain and spinal cord sections were hybridized to a TAO1-specific probe. Shown below each blot is the result of its hybridization to an actin probe. The lanes are as follows: 1, amygdala, 2, caudate nucleus, 3, corpus callosum, 4, hippocampus, 5, whole brain, 6, substantia nigra, 7, subthalamic nucleus, 8, thalamus, 9, cerebellum, 10, cerebral cortex, 11, medulla, 12, spinal cord, 13, occipital lobe, 14, frontal lobe, 15, temporal lobe, 16, putamen.

Figures 5A-5C are immunoblots. In Figure 5A, human embryonic kidney 293 cells were transiently transfected with either vector or pCMV5TAO1(HA)₃, and 24 hours later lysates were immunoblotted with a monoclonal antibody directed against the HA epitope. TAO1 is indicated by the arrow. In Figure 5B, the TAO1 proteins purified from Sf9 cells were immunoblotted with an antibody directed against the MRGS(H)₆ epitope. In Figure 5C, 50ng of (His)₆TAO1 was immunoblotted with polyclonal antisera P820 directed against a TAO1 peptide. An equal amount was blotted with the preimmune serum for P820.

Figure 6 is an autoradiogram showing the results of a representative *in vitro* linked kinase assay to estimate MEK activation by TAO1. Either 50 ng (lanes 1 and 3) or 250 ng (lanes 2 and 4) of (His)₆TAO1(1-416) was incubated with 50 ng of (His)₆MEK3 for one hour at 30° in the presence of Mg/ATP, after which a portion of the each reaction was added to a second reaction containing (His)₆p38. After a one hour incubation, the reactions were subjected to SDS-PAGE and autoradiography.

Figure 7 is an autoradiogram showing the results of a representative *in vitro* linked kinase assay to estimate MEK activation by TAO1. Only the second part of the linked assay is shown. The assay was identical to that described in Figure 6, except that GSTMEK4 was substituted for MEK3, and both (His)₆p38 and GSTSAPKβ were used as MEK4 substrates.

Figure 8 is an autoradiogram showing the results of a representative *in vitro* linked kinase assay to estimate MEK activation by TAO1. The assay was as described in Figures 6 and 7, but was performed with GSTMEK6 and (His)₆p38 as the MEK6 substrate.

Figure 9 is a histogram comparing the fold activations of MEKs 1 through 6 by (His)₆TAO1(1-416).

Figure 10 is an autoradiogram illustrating TAO1 activation of MEK3 *in vivo*. Human embryonic kidney 293 cells were transiently transfected with either vector alone, or pCMV5TAO1(HA)₃ and pCMV5mycMEK3, alone and in combination. Immunoprecipitates made with a monoclonal antibody directed against the myc epitope were subjected to *in vitro* kinase assays with (His)₆p38 as substrate. Myc-tagged MEK3 expression detected with a polyclonal anti-MEK3 antisera is shown below. In several separate experiments, MEK3 activity in the immunoprecipitates was increased 3 to 4 fold when coexpressed with TAO1.

Figure 11 is an autoradiogram illustrating the copurification of TAO1 and endogenous MEK3 from Sf9 cells. Either 100μg of Sf9 whole cell lysate, or 1μg each of the recombinant TAO1 proteins purified from Sf9 cells was Western blotted with polyclonal antisera directed against MEK3 (top panel) or MEK4 (lower panel). An identical Western blot performed with an antisera against MEK6 did not detect MEK6 protein in either the Sf9 lysate or the TAO1 preparations.

Figure 12 presents an alignment of a human retina cDNA EST (subject; SEQ ID NO:5) with nts. 2341-2754 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

Figure 13 presents an alignment of a human retina cDNA EST (subject; SEQ ID NO:6) with nts. 964-651 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

Figure 14 presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:7) with nts. 2792-2423 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

Figure 15A presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:8) with nts. 2248-2437 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1). Figure 15B presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:9) with nts. 2437-2501 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

Figure 16 presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:10) with nts. 2087-2305 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

Figure 17A presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:11) with nts. 3228-3312 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1). Figure 17B presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:12) with nts. 3200-3245 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

Figure 18 presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:13) with nts. 739-854 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

Figure 19A presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:14) with nts. 526-643 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1). Figure 19B presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:15) with nts. 187-296 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

Figure 20 presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:16) with nts. 866-733 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is generally directed to compounds and methods for modulating (*i.e.*, stimulating or inhibiting) the activity of

MAP/ERK family members such as the MAP/ERK kinase MEK3. Compounds that activate such MEKs generally stimulate MEK phosphorylation. Such compounds include Ste20p homologs referred to herein as TAO polypeptides (*i.e.*, TAO1, TAO2 or a variant of TAO1 or TAO2 that retains the ability to stimulate MEK3 phosphorylation at a level that is not substantially lower than the level stimulated by the native protein).
5 Alternatively, a compound that activates MEK3 may comprise a polynucleotide that encodes a TAO polypeptide. Within other embodiments, compositions that stimulate MEK3 phosphorylation (thereby activating MEK3) may also, or alternatively, include one or more agents that stimulate TAO polypeptide expression or kinase activity. Such
10 agents include, but are not limited to, stress-inducing agents (*e.g.*, DNA-damaging agents). Additional such agents may be identified by combining a test compound with a TAO polypeptide *in vitro* and evaluating the effect of the test compound on the kinase activity of the polypeptide using, for example, a representative assay described herein.

Compositions that inhibit the activity of MEKs generally inhibit MEK
15 phosphorylation. Such compositions may include one or more agents that inhibit or block TAO polypeptide activity, such as an antibody that inhibits the kinase activity of a TAO polypeptide, a competing peptide that represents the substrate binding domain of a TAO protein or a phosphorylation motif of the MEK3 substrate, an antisense polynucleotide or ribozyme that interferes with transcription and/or translation of a
20 TAO polypeptide, a molecule that inactivates a TAO polypeptide by binding to the polypeptide, a molecule that binds to the TAO substrate and prevents phosphorylation by a TAO polypeptide or a molecule that prevents transfer of phosphoryl groups from the kinase to the substrate. Agents that inhibit TAO polypeptide kinase activity may be identified by combining a test compound with a TAO polypeptide *in vitro* and
25 evaluating the activity of the TAO polypeptide using a TAO kinase assay.

TAO POLYNUCLEOTIDES

Any polynucleotide that encodes a TAO polypeptide, or a portion or variant thereof as described herein, is encompassed by the present invention. Such
30 polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. Additional coding or

non-coding sequences may, but need not, be present within a polynucleotide of the present invention, and a TAO polynucleotide may, but need not, be linked to other molecules and/or support materials.

Native TAO DNA sequences, or portions thereof, may be isolated using
5 any of a variety of hybridization or amplification techniques, which are well known to those of ordinary skill in the art. Within such techniques, probes or primers may be designed based on the TAO sequences provided herein, and may be purchased or synthesized. Libraries from any suitable tissue (*e.g.*, brain) may be screened. An amplified portion or partial cDNA molecule may then be used to isolate a full length
10 gene from a genomic DNA library or from a cDNA library, using well known techniques. Alternatively, a full length gene can be constructed from multiple PCR fragments.

Nucleic acid sequences corresponding to the native rat TAO polypeptides TAO1 and TAO2 are provided in SEQ ID NO:1 and SEQ ID NO:3,
15 respectively. One preferred variant of TAO1 comprises amino acids 1-416 of SEQ ID NO:1. The predicted TAO1 open reading frame encodes a polypeptide of 1001 amino acids with a calculated molecular mass of 134 kD. TAO1 comprises an amino-terminal catalytic domain and an extensive carboxy-terminal region that has several distinguishing features, such as a possible nucleotide binding site and acidic stretch just
20 carboxy-terminal to the catalytic domain, as well as two serine-rich regions. The TAO1 catalytic domain extends 263 amino acids from amino acid 25 to 288 with all 11 of the typical protein kinase subdomains conserved. There are two glutamate residues between TAO1 subdomains II and IV; the second glutamate at amino acid 76 contained in the sequence KEVK is most likely to represent subdomain III (Hanks et al., *Science*
25 241:42-52, 1988). The features of the TAO1 catalytic domain are most similar to the serine/threonine family of protein kinases; subdomain VIb with the sequence HRDIKAGN (SEQ ID NO:26) suggests that TAO1 is likely to be a serine/threonine protein kinase.

A partial sequence of the closely related gene TAO2 is provided in SEQ
30 ID NOs:3 and 4. TAO2 has a similar arrangement of an amino-terminal kinase domain and a long carboxy-terminus, but differs in that it contains an acidic insert of 17

glutamate residues carboxy-terminal to the catalytic domain, and lacks the putative nucleotide binding site of TAO1.

The rat TAO1 and TAO2 transcripts are highly expressed in brain. Lower levels of TAO1 expression can be observed in heart and lung, with no detectable
5 signal (using a Northern blot analysis as described herein) in skeletal muscle, liver, kidney, testis, epididymus and spleen.

The polynucleotides specifically recited herein, as well as full length polynucleotides comprising such sequences, other portions of full length polynucleotides, and sequences complementary to all or a portion of such full length
10 molecules, are specifically encompassed by the present invention. In addition, TAO homologs from other species are specifically contemplated, and may generally be prepared as described herein for the rat homologs. In particular, within the context of the present invention, EST database sequences derived from retinal mRNAs have been identified that correspond to the human counterpart for TAO1. The sequences of these
15 ESTs are provided in SEQ ID NOs:5-16. It will be readily apparent to those of ordinary skill in the art that a full length, native, human TAO1 polynucleotide may be identified based on such sequences, using for example, standard hybridization or amplification techniques. Such full length TAO1 sequences are contemplated by the present invention, as are polypeptides encoded by such sequences, and variants of the
20 naturally occurring sequences as discussed herein.

Polynucleotide variants of the recited sequences may differ from a native TAO polynucleotide in one or more substitutions, deletions, insertions and/or modifications. Certain variants encode a polypeptide that retains the ability to stimulate MEK3 phosphorylation at a level that is not substantially lower than the level
25 stimulated by the native protein. The effect on the properties of the encoded polypeptide may generally be assessed as described herein. Preferred variants contain nucleotide substitutions, deletions, insertions and/or modifications at no more than 20%, preferably at no more than 10%, of the nucleotide positions. Certain variants are substantially homologous to a native gene, or a portion or complement thereof. Such
30 polynucleotide variants are capable of hybridizing under moderately stringent conditions to a naturally occurring DNA sequence encoding a TAO protein (or a

complementary sequence). Suitable moderately stringent conditions include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5 X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS). Such hybridizing DNA
5 sequences are also within the scope of this invention.

It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides
10 that vary due to differences in codon usage are specifically contemplated by the present invention.

As noted above, the present invention further provides antisense polynucleotides and portions of any of the above sequences. Such polynucleotides may generally be prepared by any method known in the art, including synthesis by, for
15 example, solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* or *in vivo* transcription of DNA sequences that are incorporated into a vector downstream of a suitable RNA polymerase promoter (such as T3, T7 or SP6). Certain portions of a TAO polynucleotide may be used to prepare an encoded polypeptide, as described herein. In addition, or alternatively, a
20 portion may function as a probe (*e.g.*, to detect TAO expression in a sample), and may be labeled by a variety of reporter groups, such as radionuclides, fluorescent dyes and enzymes. Such portions are preferably at least 10 nucleotides in length, and more preferably at least 20 nucleotides in length. Within certain preferred embodiments, a portion for use as a probe comprises a sequence that is unique to a TAO gene. A
25 portion of a sequence complementary to a coding sequence (*i.e.*, an antisense polynucleotide) may also be used as a probe or to modulate gene expression. DNA constructs that can be transcribed into antisense RNA may also be introduced into cells or tissues to facilitate the production of antisense RNA.

Any polynucleotide may be further modified to increase stability *in vivo*.
30 Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather

than phosphodiesterase linkages in the backbone; and/or the inclusion of nontraditional bases such as inosine, queosine and wybutosine, as well as acetyl-, methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine and uridine.

Nucleotide sequences as described herein may be joined to a variety of other nucleotide sequences using established recombinant DNA techniques. For example, a polynucleotide may be cloned into any of a variety of cloning vectors, including plasmids, phagemids, lambda phage derivatives and cosmids. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors and sequencing vectors. In general, a vector will contain an origin of replication functional in at least one organism, convenient restriction endonuclease sites and one or more selectable markers. Additional initial, terminal and/or intervening DNA sequences that, for example, facilitate construction of readily expressed vectors may also be present. Suitable vectors may be obtained commercially or assembled from the sequences described by methods well-known in the art. Other elements that may be present in a vector will depend upon the desired use, and will be apparent to those of ordinary skill in the art.

Vectors as described herein may generally be transfected into a suitable host cell, such as a mammalian cell, by methods well-known in the art. Such methods include calcium phosphate precipitation, electroporation and microinjection.

20

TAO POLYPEPTIDES

Polypeptides within the scope of the present invention comprise at least a portion of a TAO protein (*e.g.*, TAO1 or TAO2) or variant thereof, where the portion is immunologically and/or biologically active. Preferred variants retain the ability to stimulate MEK3 phosphorylation at a level that is not substantially lower than the level stimulated by the native protein. A polypeptide may further comprise additional sequences, which may or may not be derived from a native TAO protein. Such sequences may (but need not) possess immunogenic or antigenic properties and/or a biological activity.

30

A polypeptide "variant," as used herein, is a polypeptide that differs from a native protein in substitutions, insertions, deletions and/or amino acid

modifications, such that the immunogenic and/or biological properties of the native protein are not substantially diminished. A variant preferably retains at least 80% sequence identity to a native sequence, more preferably at least 90% identity, and even more preferably at least 95% identity. Within certain preferred embodiments, such variants contain alterations at no more than 10% of the amino acid residues in the native polypeptide, such that the ability of the variant to stimulate MEK3 phosphorylation is not substantially diminished. Guidance in determining which and how many amino acid residues may be substituted, inserted, deleted and/or modified without diminishing immunological and/or biological activity may be found using any of a variety of methods and computer programs known in the art. Properties of a variant may generally be evaluated by assaying the reactivity of the variant with, for example, antibodies as described herein and/or evaluating a biological property characteristic of the native protein.

A polypeptide is "immunologically active," within the context of the present invention if it is recognized (*i.e.*, specifically bound) by a B-cell and/or T-cell surface antigen receptor. Immunological activity may generally be assessed using well known techniques, such as those summarized in Paul, *Fundamental Immunology*, 3rd ed., 243-247 (Raven Press, 1993) and references cited therein. Such techniques include screening polypeptides derived from the native polypeptide for the ability to react with antigen-specific antisera and/or T-cell lines or clones, which may be prepared using well known techniques. An immunologically active portion of a TAO protein reacts with such antisera and/or T-cells at a level that is not substantially lower than the reactivity of the full length polypeptide (*e.g.*, in an ELISA and/or T-cell reactivity assay). Such screens may generally be performed using methods well known to those of ordinary skill in the art, such as those described in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. B-cell and T-cell epitopes may also be predicted via computer analysis.

Similarly, a polypeptide is "biologically active" if the ability to phosphorylate MEK3 and/or other MEKs is not substantially diminished within a representative *in vitro* assay as described in Example 3. Preferably, the ability of the polypeptide to phosphorylate MEK3 is not substantially diminished. As used herein,

the term "not substantially diminished" means retaining an activity that is at least 90% of the activity of a native TAO protein. Appropriate assays designed to evaluate such activity may be designed based on existing assays known in the art, and on the representative assays provided herein.

5 Preferably, a variant contains conservative substitutions. A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydrophobic nature of the polypeptide to be substantially unchanged. Amino acid substitutions may generally be
10 made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and
15 alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. A variant may also, or alternatively, contain nonconservative changes.

20 In general, modifications may be more readily made in non-critical regions, which are regions of the native sequence that do not substantially change the properties of the TAO protein. Non-critical regions may be identified by modifying the TAO sequence in a particular region and assaying the activity of the resulting variant in a kinase assay, using MEK3, MEK4, MEK6 or another MEK family member as a
25 substrate, as described herein. Modifications may also be made in critical regions of a TAO protein, provided that the resulting variant retains the ability to stimulate MEK3 phosphorylation and/or an immunogenic property of the native protein. Inactive proteins may be created by modifying certain critical regions. One critical region comprises the aspartate 169 residue. Modification of that residue results in a
30 catalytically defective mutant. Another critical region encompasses the lysine 57 residue. The effect of any modification on the ability of the variant to stimulate

phosphorylation of MEK3 or other MEKs may generally be evaluated using any assay for TAO kinase activity, such as the representative assays described herein.

5 Variants of TAO proteins include constitutively active proteins. In general, activation of a TAO protein *in vivo* requires stimulation by a stimulus such as a stress-inducing agent. Constitutively active variants display the ability to stimulate MEK phosphorylation in the absence of such stimulation. Such variants may be identified using the representative *in vivo* assays for TAO kinase activity described herein.

TAO proteins may also be modified so as to render the protein
10 constitutively inactive (*i.e.*, unable to phosphorylate MEKs even when stimulated as described above). Such modifications may be identified using the representative assays described herein. Genes encoding proteins modified so as to be constitutively active or inactive may generally be used in replacement therapy for treatment of a variety of disorders, as discussed in more detail below.

15 Variants within the scope of this invention also include polypeptides in which the primary amino acid structure of a native protein is modified by forming covalent or aggregative conjugates with other polypeptides or chemical moieties such as glycosyl groups, lipids, phosphate, acetyl groups and the like. Covalent derivatives may be prepared, for example, by linking particular functional groups to amino acid
20 side chains or at the N- or C-termini.

The present invention also includes polypeptides with or without associated native-pattern glycosylation. Polypeptides expressed in yeast or mammalian expression systems may be similar to or slightly different in molecular weight and glycosylation pattern than the native molecules, depending upon the expression system.
25 Expression of DNA in bacteria such as *E. coli* provides non-glycosylated molecules. N-glycosylation sites of eukaryotic proteins are characterized by the amino acid triplet Asn-A₁-Z, where A₁ is any amino acid except Pro, and Z is Ser or Thr. Variants having inactivated N-glycosylation sites can be produced by techniques known to those of ordinary skill in the art, such as oligonucleotide synthesis and ligation or site-specific
30 mutagenesis techniques, and are within the scope of this invention. Alternatively, N-linked glycosylation sites can be added to a polypeptide.

As noted above, polypeptides may further comprise sequences that are not related to an endogenous TAO protein. For example, an N-terminal signal (or leader) sequence may be present, which co-translationally or post-translationally directs transfer of the polypeptide from its site of synthesis to a site inside or outside of the cell membrane or wall (*e.g.*, the yeast α -factor leader). The polypeptide may also comprise a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (*e.g.*, poly-His, hemagglutinin, glutathione-S-transferase or FLAG), or to enhance polypeptide stability or binding to a solid support. Protein fusions encompassed by this invention further include, for example, polypeptides conjugated to an immunoglobulin Fc region or a leucine zipper domain. All of the above protein fusions may be prepared by chemical linkage or as fusion proteins.

Also included within the present invention are alleles of a TAO protein. Alleles are alternative forms of a native protein resulting from one or more genetic mutations (which may be amino acid deletions, additions and/or substitutions), resulting in an altered mRNA. Allelic proteins may differ in sequence, but overall structure and function are substantially similar.

TAO polypeptides, variants and portions thereof may generally be prepared from nucleic acid encoding the desired polypeptide using well known techniques. To prepare an endogenous protein, an isolated cDNA may be used. To prepare a variant polypeptide, standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis may be used, and sections of the DNA sequence may be removed to permit preparation of truncated polypeptides.

In general, any of a variety of expression vectors known to those of ordinary skill in the art may be employed to express recombinant polypeptides of this invention. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA sequence that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast, baculovirus-infected insect cells and animal cells. Following expression, supernatants from host/vector systems which secrete recombinant protein or polypeptide into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as

an affinity matrix or an ion exchange resin. One or more reverse phase HPLC steps can be employed to further purify a recombinant polypeptide.

Portions and other variants having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may also be generated by synthetic means, using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. *See* Merrifield, *J. Am. Chem. Soc.* 85:2149-2146, 1963. Various modified solid phase techniques are also available (*e.g.*, the method of Roberge et al., *Science* 269:202-204, 1995). Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Applied BioSystems, Inc. (Foster City, CA), and may be operated according to the manufacturer's instructions.

In general, polypeptides and polynucleotides as described herein are isolated. An "isolated" polypeptide or polynucleotide is one that is removed from its original environment. For example, a naturally-occurring protein is isolated if it is separated from some or all of the coexisting materials in the natural system. Preferably, polypeptides provided herein are isolated to a purity of at least 80% by weight, more preferably to a purity of at least 95% by weight, and most preferably to a purity of at least 99% by weight. In general, such purification may be achieved using, for example, the standard techniques of ammonium sulfate fractionation, SDS-PAGE electrophoresis, and affinity chromatography. A polynucleotide is considered to be isolated if, for example, it is cloned into a vector that is not a part of the natural environment.

ANTIBODIES AND FRAGMENTS THEREOF

The present invention further provides antibodies, and antigen-binding fragments thereof, that specifically bind to a TAO polypeptide. As used herein, an antibody, or antigen-binding fragment, is said to "specifically bind" to a TAO polypeptide if it reacts at a detectable level (within, for example, an ELISA) with a TAO polypeptide, and does not react detectably with unrelated proteins. Antibodies

may be polyclonal or monoclonal. Preferred antibodies are those antibodies that inhibit or block TAO activity *in vivo* and within a kinase assay as described herein. Other preferred antibodies (which may be used, for example, in immunokinase assays) are those that immunoprecipitate active TAO1 and/or TAO2.

5 Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art (*see, e.g.,* Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988). In one such technique, an immunogen comprising the polypeptide is initially injected into a suitable animal (*e.g.,* mice, rats, rabbits, sheep and goats), preferably according to a predetermined schedule
10 incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

 Monoclonal antibodies specific for a TAO polypeptide may be prepared,
15 for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.* 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e.,* reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above.
20 The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection
25 technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

 Monoclonal antibodies may be isolated from the supernatants of
30 growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity

of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction.

5 Within certain embodiments, the use of antigen-binding fragments of antibodies may be preferred. Such fragments include Fab fragments, which may be prepared using standard techniques. Briefly, immunoglobulins may be purified from rabbit serum by affinity chromatography on Protein A bead columns (Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988) and digested
10 by papain to yield Fab and Fc fragments. The Fab and Fc fragments may be separated by, for example, affinity chromatography on protein A bead columns.

METHODS AND KITS FOR DETECTING TAO POLYPEPTIDES AND TAO KINASE ACTIVITY

 The present invention provides methods for detecting the level of TAO1
15 and/or TAO2 in a sample, as well as for detecting TAO kinase activity in a sample. The level of a TAO polypeptide or polynucleotide may generally be determined using a reagent that binds to the TAO protein, DNA or mRNA. To detect nucleic acid encoding a TAO protein, standard hybridization and/or PCR techniques may be employed using a nucleic acid probe or a PCR primer. Suitable probes and primers
20 may be designed by those of ordinary skill in the art based on the TAO cDNA sequences provided herein. To detect TAO protein, the reagent is typically an antibody, which may be prepared as described herein.

 There are a variety of assay formats known to those of ordinary skill in the art for using an antibody to detect a polypeptide in a sample. *See, e.g.*, Harlow and
25 Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. For example, the antibody may be immobilized on a solid support such that it can bind to and remove the polypeptide from the sample. The bound polypeptide may then be detected using a second antibody that binds to the antibody/peptide complex and contains a detectable reporter group. Alternatively, a competitive assay may be
30 utilized, in which polypeptide that binds to the immobilized antibody is labeled with a reporter group and allowed to bind to the immobilized antibody after incubation of the

antibody with the sample. The extent to which components of the sample inhibit the binding of the labeled polypeptide to the antibody is indicative of the level of polypeptide within the sample. Suitable reporter groups for use in these methods include, but are not limited to, enzymes (*e.g.*, horseradish peroxidase), substrates, 5 cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin.

For detecting an active TAO protein in a sample, an immunokinase assay may be employed. Briefly, polyclonal or monoclonal antibodies may be raised against a unique sequence of a TAO protein (such as amino acid residues 296-315, 403- 10 418, 545-563 or 829-848) using standard techniques. A sample to be tested, such as a cellular extract, is incubated with the anti-TAO antibodies to immunoprecipitate a TAO protein, and the immunoprecipitated material is then incubated with a substrate (*e.g.*, MEK3) under suitable conditions for substrate phosphorylation. The level of substrate phosphorylation may generally be determined using any of a variety of assays, as 15 described herein.

TAO kinase assays, for use in evaluating the polypeptide variants and other agents discussed herein, include any assays that evaluate a compound's ability to phosphorylate MEK3 or other MEKs, thereby rendering the MEK active (*i.e.*, capable of phosphorylating *in vivo* substrates such as p38). MEKs such as MEK3 for use in 20 such methods may be endogenous proteins or variants thereof, may be purified or recombinant, and may be prepared using any of a variety of techniques that will be apparent to those of ordinary skill in the art. For example, cDNA encoding MEK3 may be cloned by PCR amplification from a suitable human cDNA library, using polymerase chain reaction (PCR) and methods well known to those of ordinary skill in 25 the art. MEK3 may be cloned using primers based on the published sequence (Derijard et al., *Science* 267:682-685, 1995). MEK3 cDNA may then be cloned into a bacterial expression vector and the protein produced in bacteria, such as *E. coli*, using standard techniques. The bacterial expression vector may, but need not, include DNA encoding an epitope such as glutathione-S transferase protein (GST) such that the recombinant 30 protein contains the epitope at the N- or C-terminus.

A TAO kinase assay may generally be performed as described herein. Briefly, a TAO polypeptide may be incubated with MEK3 and [γ - ^{32} P]ATP in a suitable buffer (such as 50 mM HEPES pH 8, 10 mM MgCl₂, 1 mM DTT, 100 μ M ATP) for 60 minutes at 30°C. In general, approximately 50 ng to 1 μ g of the polypeptide and 50 ng
5 recombinant MEK3, with 2-7 cpm/fmol [γ - ^{32} P]ATP, is sufficient. Proteins may then be separated by SDS-PAGE on 10% gels and subjected to autoradiography. Incorporation of [^{32} P]phosphate into MEK3 may be quantitated using techniques well known to those of ordinary skill in the art, such as with a phosphorimager. To evaluate the substrate specificity of polypeptide variants, a kinase assay may generally be performed as
10 described above except that other MEK substrates (*i.e.*, MEK1, 2, 4 or 6) are substituted for the MEK3.

To determine whether MEK3 phosphorylation results in activation, a coupled *in vitro* kinase assay may be performed using a substrate for MEK3, such as p38, with or without an epitope tag. p38 for use in such an assay may be prepared as
15 described in Han et al., *J. Biol. Chem.* 271:2886-2891, 1996. Briefly, following phosphorylation of MEK3 as described above, the MEK3 (*e.g.*, 0.1-10 ng) may be incubated with p38 (*e.g.*, 10 μ g/ml) and [γ - ^{32} P]ATP in a kinase buffer as described herein. It should be noted that alternative buffers may be used and that buffer composition can vary without significantly altering kinase activity. Reactions may be
20 separated by SDS-PAGE, visualized by autoradiography and quantitated using any of a variety of known techniques. Activated MEK3 will be capable of phosphorylating p38 at a level that is at least 5% above background using such an assay.

The present invention further provides kits for detecting TAO polypeptides and TAO kinase activity. Such kits may be designed for detecting the
25 level of a TAO polypeptide or polynucleotide, or may detect phosphorylation of MEK3 in a direct kinase assay or a coupled kinase assay, in which the level of phosphorylation and/or the kinase activity of MEK3 may be determined. TAO polypeptides and TAO kinase activity may be detected in any of a variety of samples, such as eukaryotic cells, bacteria, viruses, extracts prepared from such organisms and fluids found within living
30 organisms. In general, the kits of the present invention comprise one or more containers enclosing elements, such as reagents or buffers, to be used in the assay.

A kit for detecting the level of TAO polypeptide or polynucleotide typically contains a reagent that binds to TAO1 and/or TAO2 protein, DNA or RNA. To detect nucleic acid encoding a TAO polypeptide, the reagent may be a nucleic acid probe or a PCR primer. To detect a TAO protein, the reagent is typically an antibody.

5 The kit also contains a reporter group suitable for direct or indirect detection of the reagent (*i.e.*, the reporter group may be covalently bound to the reagent or may be bound to a second molecule, such as Protein A, Protein G, immunoglobulin or lectin, which is itself capable of binding to the reagent). Suitable reporter groups include, but are not limited to, enzymes (*e.g.*, horseradish peroxidase), substrates, cofactors,

10 inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. Such reporter groups may be used to directly or indirectly detect binding of the reagent to a sample component using standard methods known to those of ordinary skill in the art.

A kit for detecting TAO kinase activity based on measuring the phosphorylation of MEK3 generally comprises MEK3 in combination with a suitable

15 buffer. A kit for detecting TAO kinase activity based on detecting MEK3 activity generally comprises MEK3 in combination with a suitable MEK3 substrate, such as p38. Optionally, the kit may additionally comprise a suitable buffer and/or material for purification of MEK3 after activation and before combination with substrate. Such kits

20 may be employed in direct or coupled kinase assays, which may be performed as described above.

METHODS FOR IDENTIFYING BINDING AGENTS AND MODULATING AGENTS

The present invention further provides methods for identifying

25 antibodies and other compounds that bind to and/or modulate the activity of a TAO polypeptide. To evaluate the effect of a candidate modulating agent on TAO polypeptide activity, a kinase assay may be performed as described above, except that the candidate modulating agent is added to the incubation mixture. Briefly, the reaction components, which include the composition to be tested and the TAO polypeptide or a

30 polynucleotide encoding the kinase, are incubated under conditions sufficient to allow the components to interact. Subsequently, the effect of composition on kinase activity

or on the level of a polynucleotide encoding the kinase is measured. The observed effect on the kinase may be either inhibitory or stimulatory. The increase or decrease in kinase activity can be measured by, for example, adding a radioactive compound such as ^{32}P -ATP to the mixture of components, and observing radioactive incorporation into
5 MEK3 or other suitable substrate for a TAO polypeptide, to determine whether the compound inhibits or stimulates kinase activity. A polynucleotide encoding the kinase may be inserted into an expression vector and the effect of a composition on transcription of TAO mRNA can be measured, for example, by Northern blot analysis.

Within such assays, the candidate agent may be preincubated with a
10 TAO polypeptide before addition of ATP and substrate. Alternatively, the substrate may be preincubated with the candidate agent before the addition of kinase. Further variations include adding the candidate agent to a mixture of TAO polypeptide and ATP before the addition of substrate, or to a mixture of substrate and ATP before the addition of TAO polypeptide. Any of these assays can further be modified by
15 removing the candidate agent after the initial preincubation step. In general, a suitable amount of antibody or other candidate agent for use in such an assay ranges from about 0.1 μM to about 10 μM . The effect of the agent on TAO kinase activity may then be evaluated by quantitating the incorporation of [^{32}P]phosphate into MEK3, as described above, and comparing the level of incorporation with that achieved using the TAO
20 polypeptide without the addition of the candidate agent.

TAO kinase activity may also be measured in whole cells transfected with a reporter gene whose expression is dependent upon the activation of MEK3. For example, polynucleotides encoding a TAO polypeptide and a substrate (*e.g.*, MEK3) may be cotransfected into a cell. The substrate may then be immunoprecipitated, and
25 its activity evaluated in an *in vitro* assay. Alternatively, cells may be transfected with a ATF2-dependent promoter linked to a reporter gene such as luciferase. In such a system, expression of the luciferase gene (which may be readily detected using methods well known to those of ordinary skill in the art) depends upon activation of ATF2 by p38, which may be achieved by the stimulation of MEK3 with a TAO polypeptide.
30 Candidate modulating agents may be added to the system, as described below, to evaluate their effect on TAO polypeptide activity.

Alternatively, a whole cell system may employ only the transactivation domain of ATF2 fused to a suitable DNA binding domain, such as GHF-1 or GAL4. The reporter system may then comprise the GH-luciferase or GAL4-luciferase plasmid. Candidate TAO protein modulating agents may then be added to the system to evaluate
5 their effect on ATF2-specific gene activation.

In other aspects of the subject invention, methods for using the above polypeptides to phosphorylate and activate MEK3, peptide derivatives thereof or other MEK family members are provided. MEK substrate for use in such methods may be prepared as described above. In one embodiment, MEK3 may be phosphorylated *in*
10 *vitro* by incubation with a TAO polypeptide and ATP in a suitable buffer as described above. In general, the amounts of the reaction components may range from about 0.1 µg to about 10 µg of TAO polypeptide, from about 0.1 µg to about 10 µg of recombinant MEK3, and from about 100 nM to about 1 mM (preferably about 100 pmol - 30 nmol) of ATP. Phosphorylated proteins may then be purified by binding to
15 GSH-sepharose and washing. The extent of MEK3 phosphorylation may generally be monitored by adding [γ -³²P]ATP to a test aliquot, and evaluating the level of MEK3 phosphorylation as described above. The activity of the phosphorylated MEK3 may be evaluated using a coupled *in vitro* kinase assay, as described above.

Once activated *in vitro*, MEK3 may be used, for example, to identify
20 agents that inhibit the kinase activity of MEK3. Such inhibitory agents, which may be antibodies or drugs, may be identified using the coupled assay described above. Briefly, a candidate agent may be included in the mixture of MEK3 and p38, with or without pre-incubation with one or more components of the mixture, as described above. In general, a suitable amount of antibody or other agent for use in such an assay
25 ranges from about 0.1 µM to about 10 µM. The effect of the agent on MEK3 kinase activity may then be evaluated by quantitating the incorporation of [³²P]phosphate into p38, as described above, and comparing the level of incorporation with that achieved using activated MEK3 without the addition of a candidate agent.

Within other aspects, TAO polypeptides may be used to identify one or
30 more native upstream kinases (*i.e.*, kinases that phosphorylate and activate TAO1 and/or TAO2 *in vivo*, or other signaling molecules that regulate TAO activity). TAO

polypeptides may be used in a yeast two-hybrid system to identify interacting proteins. Alternatively, an expression library may be screened to identify cDNAs that encode proteins which phosphorylate a TAO polypeptide. Other methods for identifying such upstream kinases may also be employed, and will be apparent to those of ordinary skill
5 in the art.

PHARMACEUTICAL COMPOSITIONS

For administration to a patient, one or more polypeptides, polynucleotides, antibodies and/or modulating agents are generally formulated as a
10 pharmaceutical composition, which may be a sterile aqueous or non-aqueous solution, suspension or emulsion, and which additionally comprises a physiologically acceptable carrier (*i.e.*, a non-toxic material that does not interfere with the activity of the active ingredient). Any suitable carrier known to those of ordinary skill in the art may be employed in a pharmaceutical composition. Representative carriers include
15 physiological saline solutions, gelatin, water, alcohols, natural or synthetic oils, saccharide solutions, glycols, injectable organic esters such as ethyl oleate or a combination of such materials. Such compositions may also comprise buffers (*e.g.*, neutral buffered saline or phosphate buffered saline), carbohydrates (*e.g.*, glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as
20 glycine, antioxidants, antimicrobial compounds, chelating agents such as EDTA or glutathione, adjuvants (*e.g.*, aluminum hydroxide), inert gases and/or preservatives. Compositions of the present invention may also be formulated as a lyophilizate. Pharmaceutical compositions may also contain other compounds, which may be biologically active or inactive.

25 The compositions described herein may be administered as part of a sustained release formulation (*i.e.*, a formulation such as a capsule that effects a slow release of compound following administration). Such formulations may generally be prepared using well known technology and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-
30 release formulations may contain a polypeptide, polynucleotide or modulating agent dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate

controlling membrane. Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of release. The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected
5 duration of release and the nature of the condition to be treated or prevented.

Certain pharmaceutical compositions contain DNA encoding a polypeptide, antibody fragment or other modulating agent as described above (such that a TAO polypeptide, a variant thereof or a modulating agent is generated *in situ*) or an antisense polynucleotide. In such pharmaceutical compositions, the DNA may be
10 present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid, bacterial and viral expression systems, as well as colloidal dispersion systems, including liposomes. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal). The DNA may also be "naked," as
15 described, for example, in Ulmer et al., *Science* 259:1745-1749, 1993.

Various viral vectors that can be used to introduce a nucleic acid sequence into the targeted patient's cells include, but are not limited to, vaccinia or other pox virus, herpes virus, retrovirus, or adenovirus. Techniques for incorporating DNA into such vectors are well known to those of ordinary skill in the art. Preferably,
20 the retroviral vector is a derivative of a murine or avian retrovirus including, but not limited to, Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A retroviral vector may additionally transfer or incorporate a gene for a selectable marker (to aid in the identification or selection of transduced cells) and/or a gene that
25 encodes the ligand for a receptor on a specific target cell (to render the vector target specific). For example, retroviral vectors can be made target specific by inserting a nucleotide sequence encoding a sugar, a glycolipid, or a protein. Targeting may also be accomplished using an antibody, by methods known to those of ordinary skill in the art.

Viral vectors are typically non-pathogenic (defective), replication
30 competent viruses, which require assistance in order to produce infectious vector particles. This assistance can be provided, for example, by using helper cell lines that

contain plasmids that encode all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR, but that are missing a nucleotide sequence which enables the packaging mechanism to recognize an RNA transcript for encapsulation. Such helper cell lines include (but are not limited to) Ψ2, PA317 and
5 PA12. A retroviral vector introduced into such cells can be packaged and vector virion produced. The vector virions produced by this method can then be used to infect a tissue cell line, such as NIH 3T3 cells, to produce large quantities of chimeric retroviral virions.

Another targeted delivery system for TAO polynucleotides is a colloidal
10 dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*i.e.*, an artificial membrane vesicle). It has been shown that large unilamellar vesicles (LUV), which range in size
15 from 0.2-4.0 μm can encapsulate a substantial percentage of an aqueous buffer containing large macromolecules. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, et al., *Trends Biochem. Sci.* 6:77, 1981). In addition to mammalian cells, liposomes have been used for delivery of polynucleotides in plant, yeast and bacterial
20 cells. In order for a liposome to be an efficient gene transfer vehicle, the following characteristics should be present: (1) encapsulation of the genes of interest at high efficiency while not compromising their biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4)
25 accurate and effective expression of genetic information (Mannino, et al., *Biotechniques* 6:882, 1988).

The targeting of liposomes can be classified based on anatomical and mechanistic factors. Anatomical classification is based on the level of selectivity, for example, organ-specific, cell-specific, and organelle-specific. Mechanistic targeting
30 can be distinguished based upon whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to distribute to cells of the reticuloendothelial

system (RES) in organs which contain sinusoidal capillaries. Active targeting, on the other hand, involves alteration of the liposome by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of the liposome in order to achieve targeting to organs and cell types other than the naturally occurring sites of localization.

Routes and frequency of administration, as well as polypeptide, modulating agent or nucleic acid doses, will vary from patient to patient. In general, the pharmaceutical compositions may be administered intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity or transdermally. Between 1 and 6 doses may be administered daily. A suitable dose is an amount of polypeptide or DNA that is sufficient to show improvement in the symptoms of a patient afflicted with a disease associated with a stress-responsive MAP kinase pathway. Such improvement may be detected based on a determination of relevant cytokine levels (*e.g.*, IL-2, IL-8), by monitoring inflammatory responses (*e.g.*, edema, transplant rejection, hypersensitivity) or through an improvement in clinical symptoms associated with the disease. In general, the amount of polypeptide present in a dose, or produced *in situ* by DNA present in a dose, ranges from about 1 µg to about 250 µg per kg of host, typically from about 1 µg to about 60 µg. Suitable dose sizes will vary with the size of the patient, but will typically range from about 10 mL to about 500 mL for 10-60 kg animal.

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THERAPEUTIC APPLICATIONS

The above polypeptides, polynucleotides and/or modulating agents may be used to phosphorylate (and thereby activate) MEK3, or to inhibit such phosphorylation, in a patient. As used herein, a "patient" may be any mammal, including a human, and may be afflicted with a disease associated with a stress-responsive MAP kinase pathway, or may be free of detectable disease. Accordingly, the treatment may be of an existing disease or may be prophylactic. Diseases associated with a stress-responsive MAP kinase pathway include any disorder which is etiologically linked to a TAO protein kinase activity, including immune-related diseases (*e.g.*, inflammatory diseases, autoimmune diseases, malignant cytokine production or endotoxic shock), cell growth-related diseases (*e.g.*, cancer, metabolic

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diseases, abnormal cell growth and proliferation or cell cycle abnormalities) and cell regeneration-related diseases (e.g., cancer, degenerative diseases, trauma, environmental stress by heat, UV or chemicals or abnormalities in development and differentiation). Immunological-related cell proliferative diseases such as osteoarthritis, 5 ischemia, reperfusion injury, trauma, certain cancers and viral disorders, and autoimmune diseases such as rheumatoid arthritis, diabetes, multiple sclerosis, psoriasis, inflammatory bowel disease, and other acute phase responses may also be treated.

Treatment includes administration of a composition or compound which 10 modulates the kinase activity of TAO1 and/or TAO2. Such modulation includes the suppression of TAO expression and/or activity when it is over-expressed, or augmentation of TAO expression and/or activity when it is under-expressed. Modulation may also include the suppression of phosphorylation of MEK3 or related kinases.

15 As noted above, antibodies, polynucleotides and other agents having a desired effect on TAO expression and/or activity may be administered to a patient (either prophylactically or for treatment of an existing disease) to modulate the activation of MEK3 *in vivo*. For example, an agent that decreases TAO activity *in vivo* may be administered to prevent or treat inflammation, autoimmune diseases, cancer or 20 degenerative diseases. In particular, such agents may be used to prevent or treat insulin-resistant diabetes, metabolic disorders and neurodegenerative diseases. In general, for administration to a patient, an antibody or other agent is formulated as a pharmaceutical composition as described above. A suitable dose of such an agent is an amount sufficient to show benefit in the patient based on the criteria noted above.

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The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 15 Cloning and Sequencing cDNA Encoding TAO1 and TAO2

This Example illustrates the cloning of cDNA molecules encoding the rat Ste20p-related protein kinases TAO1 and TAO2, and the identification of the human TAO1 homolog.

First-strand cDNA from adult rat brain was used as the template in the
10 first round of PCR with degenerate oligonucleotide primers derived from the Ste20p sequence, 5'-GACGCTGGATCCAA(AG)AT(ACT)GGICA(AG)GGIGC-3' (SEQ ID NO:19) and 5'-GGIGTICC(AG)TTIGTIGCIAT-3' (SEQ ID NO:20). A portion of the product of this reaction was used as the template in a second round of PCR with nested primers, also derived from the Ste20p sequence,
15 5'-AA(AG)GA(AG)CAIATI(CA)TIAA(CT)GA(AG)AT-3' (SEQ ID NO:21) and 5'-GACGCTGAATTAC(CT)TCIGGIGCCATCCA-3' (SEQ ID NO:22). The resulting 420 base product was labeled with [α -³²P]dCTP by random-priming, and used to probe approximately 1x10⁶ plaques of an oligo(dT) and random-primed λ ZAP library generated from adult rat forebrain RNA. In excess of 100 positive clones were
20 obtained; of those sequenced, all contained regions of overlap with the original PCR product. A full length TAO1 sequence was assembled from two overlapping cDNAs, using the SacI site at nucleotide 50 to insert a fragment of TAO1 cDNA including nucleotides 50 to 3003. The full length TAO1 sequence is shown in Figure 1 and SEQ ID NO:1.

25 The TAO1 open reading frame encodes 1001 amino acids, with a calculated molecular mass of 134kDa. The presumed initiator codon begins at base 121 and is preceded by an in-frame stop codon at base 106. The longest 5' UTR obtained was 600 nucleotides in length, and the longest 3' UTR was 1200 nucleotides. None of the clones analyzed contained a poly-A track.

30 As is the case with most protein kinases, TAO1 can be divided into regions based on amino acid sequence comparison to other protein kinases. TAO1 is

composed of an amino-terminal catalytic domain and an extensive carboxy-terminal region that has several distinguishing features, such as a possible nucleotide binding site and acidic stretch just carboxy-terminal to the catalytic domain, as well as two serine-rich regions. TAO1 does not appear to contain the leucine zipper motifs found
5 in the MLK subfamily of kinases.

The TAO1 catalytic domain extends 263 amino acids from amino acid 25 to 288 with all 11 of the typical protein kinase subdomains conserved. There are two glutamate residues between TAO1 subdomains II and IV; the second glutamate at amino acid 76 contained in the sequence KEVK is most likely to represent subdomain
10 III (Hanks et al., *Science* 241:42-52, 1988). The features of the TAO1 catalytic domain are most similar to the serine/threonine family of protein kinases; subdomain VIb with the sequence HRDIKAGN suggests that TAO1 is likely to be a serine/threonine protein kinase.

When using FASTA (GCG, Wisconsin Package) to align TAO1 with
15 sequences from the databases, the TAO1 catalytic domain shows the highest degree of identity to a *C. elegans* putative serine/threonine protein kinase (accession number U32275), to which it has 63% identity and 79% similarity. That sequence appears to represent the *C. elegans* homolog of TAO1, and is shown as ceTAO in Figure 2. The TAO1 catalytic domain is 39% identical to Ste20p and 40% identical to the catalytic
20 domains of the p21-activated kinases PAK1 and PAK2. The catalytic domain of TAO1 is only 31% identical to the mixed lineage kinase MLK1, and 33% identical to dual leucine zipper-bearing kinase (DLK), also known as MLK2. Thus, TAO1 appears to be more closely related to the STE20-like kinases than to the MLK family. TAO1 is also related to germinal center kinase (GCK) and mammalian Ste20-like kinase 1 (MST1),
25 with 42% and 45% identity respectively in the catalytic domains. The TAO1 sequence has similarity with that of the MEK kinase MEKK1. Although the overall identity between the catalytic domains of TAO1 and MEKK is only 33%, the identity of the carboxy-terminal half of their catalytic domains is higher (42%).

In the process of screening the cDNA library for clones near the 5' end
30 of TAO1, multiple clones representing a second closely related gene (TAO2) were identified. The TAO2 sequence is provided in SEQ ID NO:3, with the predicted amino

acid sequence shown in SEQ ID NO:4 and Figure 2. TAO2 is highly related to TAO1, and has a similar arrangement of an amino-terminal kinase domain and a long carboxy-terminus, but differs in that it contains an acidic insert of 17 glutamate residues carboxy-terminal to the catalytic domain, and lacks the putative nucleotide binding site of TAO1.

Sequences from EST databases derived from retinal mRNAs revealed the human counterpart for TAO1. The EST sequences identified are provided in SEQ ID NOs:5-16, and the alignments of these sequences with the rat TAO1 sequence are provided in Figures 12-20.

The Fasta program was used to compare the percent amino acid identities of several protein kinase catalytic domains, and the results are presented in Table 1, below.

Table 1

	TAO1						
TAO2	90	TAO2					
ceTAO	65	61	ceTAO				
STE20d	40	39	37	STE20			
GCK	43	42	35	40	GCK		
MLK1	32	30	27	30	29	MLK1	
MST1	47	43	42	42	47	28	MST1
MEKK1	34	33	27	30	30	30	29

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To assess the expression of TAO1 in transfected cells, full-length, HA-tagged TAO1 cDNA was transfected into human embryonic kidney 293 cells. A protein of approximately 140kDa could be detected by Western blotting with an

antibody directed against the HA epitope (Figure 5A). The observed molecular mass of the protein is in good agreement with the mass predicted from the cDNA sequence.

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Example 2

In vivo Expression of TAO1 and TAO2

This Example illustrates the expression of TAO1 and TAO2 in a variety of adult rat and human tissues, as determined by Northern blot analysis.

Total RNA isolated from various adult male rat tissues was selected for poly-A⁺ RNA with oligo(dT)cellulose (Collaborative Biomedical Products) according to the manufacturers protocols, and 5µg of each RNA was subjected to Northern analysis. The PCR-generated 420 base fragment derived from the catalytic domain of TAO1 (described above) was labeled with [α -³²P]dCTP by random-priming and used to probe the Northern blot. Hybridization was at 42°C, followed by washing at 55°C in 0.2%SSC/0.1%SDS. Integrity of the mRNA was confirmed by hybridization to an actin probe. The TAO1 probe hybridized predominately to an mRNA species of approximately 12kb, and less strongly to another of approximately 10kb (Figure 3A). Of the rat tissues examined, brain clearly showed the strongest hybridization signal. On prolonged exposure, heart and lung revealed weak hybridization signals, while in skeletal muscle, liver, kidney, testis, epididymus, and spleen no signal was detected.

To assess the expression pattern of TAO2, the rat tissue Northern blot was stored until the hybridization signal for TAO1 was not seen on a two week exposure at -80°C. A fragment from the catalytic domain of TAO2 was labeled with [α -³²P]dCTP by random priming, and used to probe the Northern under the same hybridization and washing conditions described above for TAO1.

When the same rat tissue Northern blot was probed with a fragment of the catalytic domain of TAO2, the strongest hybridization signal was also seen in brain. The size of the transcript hybridizing to the TAO2 probe was smaller than that seen for TAO1, at 5kb (Figure 3B).

A probe from the non-catalytic carboxy-terminus of TAO1 (corresponding to nucleotides 1555 to 2632 of TAO1 (*see* Figure 1)) was used for all

additional Northern analyses because it is less likely to hybridize to TAO2 mRNA. This probe from the carboxy-terminus of TAO1 was used to assess the expression pattern in sections of human brain (Clontech). Hybridizations were performed at 68°C in Clontech ExpressHyb buffer, and washed at 55°C as per the manufacturer's
5 instructions.

The strongest hybridization signals were seen in amygdala, corpus callosum, hippocampus, and substantia nigra, and each of these was stronger than that seen in whole brain (Figure 4A). Weaker signals were seen in caudate nucleus, subthalamic nucleus and thalamus. A second human brain Northern hybridized to the
10 same probe showed strong hybridization signals in cerebellum, putamen and occipital, frontal and temporal lobes, but much weaker signals in cerebral cortex, medulla and spinal cord (Figure 4B).

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Example 3

Kinase Activity and Substrate Specificity of TAO1

This Example illustrates the kinase activity and substrate specificity of TAO1, in *in vitro* and *in vivo* assays.

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To determine whether TAO1 is active as a protein kinase, two constructs were employed. pCMV5TAO1-HA₃ and pCMV5TAO1(1-416)-HA₃ were generated by cloning the cDNAs encoding these TAO1 polypeptides into the pCMV5 mammalian expression vector. Oligonucleotide primers were used with TAO1 cDNA as template to amplify a 1247 base pair DNA product encoding amino acids 1 to 416. This
25 fragment contains all 11 of the kinase subdomains (with the initial methionine deleted). The resulting constructs were transfected into human embryonic kidney 293 cells, and the recombinant, tagged proteins immunoprecipitated with an antibody directed against the HA epitope.

In vitro kinase assays were generally performed as follows. Kinase
30 assays contained: 50mM Hepes, pH 8, 10mM MgCl₂, 1mM DTT, 100μM ATP, [γ-³²P]ATP (at a final concentration of 2-7 cpm/fmol), and unless otherwise noted, reactions were incubated at 30°C for 60 minutes in a 30μl volume. Protein kinase

substrates such as myelin basic protein were added at a final concentration of 0.5 mg/ml. Reactions were halted by the addition of 10 μ l 5X Laemmli buffer, followed by boiling, and 20 μ l were analyzed by SDS-PAGE and autoradiography. For linked kinase assays, 50-250ng of recombinant TAO1 protein was incubated with 50ng of each of the bacterially expressed MEK proteins in a 30 μ l reaction volume for 60 minutes at 30°C, and then 5 μ l of this reaction was added to a second reaction mix containing bacterially expressed (His)₆p38 or GST-SAPK β at a final concentration of 10 μ g/ml. Recombinant MEK proteins were kindly provided by Andrei Khokhlatchev and Megan Robinson, and may be prepared as described by Robinson et al., *J. Biol. Chem.* 271:29734-29739, 1996 and references cited therein. Within such assays, both TAO1(1-416) and full-length TAO1 were able to phosphorylate MBP in immune complex kinase reactions.

To quantitate the activity of more highly purified TAO1, TAO1(1-416), full-length TAO1 and full-length TAO1(D169A) were expressed with an amino-terminal hexa-histidine tag in Sf9 cells. TAO1(D169A) is a catalytically defective TAO1 mutant, which was created by changing aspartic acid 169 to an alanine (D169A) with PCR, and cloning the resulting construct into the pCMV5 mammalian expression vector. These constructs were prepared with either a single hemagglutinin (HA) epitope tag at the amino-terminus, a triple HA epitope tag at the carboxy-terminus, or a myc epitope tag at the amino-terminus.

The recombinant, hexa-histidine tagged TAO1, TAO1(1-416), and TAO1(D169A) were expressed in *Spodoptera frugiperda* (Sf9) cells. Cells were lysed by douncing in 50mM sodium phosphate, pH 8.5, 1mM DTT, 1mM PMSF, and 1mg/ml each leupeptin, pepstatin A, and aprotinin. After centrifugation at 30,000xg for 30 minutes, the supernatant was applied to a Ni²⁺- NTA agarose (Qiagen) column pre-equilibrated with the same buffer. The column was then washed with 50 column volumes of buffer, and eluted with a 20 ml gradient of 0 to 250mM imidazole, all in the above buffer. Fractions containing recombinant TAO1 proteins were detected in fractions by Western blotting with an antibody to the MRGS(H)₆ epitope (Qiagen), and appropriate fractions were pooled and dialyzed to remove the imidazole.

(His)₆TAO1(1-416) expressed as a single 57kDa band (Figure 5B). Both the (His)₆TAO1 and (His)₆TAO1(D169A) recombinant proteins migrated as 140kDa bands, although the D169A mutant appears to be more subject to degradation. (His)₆TAO1(1-416) phosphorylates MBP with a specific activity of 1 $\mu\text{molmin}^{-1}\text{mg}^{-1}$ in the presence of 1mM ATP. Full-length (His)₆TAO1 exhibits MBP phosphorylating activity that is comparable to the 1-416 truncation mutant, while the activity of TAO1(D169A) is reduced to 90% of that of the wild-type protein. (His)₆TAO1(1-416) was also able to phosphorylate α -casein, histone 1, and histone 7.

To determine whether TAO1 activates one or more of the known MEKs, (His)₆TAO1(1-416) was incubated with bacterially produced MEK for one hour in the presence of Mg^{2+} and [γ -³²P]ATP. A portion of this reaction was then transferred to a similar reaction containing the appropriate bacterially expressed MEK substrate, (His)₆ERK2K52R for MEK1 and MEK2, (His)₆p38 for MEK3 and MEK6, and (His)₆p38 and GST-SAPK β for MEK4. After a one hour incubation, the phosphoproteins were separated by SDS-PAGE. Autoradiography revealed that (His)₆TAO1(1-416) phosphorylated and activated (His)₆MEK3, and enhanced the ability of MEK3 to phosphorylate p38 by approximately 100-fold (Figure 6).

(His)₆TAO1(1-416) activated GST-MEK4 5-fold toward (His)₆p38, and 150-fold towards GST-SAPK β (Figure 7). The difference in fold activation seen for MEK4 towards the two substrates probably reflects the difference in basal kinase activity of MEK4 towards p38 and SAPK β *in vitro*. TAO1 also increased the ability of GST-MEK6 to phosphorylate (His)₆p38, by 5-fold (Figure 8). Recombinant GST-MEK5 was not phosphorylated by (His)₆TAO1(1-416).

Recombinant (His)₆TAO1 and (His)₆TAO1(D169A) were also examined for their ability to activate the same MEK proteins. (His)₆TAO1 showed a reduced ability to activate MEK3 as compared to that of the carboxy-terminal truncation mutant (His)₆TAO1(1-416). In multiple experiments, the full-length TAO protein displayed from 0 to 30% of the MEK3 activating ability of (His)₆TAO1(1-416), and (His)₆TAO1(D169A) was unable to activate any of the MEK proteins above basal activities.

The degree of activation of each of the MEK proteins by (His)₆TAO1(1-416) *in vitro* is comparable to that seen by a bacterially produced amino-terminal truncation of MEKK1 (Xu et al., *Proc. Natl. Acad. Sci. USA* 92:6808-6812, 1995; Robinson et al., *J. Biol. Chem.* 271:29734-29739, 1996). To distinguish
5 the MEK-activating ability of TAO1 from that of MEKK, the ability of (His)₆TAO1(1-416) to activate MEK1 and MEK2 was assessed. As shown in Figure 9, (His)₆TAO1(1-416) was completely unable to increase the activity of MEK1 or MEK2 towards the substrate (His)₆ERK2 under the same conditions that TAO1 activates MEK3, MEK4, and MEK6. Thus, while TAO1 displays MEKK-like activity in its
10 ability to activate various MEKs, TAO1 is differentiated from MEKK by its inability to recognize MEK1 and MEK2. Figure 9 shows the fold activation of the various MEKs by TAO1.

To assess the ability of TAO1 to activate the various MEKs *in vivo*, full-length HA-tagged TAO1 was co-transfected into 293 cells with myc-tagged
15 MEK3, or myc-tagged TAO1 was co-transfected with HA-tagged MEK4 or HA-tagged MEK6. The pCMV5myc-MEK3 construct was generated by inserting the MEK3 coding sequence (provided by K.L. Guan, University of Michigan, which may be prepared as described by Robinson et al., *J. Biol. Chem.* 271:29734-29739, 1996) into the pCMV5Myc vector, such that the Myc epitope is at the amino-terminus of MEK3.
20 The MEKs were then immunoprecipitated and added to immune complex kinase assays with the appropriate substrate and Mg²⁺/ATP. In multiple experiments, myc-tagged MEK3 showed a 3-fold higher activity toward p38 when immunoprecipitated from 293 cells co-expressing TAO than from cells not transfected with TAO (Figure 10). In contrast, TAO was not able to increase the activity of immunoprecipitated HA-tagged
25 MEK4 towards GST-SAPK β , or that of HA-tagged MEK6 toward p38.

In transfected cells, TAO1 activates MEK3 3-fold, but neither MEK4 nor MEK6. The selectivity in transfected cells may arise from the ability of TAO1 to bind MEK3. The endogenous MEK3 from Sf9 cells copurifies with recombinant TAO1 expressed in the cells. These findings suggest that TAO1 may be an important
30 regulator of the p38 pathway.

To determine which MEK3 residues are phosphorylated by TAO, an *in vitro* kinase reaction was performed with (His)₆TAO1(1-416) and (His)₆MEK3; the 57kDa band corresponding to TAO1 and the 30 kDa band corresponding to MEK3 were excised and treated as described. Phosphoproteins were separated by SDS-PAGE, transferred to Immobilon-P membrane (Millipore) electrophoretically, and visualized by autoradiography. Bands of interest were excised and hydrolyzed in 6M HCl for 60 minutes at 110°C. The hydrolysate was dried under vacuum, and resuspended in a 2.2% formic acid, 12% acetic acid solution at an activity of 2000cpm/μl. Then 1μl of each sample was mixed with 1μg each of the three phosphoamino acid standards, and spotted onto cellulose thin-layer chromatography plates. Electrophoresis was performed in 0.5% pyridine, 5% acetic acid at 1200 volts for 60 minutes. After air drying the plates, the standards were visualized with 0.25% ninhydrin in acetone. Autoradiography revealed only phosphoserine and phosphothreonine in both (His)₆TAO1(1-416) and (His)₆MEK3 (Figure 11).

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Example 4

Co-Purification of MEK3 and TAO1

20 This Example shows that TAO1 and MEK3 co-purify.

Although the ability of (His)₆TAO1 to activate MEK3 was always reduced in comparison with that of (His)₆TAO1(1-416), several assays showed that the ability of (His)₆TAO1 to lead to an increase in the phosphorylation of p38 in the linked kinase assays was partly independent of the addition of MEK. (His)₆TAO1(1-416) does not phosphorylate p38. Therefore, Western analyses were performed to determine if one or more MEKs might be present in the TAO1 preparations purified from Sf9 cells.

(His)₆TAO1, (His)₆TAO1(1-416), and (His)₆TAO1(D169A) were subjected to Western analysis with antisera specific to MEK3, MEK4, and MEK6. Four different polyclonal antisera were raised to these three TAO1 peptides in rabbits. The peptide TKDAVRELDNLQYRKMKKLL (SEQ ID NO:23) corresponding to the amino acids 296 to 315 yielded antisera P820. The peptide

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KKELNSFLESQKREYKLRK (SEQ ID NO:24) of amino acids 545 to 563 yielded the antiserum R562. Finally, the peptide RELRELEQQRVSLRRALLEQK (SEQ ID NO:25) of amino acids 829 to 848 resulted in the antisera R564 and R565. These peptides were conjugated to *Limulus* hemocyanin (Boulton and Cobb, *Cell. Regul.* 2:357-371, 1991) and dialyzed into phosphate-buffered saline. A total of five boosts were performed, after which the rabbits were exsanguinated and the serum collected. The antisera were screened for reactivity by Western blotting of recombinant TAO1 expressed in Sf9 cells. Five antisera were found to consistently recognize the recombinant TAO1 protein in Western blots. Free peptide was able to block the specific recognition of TAO1 protein by the antisera. None of the five antisera detected the presence of TAO1 in lysates of 293, NIH3T3, NG-108, or COS cells.

For immunoblot analysis, either 50ng of recombinant TAO1 protein or 100 µg of cell lysate was subjected to SDS-PAGE, then transferred to nitrocellulose membranes. The membranes were blocked with 5% nonfat powdered milk in TBST (20mM Tris, pH 8, 500mM NaCl, 0.05% Tween 20) for one hour, then incubated with the polyclonal antisera at 1:500 dilution in TBST plus 0.25% milk for one hour. After three washes with TBST, the membranes were incubated with a 1:2500 dilution of horseradish peroxidase-conjugated goat-anti-rabbit IgG in TBST plus 0.25% milk for one hour. Membranes were washed again in TBST then visualized with the ECL system (Amersham).

MEK3 was clearly seen in the (His)₆TAO1 preparation, and to a lesser extent in the (His)₆TAO1(D169A) preparation (Figure 11). MEK4 was detected in the Sf9 cell lysates, but not in the TAO1 preparations, while MEK6 was detected in neither.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

Claims

1. A polypeptide comprising an amino acid sequence provided in SEQ ID NO:2, or a variant thereof in which the ability to phosphorylate MEK3 is not substantially diminished.
2. A polypeptide according to claim 1, wherein the polypeptide comprises a sequence that differs from a sequence recited in SEQ ID NO:2 only in conservative substitutions and/or modifications at no more than 10% of the amino acid residues.
3. A constitutively active variant of a polypeptide according to claim 1.
4. A polypeptide according to claim 1, wherein the polypeptide comprises amino acids 1-416 of SEQ ID NO:2.
5. A polypeptide comprising an amino acid sequence provided in SEQ ID NO:2 modified at no more than 10% of the amino acid residues, such that said polypeptide is rendered constitutively inactive.
6. A polypeptide capable of phosphorylating MEK3, wherein the polypeptide does not detectably phosphorylate MEK1 or MEK2.
7. A polypeptide comprising an amino acid sequence provided in SEQ ID NO:4, or a variant thereof in which the ability to phosphorylate MEK3 is not substantially diminished.
8. A polypeptide according to claim 7, wherein the polypeptide comprises a sequence that differs from a sequence recited in SEQ ID NO:4 only in conservative substitutions and/or modifications at no more than 10% of the amino acid residues.

9. A constitutively active variant of a polypeptide according to claim 7.
10. A polypeptide comprising an amino acid sequence provided in SEQ ID NO:4 modified at no more than 10% of the amino acid residues, such that said polypeptide is rendered constitutively inactive.
11. An isolated polynucleotide encoding a polypeptide according to any one of claims 1-10.
12. An isolated polynucleotide according to claim 11, wherein the polynucleotide comprises a nucleotide sequence provided in SEQ ID NO:1.
13. An isolated polynucleotide according to claim 11, wherein the polynucleotide comprises a nucleotide sequence provided in SEQ ID NO:3.
14. An isolated polynucleotide according to claim 11, wherein the polynucleotide encodes amino acids 1-416 of SEQ ID NO:2.
15. A recombinant expression vector comprising a polynucleotide according to claim 11.
16. A host cell transformed or transfected with an expression vector according to claim 15.
17. A polynucleotide comprising at least 10 nucleotides as recited within, or complementary to, SEQ ID NO:1 or SEQ ID NO:3.
18. A pharmaceutical composition, comprising:
 - (a) a polypeptide according to any one of claims 1-10; and
 - (b) a physiologically acceptable carrier.
19. A pharmaceutical composition, comprising:

- (a) a polynucleotide according to claim 11; and
- (b) a physiologically acceptable carrier.

20. A pharmaceutical composition, comprising:

- (a) a polynucleotide according to claim 17; and
- (b) a physiologically acceptable carrier.

21. A method for phosphorylating a MEK polypeptide, comprising contacting a MEK polypeptide with a polypeptide according to claim 1 or claim 3, wherein the MEK polypeptide comprises MEK3, MEK4 or MEK6 or a variant thereof, and thereby phosphorylating the MEK polypeptide.

22. A method for activating a member of a stress-responsive MAP kinase pathway in an organism, comprising administering to an organism a polypeptide according to claim 1 or claim 3, thereby activating a member of a stress-responsive MAP kinase pathway.

23. The method of claim 22 wherein the member of the stress-responsive MAP kinase pathway is MEK3.

24. A method for phosphorylating a MEK polypeptide comprising contacting a MEK polypeptide with a polypeptide according to claim 7 or claim 9, wherein the MEK polypeptide comprises MEK3, MEK4 or MEK6 or a variant of any of the foregoing MEKs, and thereby phosphorylating the MEK polypeptide.

25. A method for activating a member of a stress-responsive MAP kinase pathway in an organism, comprising administering to an organism a polypeptide according to claim 7 or claim 9, thereby activating a member of the stress-responsive MAP kinase pathway.

26. The method of claim 25 wherein the member of the stress-responsive MAP kinase pathway is MEK3.

27. A method for screening for an agent that modulates signal transduction via a stress-responsive MAP kinase pathway, comprising:

(a) contacting a candidate agent with a polypeptide according to any one of claims 1, 3, 7 or 9; and

(b) subsequently measuring the ability of said polypeptide to modulate the activity of a MEK3 polypeptide, and thereby evaluating the ability of the compound to modulate signal transduction via a stress-responsive MAP kinase pathway.

28. A monoclonal antibody or antigen-binding fragment thereof that specifically binds to a polypeptide according to claim 1 or claim 7.

29. A monoclonal antibody according to claim 28, wherein said antibody inhibits the phosphorylation of MEK3 by said polypeptide.

30. A pharmaceutical composition, comprising:

(a) an antibody or antigen-binding fragment thereof according to claim 28; and

(b) a physiologically acceptable carrier.

31. A method for treating a patient afflicted with a disease associated with a stress-responsive MAP kinase pathway, comprising administering to a patient a compound that modulates the phosphorylation of MEK3.

32. The method of claim 31 wherein said compound is a monoclonal antibody or antigen-binding fragment thereof.

33. The method of claim 31 wherein said compound comprises a nucleotide sequence.

34. The method of claim 31, wherein the compound inhibits the phosphorylation of MEK3 and the disease is selected from the group consisting of inflammation, autoimmune diseases, cancer and degenerative diseases.

35. The method of claim 31, wherein the compound enhances the phosphorylation of MEK3 and the disease is selected from the group consisting of insulin-resistant diabetes, metabolic disorders and neurodegenerative diseases.

36. A method for determining the presence or absence of TAO kinase activity in a sample, comprising evaluating the ability of the sample to phosphorylate a MEK3 polypeptide, thereby determining the presence or absence of TAO kinase activity in the sample.

37. A kit for detecting TAO kinase activity in a sample, comprising a MEK3 polypeptide in combination with a suitable buffer.

38. An isolated polynucleotide comprising one or more sequences recited in any one of SEQ ID NOs:5-16, or a variant thereof, wherein the polynucleotide encodes a polypeptide capable of phosphorylating MEK3.

39. A polynucleotide according to claim 37, wherein the polynucleotide comprises a sequence that differs from a sequence recited in anyone of SEQ ID NOs:5-16 only in conservative substitutions and/or modifications at no more than 10% of the amino acid residues.

40. A polynucleotide according to claim 38, wherein the polynucleotide encodes a polypeptide that is a constitutively active variant of a TAO polypeptide.

41. A polypeptide encoded by a polynucleotide according to any one of claims 38-40.

42. A recombinant expression vector comprising a polynucleotide according to claim 38.

43. A host cell transformed or transfected with an expression vector according to claim 42.

44. A polynucleotide comprising at least 10 nucleotides as recited within, or complementary to, a polynucleotide according to claim 38.

45. A pharmaceutical composition, comprising:

- (a) a polypeptide according to claim 41; and
- (b) a physiologically acceptable carrier.

46. A pharmaceutical composition, comprising:

- (a) a polynucleotide according to claim 38; and
- (b) a physiologically acceptable carrier.

47. A method for phosphorylating a MEK polypeptide comprising contacting a MEK polypeptide with a polypeptide according to claim 41, wherein the MEK polypeptide comprises MEK3, MEK4 or MEK6 or a variant of any of the foregoing MEKs, and thereby phosphorylating the MEK polypeptide.

48. A method for activating a member of a stress-responsive MAP kinase pathway in an organism, comprising administering to an organism a polypeptide according to claim 41, thereby activating a member of a stress-responsive MAP kinase pathway.

49. The method of claim 48 wherein the member of the stress-responsive MAP kinase pathway is MEK3.

50. A method for screening for an agent that modulates signal transduction via a stress-responsive MAP kinase pathway, comprising:

- (a) contacting a candidate agent with a polypeptide according to claim 41; and
- (b) subsequently measuring the ability of said polypeptide to modulate the activity of a MEK3 polypeptide, and thereby evaluating the ability of the compound to modulate signal transduction via a stress-responsive MAP kinase pathway.

51. A monoclonal antibody or antigen-binding fragment thereof that specifically binds to a polypeptide according to claim 41.

52. A monoclonal antibody according to claim 51, wherein said antibody inhibits the phosphorylation of MEK3 by said polypeptide.

53. A pharmaceutical composition, comprising:

(a) an antibody or antigen-binding fragment thereof according to claim 51;

and

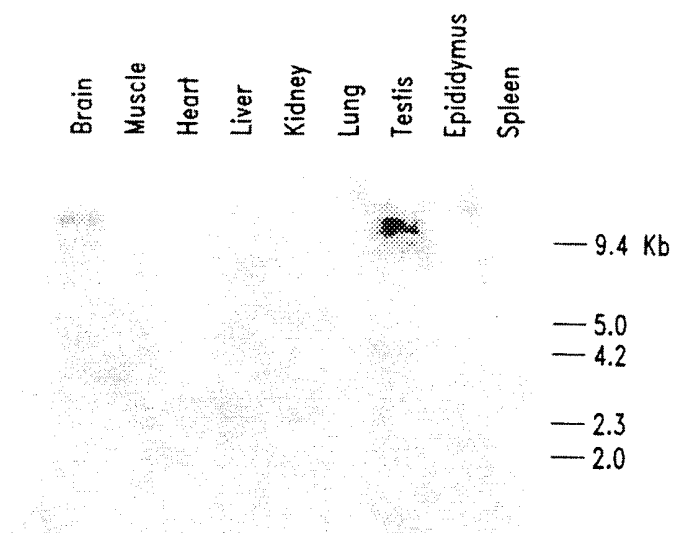
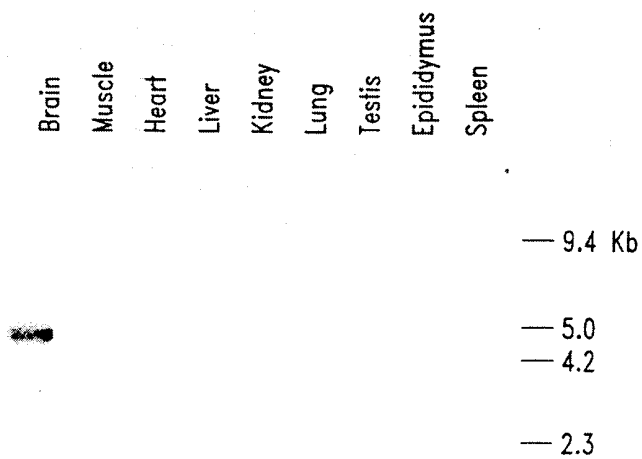
(b) a physiologically acceptable carrier.

1 TCTGCAGTATGGTAGATTATTTATGCAATTTATGCCAGTGTGGCTTCATTCATACAGATGAACCAAGCTTTGGGATAGCAGTA TAAATTAGAATCAGACAGCTGACTGCTC
 115 AGCAGGATGCCATCAACTAACAGAGCAGGCAGTCTAAAGGACCTGAAATGCGAGAGCTCTTCTTCAAGAAGATCCGAAAAACTTTACAGATCTCAGAGAAATCGGCCAT
 M P S T N R A G S L K D P E I A E L F P K E D P E K L F T D L R E I G H 36
 229 GGGAGCTTTGGAGCAGTTATTTTGCACGAGATGTGGTACTAATGAAGTGGTGGCCATCAAGAAAATGTCTTATAGTGGAAAGCACTCTACTGAGAAAATGGCAGGATATTATT
 G S F G A V Y F A R D V R T N E V V A I K K M S Y S G K 2 S T E K W Q D I I 74
 343 AAGGAATCAAGTTTCTACAAAGAATAAAACATCCCAACAGTATAGAAATCAAAAGGCTGCTATTTACGTGAACACACAGCATGGCTTGTAAATGGAATATTGTTTAGCATCTGCT
 K E V K F L Q R I K H P N S I E Y K G C Y L R E H T A W L V M E Y C L G S A 112
 457 TCGGATTTACTAGAAGTTTCAATAAAAGCCATTACAAGAAGTGGAAATAGCAGCAATTA CACA TGGTCTCTCCAGGATTAGCTTA TTTACATTCTCATACCATGATCCATAGA
 S D L L E V H K K P L Q E V E I A A I T H G A L Q G L A 7 L H S H T M I H R 150
 571 GATATCAAAGCAGGAAATATCTCTGACAGAACCAAGCCAAAGTGAACCTTGCTGACTTTGGATCTGCTTCCATGGCTCCCTCCCTGCAATTCCTTTTGTGGAAACACCATATTGG
 D I K A G N I L L T E P G Q V K L A D F G S A S M A S P A N S F V G T P Y W 188
 685 ATGGCCCCAGAAATATTTAGCCATGGATGAAGGACAAATATGATGGCAAGTTGATGATGGTCTCTTGGAAATACATGATTTGAATTAGCCGAGAGGAAGCCTCTTTATTT
 M A P E V I L A M D E G Q Y D G K V D V W S L G I T C I E L A E R K P P L F 266
 799 AATATGAATGCAATGAGTGCCTTATATGACATAGCCCAAAATGAAATCCCTTACACTACAGTCTAAATGAATGGTCTGATTATTTTGGAAATCTTTGTAGATTCTTGCCTCCAGAAA
 N M N A M S A L Y H I A Q N E S P T L Q S N E W S D Y F F N F V D S C L Q K 264
 913 ATCCCTCAAGATCGCCTTACATCAGAGGAACCTTTTAAAGCACAATGTTTGTCTTGGAGAGCGCCTGAAACAGTGTAAATAGATCTTATTTCAAAGGACAAAGGATCGAGTAAGA
 I P Q D R P T S E P T I K H M F V L R E R P E T V L I D L I Q R T K D A V R 302
 1027 GAGCTGGACAATCTACATATGGAAGATGAAGAACTCCTTTTCCAGGAGGCACATAATGACACAGCAGTAGAAGCACAGGAAGAGGAGGAGCAAGATCATGGTGGTGGC
 E L D N L Q Y R K M K K L L F Q E A H N G P A V E A Q E E E E E Q D H G G G 340
 1141 CGGACGGAACAGTAAATAGTGTGGAAGCAATCAGTCTATCCCACTATGCTTATGCTGAGTGGCAAGAGCAGCAGTGTCTAATAGTCTTCCAGATGCATCGGATGACAAG
 R L R L D K D L E T Q R N N F A A E M E K L I K K H Q A S M E K E A K V M A 378
 1255 AGTGAGCTAGACATGATGGAGGAGACCATACAGTGTGTCTAACTGTCTGCTATCCACTTAAACCTGAGGAGGAAAAATTAAGAAGAAGGAGATCCTAGAAACAGAGCA
 S E L D M M E G D H T V M S N S S V I H L K P E E E N Y 2 E E G D P R T R A 416
 1368 TCAGCTCCACAGTCTCCACCTCAAGTGTCTGTCACAAATCACA TTA TGTAA TAGAGAACACTTTGCAACTATACGAAACAGCATCACTGGTTTACAAGACAGATGCAAGAACAT
 S A P Q S P P Q V S R H K S H Y R N R E H F A T I R T A S L V T R Q M Q E H 454
 1483 GAGCAGGACTCTGAACTTAGAGAACAGATGCTGCTTAAAGCGGATGAGGCGACAGCATGCAAGCAGCTGATGCTCTGGAATAAATGAAGGAGAGAAATGGAACGAACAT
 E Q D S E L R E Q M S G Y K R M R R Q H Q K Q L M T L E N K L K A E M D E H 492
 1597 CGGCTCAGATTAGACAAAGATCTTGAACCTCAGCGCAACAAATTCGCTGACAGAAATGGAGAACTTATTAAGAAACACCAAGCTTCTATGGAAGAGAGGCTAAAGTGAATGGCC
 R L R L D K D L E T Q R N N F A A E M E K L I K K H Q A S M E K E A K V M A 530
 1711 AACGAGGAGAAAAATTCACAAACATTCAGGCTCAACAGAGAAAGAACTGATAGCTTTTGGAGCTCTCAAAAAAGAGAAATAAATTCGAAAGAGCAGCTTAAAGGAG
 N E E K K F Q Q H I Q A Q Q K K E L N S F L E S Q K R E Y K L R K E Q L K E 568
 1825 GAGCTGAATGAAGAACAGAGCAGCACTTAAAAAGAGAGGAGAAATGGCTTTCAAGCAGAGAGAGAAATATTCACATTTTCAAGCAGAGAGAGAGCTAATTTCTTTCGAGCT
 E L N E N Q S T P K K E K Q E W L S K Q K E N I Q H F Q A E E E A N L L R R 606
 1939 CAAAGGCAGTATCTAGAGCTAGAATGCTGCTGCTTCAAAAGAGAGAAATGTTACTTGGTGGCCATAATGGAAACAGGACCTTCTCAAGGAGAGTAAACAAAAAGCAGACTCAG
 Q R Q Y L E L E C R R F K R R M L L G R H N L E Q D L V P E E L N K R Q T Q 644
 2053 AAGGACTTAGAACAATGCTTACTGCGACAGCATGAAATCCATGCAAGAACTGGAGTTTGGCCACTCAACACTATTCAGAGATGGCTGTGAGTTGATCAGACTGCAACAT
 K D L E H A M L R Q H E S Q E L E F R H L N T I Q K M R C E L I R L Q H 682
 2167 CAACTGAGCTTACTAACCAGCTGGAATACCAATAGAGAGAGGAAACGGGAACATAGAGCGGAAACATGTCATGGAAGTTTCGACACAGCCTAAGAGTTTGAAGCTTAAGAACTC
 Q T E L T N Q L E Y N K R R E R E L R R K H V M E V R Q 2 P K S L K S K E L 720
 2281 CAAATAAAAAGCAGTTTCAGGATACCTGCAAAATTCAAACAGACAGTACAAAGCATTAAGGAATCACTTACTGAGACTACACCAAGAGTGAACCAAGCTGTCTTGAAA
 Q I K K Q F Q D T C K I Q T R Q Y K A L R N H L L E T T F K S E H K A V L K 758
 2395 AGACTCAAGGAGGAAACAGACTCGGAAGTTAGCCATCTTGGCTGAGCAGTATGATCATAGCATTAATGAAAATGCTCTCCACACAGCTCTGGCTTTGGATGAAGCAAGGAAGCA
 R L K E E Q T R K L A I L A E Q Y D H S I N E M L S T Q A L R L D E A Q E A 796
 2509 GAATGCCAGCTTTTGAAGATGACCTACAGCAGGAACCTGGAGCTGTGTAATGATATCAGAGCAAAATCAAGATGCAAGCTGAGGCCAACATGATGAGAGCTTCGAGAGCTG
 E C Q V L K M Q L Q Q E L E L L N A Y Q S K I K M Q A E A Q H D R E L R E L 834
 2623 GAACAAAGGCTCTCCCTTCGGAGAGCACTCTTAGAACAGAGATTTGAAGAAGAGATGTTGGCTTTTCAGAAATGAACGCAAGAAATAGCTAGCCTGCTCGAGCGCCAGGCC
 E Q R V S L R R A L L E Q K I E E E M L A L Q N E R T E I R S L L E R Q A 872
 2737 AGAGAAATGAAGCTTTTGAATCTGAAAGCATGAGATTAGTTTATGTAACATGCTCTTCTTAAATCTCTCCCTGAGGCATTAACCCACAGCTACCCAGGAGCTTCTAGCTGG
 R E I E A F D S E S M R L G F S N M V L S N L S P E A F S H S Y P G A S S W 910
 2851 TCTCACAATCCTACTGGGGTTCAAGGACCTCAGTGGGTCTATCCCATGGGTGGCAACCAAGCTTGGGGTCAATCCGATGCAAGGCGGACCCCAACCATGGGGTCAACCCCTCA
 S H N P T G S G S G P H W G H P M G G T P Q A W G H P M Q 3 G P Q P W G H P S 948
 2965 GGGCCAAATGCAAGGGTACCTCGAGGTAGCAGTATAGGAGTCCGCAATAGCCCCAGGCTCTGAGCGGACAGCTTCTGGGGGATGAGCGGAACAGGGCATGAGCAGAGCAAG
 G P M Q G V P R G S S I G V R N S P Q A L R R T A S G G R T E Q G M S R S T 986
 3079 AGTGTCACTTCAAAATATCCAAATGGGTCAACATGCTTACACATAAATTTGAAGTGGCAATTCGCTGGAGCTGCTGCTCAAAAGAAACTGCCTACAGACATCAGCACAG
 S V T S Q I S N G S H M S Y T * * 1001
 3193 CAGCCTCTCACTTGGGTACTACCGGGTGAAGCTGTGCATATGGTATATTTTATTCGCTCTTTGTAAGCGTTATGTTTGTGTTTACTAAATGGGATGTCATAGTATTTGGCT

FIG. 1

	I	II	III	
TA01	MPSTNRAGSLKDPETAELEFFKEDPEKLFDTLREIGHGSFGAVYFARDVRNNEVVAIKMSYSCKQSTEKW--QDIKEV	77		
TA02	MPAGGRAGSLKDPDVAELFFKDDPEKLFSDREIGHGSFGAVYFARDVRNNEVVAIKMSYSCKQSTEKW--QDIKEV	77		
ceTOA	-MAPAVLQKPGVIKDPISIAALFNSNKDPEQDLREIGHGSFGAVYFAYDKKNEQTVAIKMNFSGKQAVEKW--NDILKEV	76		
STE20	REERERRKKQLYAKLNEICSDGDPSTKYANLVKIGGASGGVYTA YEIGTNVSVAIKQMNLE-KQPKKELIINEILVMK	670		
	IV	V	VI	
TA01	KFLQRIKHPNSIEYKGCYLREHTA ~~W~~ LVMEYCLGSASDLLLVHKKPLQVEVEIAAITHGALQGLAYLHSHMTMIHRDIKAGN	156		
TA02	RFLQKLHPNTIQYRCGYLREHTA ~~W~~ LVMEYCLGSASEFLEVHKKPLQVEVEIAAVTHGALQGLAYLHSHNMIMHRDVKAGN	156		
ceTOA	SFLNTVVHPHIVIDYKACFLKDTTC ~~W~~ LVMEYCLGSAAIDIVLPRKGMREVEIAAICSQITLDALRYLSLKRIHRDIKAGN	155		
STE20	G----SKHPNIVNFIDSYVLKGD ~~W~~ LVMEYMEGFS ~~LDV~~ -VTHCILTEGQIGAVCREITLSGLEFLHSGKGV ~~LHRDIKSDN~~	744		
	VII	VIII	IX	
TA01	ILLTEPGQV ~~KLADFG~~ SAS----MASPANSFVCTPY ~~WMAPEV~~ ILAMDEGOYDGKVDV ~~W~~ SLGITCTIELAERKPP ~~LFNMNA~~	230		
TA02	ILLSEPGLV ~~KLGD~~ FGSAS----IMAPANSFVCTPY ~~WMAPEV~~ ILAMDEGOYDGKVDV ~~W~~ SLGITCTIELADRKP ~~PLENMNA~~	230		
ceTOA	ILLS ~~SDHAIVKLAD~~ FGSAS----LVDPAQTFICGTPFF ~~MAPEV~~ ILAMDEGHYTD ~~RAD~~ I ~~W~~ SLGITCTIELAERRPP ~~LF~~ SMNA	229		
STE20	ILLSMEGDI ~~KLTD~~ FGFCAQINEINLKRTTWGTPY ~~WMAPEV~~ VS ~~RKE~~ ---YGP ~~KVD~~ I ~~W~~ SLGIMIEMIEGEPPY ~~ILNETP~~	819		
	X	XI		
TA01	MSALYHIAQNESPTLQSN ~~EWSDYFRN~~ -----FVDSCLQKIPQDRPTSE	273		
TA02	MSALYHIAQNESPALQSGHWSEYFRN-----FVDSCLQKIPQDRPTSE	273		
ceTOA	MSALYHIAQNDPPTLSPIDTSEQPEWSLEFVQFIDKCLRKPAEERMSAE	279		
STE20	LRALYLIATNGTPKLKEPENLSS---SLKK--FLDWCLC ~~VEPE~~ DRASAT	863		

Fig. 2

*Fig. 3A**Fig. 3B*

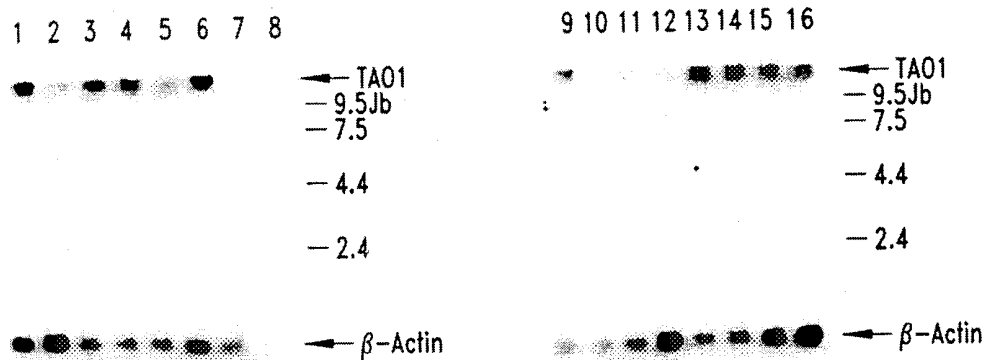


Fig. 4A

Fig. 4B

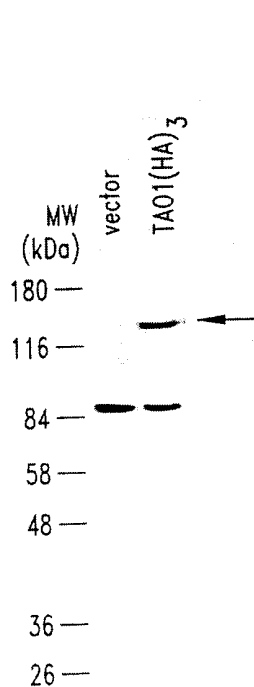


Fig. 5A

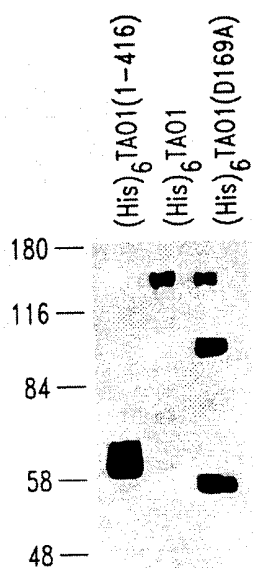


Fig. 5B

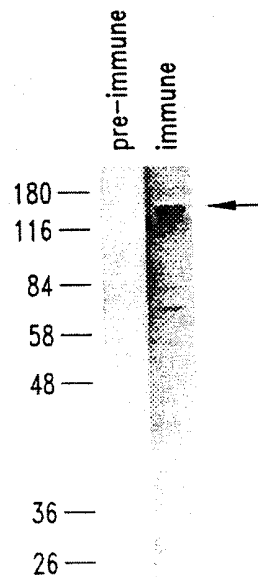
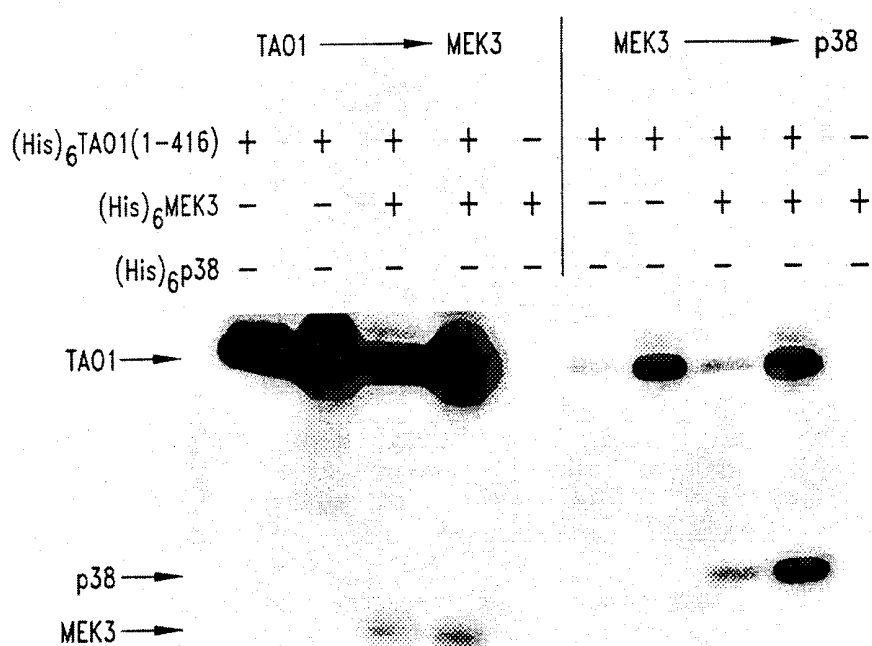


Fig. 5C

*Fig. 6*

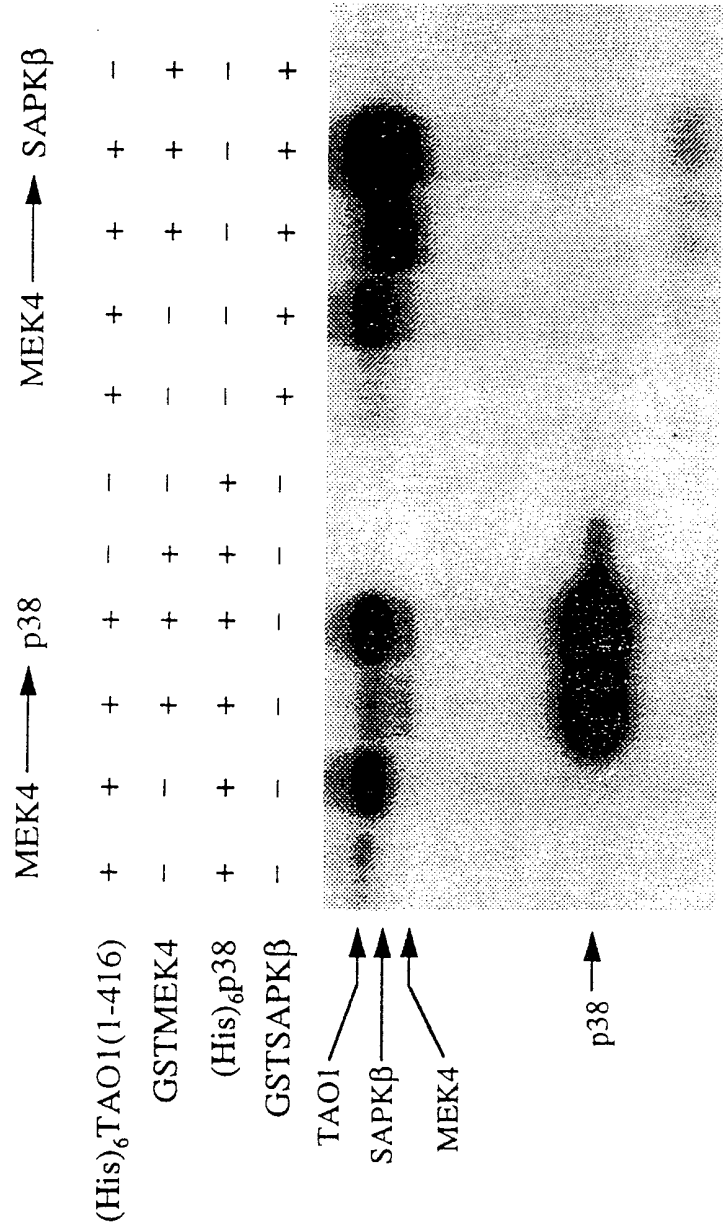


FIG. 7

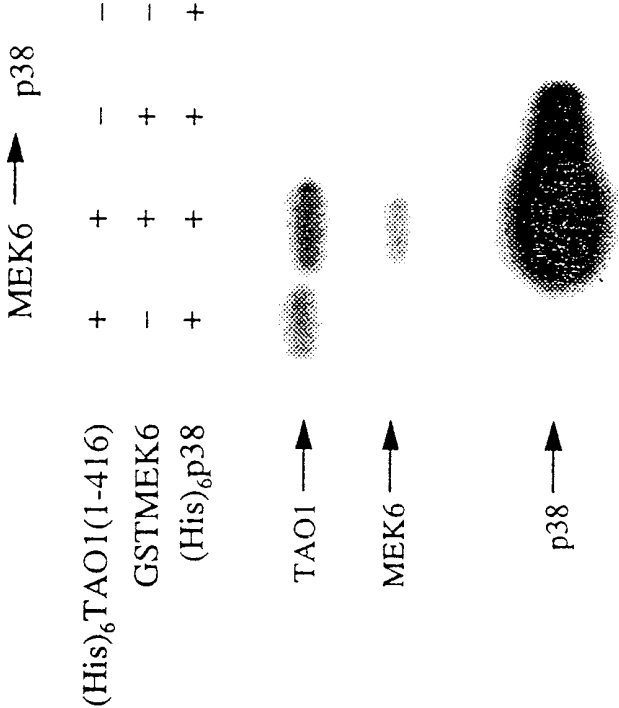


FIG. 8

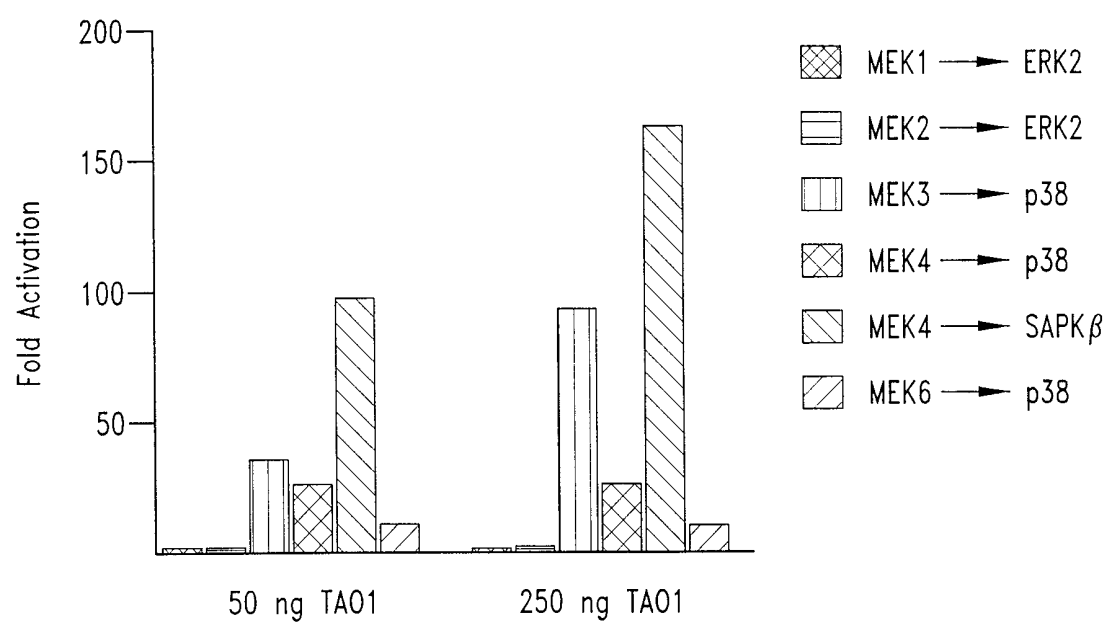
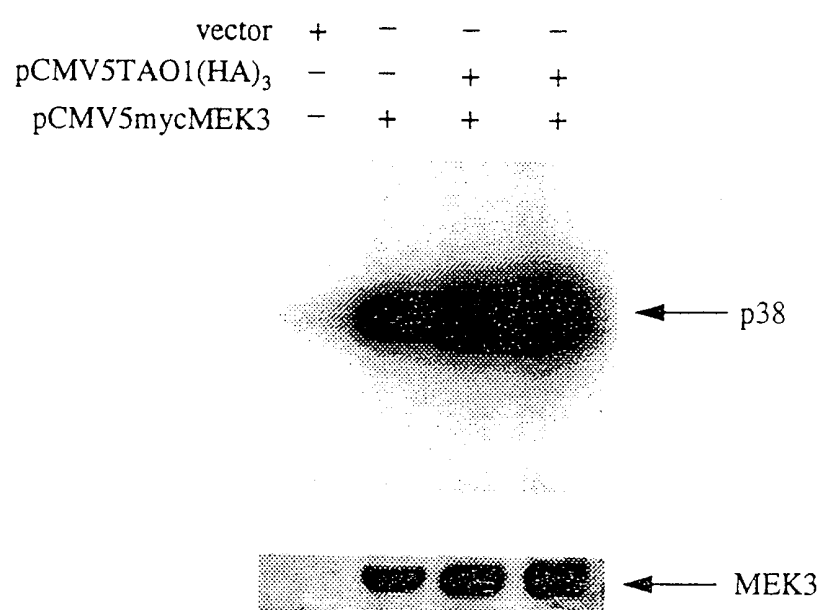
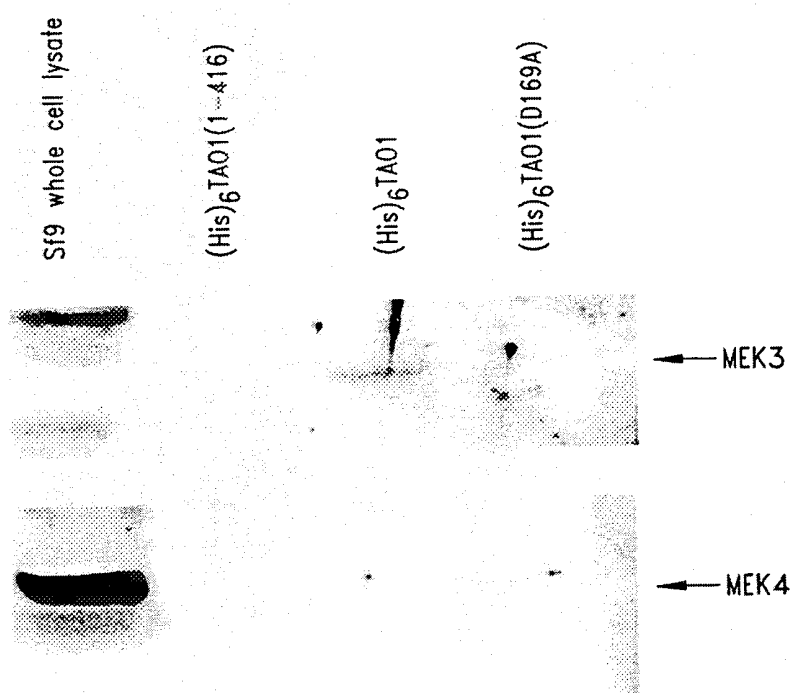


Fig. 9

**FIG. 10**

*Fig. 11*


```
Query: 964 GAACAAACATGTGCTTTAAAGTTCCTCTGATGTAGGGCGATCTTGAGGGATTTTCTGGA 905
      |||||  ||| ||| |||| | ||||| || | || |||| | ||||| |
Sbjct: 79 GAACAAAGTCATGCCCTTAATAGTTCTGCTGATGTTGGCCTTTCCTGAGGTATTTCTGCA 138

Query: 904 GGCAAGAATCTACAAAGTTTCGAAAATAATCAGACCATTTCATTAGACTGTAGTGTAGGGG 845
      ||| ||| |||| | ||| | || | ||||| ||||| || ||||
Sbjct: 139 AGCAGTAATCAACAAATCTCTAAAGGAGTCTGTCCATTTCATTAGACTGTAACTTGGGG 198

Query: 844 ATTCATTTTGGGCTATGTGATATAAGGCACTCATTGCATTCATATTAATAAAGGAGGCT 785
      | |||| | |||| | ||||| ||||| ||||| ||||| || || | || ||||
Sbjct: 199 AGTCATTCTGGGCAATGTGATATAAGGCACTCATTGCATTCATGTTGAAAAGGGCGGCT 258

Query: 784 TCCTCTCGGCTAATTCAATACATGTTATTCCAAGAGACCATACATCAACTTTGCCATCAT 725
      ||| || || | ||||| |||| | || |||| | |||| | ||||| |||||
Sbjct: 259 TCCGTTCCGCCAATTCAATACAAGTGATGCCAAGTGACCAATATCAACTTTCCCATCAT 318

Query: 724 ATTGTCCTTCATCCATGGCTAAAATTACTTCTGGGGCCATCCAATATGGTGTTCACAAA 665
      | ||||| ||||| |||| | || |||| | |||| | || |||| | |||| |
Sbjct: 319 ACTGTCCTTCATCCATAGCTAAGATCACCTCTGGAGCCATCCAGTAAGGTGTGCCACGA 378

Query: 664 AAGAATTGGCAGGG 651
      ||| |||| |
Sbjct: 379 AGGAGTTGGCCAGG 392
```

Fig. 13


```
Query:   2248 CAGCAGCCTAAGAGTTTGAAGTCTAAAGAACTCCAATAAAAAAGCAGTTTCAGGATACC 2307
          ||| |||| | | | | | | | | | | | | | | | | | | | | |
Sbjct:    3 CAACAGCAGAAAAAATTAAAGGCCATGGAATGCAAAATTA AAAACAGTTTCAGGACACT 62

Query:   2308 TGCAAAATTCAAACCAGACAGTACAAGCATTAAGGAATCACCTACTGGAGACTACACCA 2367
          ||||| | || |||| ||||| ||||| | | ||||| | |||| | ||| |||
Sbjct:   63 TGCAAAGTACAGACCAAACAGTATAAAGCACTCAAGAATCACCAAGTTGGAAGTTACTCCA 122

Query:   2368 AAGAGTGAGCACAAAGCTGTTCTGAAAAGACTCAAGGAGGAACAGACTCGGAAGTTAGCC 2427
          |||| ||||||||| | | | | | | | | | | | | | | | | | | | |
Sbjct:   123 AAGAATGAGCACAAAACAATCTTAAAGACACTGAAAGATGAGCAGACAAGAAAATTGCC 182

Query:   2428 ATCTTGGCTG 2437
          || |||| |
Sbjct:   183 ATTTTGGCAG 192
```

Fig. 15A

```

Query: 2437 GAGCAGTATGATCATAGCATTAAATGAAATGCTCTCCACACAAGCTCTGCGTTTGGATGAA 2496
          |||||
Sbjct: 193 GAGCAGTATGAACAGAGTATAAATGAAATGATGGCCTCTCAAGCGTTACGGCTAGATGAG 252

Query: 2497 GCACA 2501
          |||
Sbjct: 253 GCTCA 257

```

Fig. 15B

```
Query: 2087 ATGAATCCATGCAAGAACTGGAGTTTCGCCACCTCAACACTATTCAGAAGATGCGCTGTG 2146
          | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct: 31 ACGAGTCCCCCGAGAGCTAGAGTACAGGCAGCTGCACACGTTACAGAAGCTACGCATGG 90

Query: 2147 AGTTGATCAGACTGCAACATCAAACCTGAGCTTACTAACCAGCTGGAATACAATAAGAGAA 2206
          | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct: 91 ATCTGATCCGTTTACAGCACCAGACGGAACCTGGAAAACAGCTGGAGTACAATAAGAGGC 150

Query: 2207 GGGAAACGGGAACCTAAGACGGAACATGTCATGGAAGTTCGACAGCAGCCTAAGAGTTTGA 2266
          | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct: 151 GAGAAAGAGAACTGCACAGAAAGCATGTCATGGAACCTCGGCAACAGCCAAAAAAGCTTAA 210

Query: 2267 AGTCTAAAGAACTCCAATAAAAAAGCAGTTTCAGGATA 2305
          | | | | | | | | | | | | | | | | | | | | | |
Sbjct: 211 AGGCCATGGAANTGCAATTTAAAAACAGTTCAGGAAA 249
```

Fig. 16

```
Query: 3228 GTGCATATGGTATATTTTATTCGTCTTTGTAAAGCGTTATGTTTTGTGTTTACTAATTGG 3287
          |||
Sbjct: 31 GTGCATATGGTATATTTNATTCATTTTGTAAAGCGTCTGTTTTGTGTTTACTAATTGG 90

Query: 3288 GATGTCATAGTATTTGGCTGCCGGG 3312
          |||
Sbjct: 91 GATGTCATAGTACTTGGCTGCCGGG 115
```

Fig. 17A

```
Query: 3200 CTCACCTGGGTACTACCGGGTGAAGCTGTGCATATGGTATATTT 3245
          |||
Sbjct: 1 CTCACCTGGGTACTACAGTGTGAAGCTGAGTGCATATGGTATATT 46
```

Fig. 17B

```

Query:   739  GATGTATGGTCTCTTGAATAACATGTATTGAATTAGCCGAGAGGAAGCCTCCTTTATT  798
          ||| ||||| | || || ||||| || | ||||| |  || || || ||
Sbjct:   14   GATATTTGGTCATTGGGTATCACGTGTATAGAGCTGGCCGAACGTCGTCCACCATGTC  73

Query:   799  AATATGAATGCAATGAGTGCCTTATATCACATAGCCCAAATGAATCCCTACACT  854
          | ||||| ||||| |||| | || || || ||||| || || || ||
Sbjct:   74   AGTATGAATGCAATGTCTGCCCTCTACCATATTGCTCAAATGATCCTCCAACCTCT  129

```

Fig. 18

```

Query:   526  CTCAGGGATTAGCTTATTTACATTCTCATACCATGATCCATAGAGATATCAAAGCAGGA  585
          || || || || || || || || || || || || || || || || || || ||
Sbjct:   449  CTGAAAGGCCTGGATTATCTGCACTCAGAGCGCAAGATCCACCGAGATATCAAAGCTGCC  508

Query:   586  AATATCCTTCTGACAGAACCAGGCCAAGTGAACTTGCTGACTTTGGATCTGCTTCCA  643
          || || || || || || || || || || || || || || || || || ||
Sbjct:   509  AACGTGCTGCTCTCGGAGCAGGGTGATGTGAAGATGGCAGACTTCGGTGTGGCTGGCA  566

```

Fig. 19A

```

Query: 187 GATCCGAAAACTCTTCACAGATCTCAGAGAAATCGGCCATGGGAGCTTTGGAGCAGTT 246
      |||||
Sbjct: 119 GACCCAGAGGAACCTTCAACCAAGCTTGACCGCATTGGCAAAGGCTCATTGGGGAGGTG 178

Query: 247 TATTTTGCACGAGATGTGCGTACTAATGAAGTGGTGGCCATCAAGAAAAT 296
      |||||
Sbjct: 179 TACAAGGGGATCGACAACCACACCAAGGAAGTGGTGGCCATCAAGATCAT 228

```

Fig. 19B

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANTS: Cobb, Melanie
Hutchinson, Michele
Chen, Zhu
Berman, Kevin
- (ii) TITLE OF INVENTION: TAO PROTEIN KINASES AND METHODS OF USE
THEREFOR
- (iii) NUMBER OF SEQUENCES: 26
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: SEED and BERRY LLP
 - (B) STREET: 6300 Columbia Center, 701 Fifth Avenue
 - (C) CITY: Seattle
 - (D) STATE: Washington
 - (E) COUNTRY: USA
 - (F) ZIP: 98104
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: US
 - (B) FILING DATE: 14-APR-1998
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Maki, David J.
 - (B) REGISTRATION NUMBER: 31,392
 - (C) REFERENCE/DOCKET NUMBER: 860098.421
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (206) 622-4900
 - (B) TELEFAX: (206) 682-6031

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 3312 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 121..3123

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

TCTGCAGTAT GGTAGATTAT TATTTATGCA TTTATGCCAG TGTGGCTTCA TTCATACAGA	60
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ATG CCA TCA ACT AAC AGA GCA GGC AGT CTA AAG GAC CCT GAA ATC GCA Met Pro Ser Thr Asn Arg Ala Gly Ser Leu Lys Asp Pro Glu Ile Ala 1 5 10 15	168
GAG CTC TTC TTC AAA GAA GAT CCG GAA AAA CTC TTC ACA GAT CTC AGA Glu Leu Phe Phe Lys Glu Asp Pro Glu Lys Leu Phe Thr Asp Leu Arg 20 25 30	216
GAA ATC GGC CAT GGG AGC TTT GGA GCA GTT TAT TTT GCA CGA GAT GTG Glu Ile Gly His Gly Ser Phe Gly Ala Val Tyr Phe Ala Arg Asp Val 35 40 45	264
CGT ACT AAT GAA GTG GTG GCC ATC AAG AAA ATG TCT TAT AGT GGA AAG Arg Thr Asn Glu Val Val Ala Ile Lys Lys Met Ser Tyr Ser Gly Lys 50 55 60	312
CAG TCT ACT GAG AAA TGG CAG GAT ATT ATT AAG GAA GTC AAG TTT CTA Gln Ser Thr Glu Lys Trp Gln Asp Ile Ile Lys Glu Val Lys Phe Leu 65 70 75 80	360
CAA AGA ATA AAA CAT CCC AAC AGT ATA GAA TAC AAA GGC TGC TAT TTA Gln Arg Ile Lys His Pro Asn Ser Ile Glu Tyr Lys Gly Cys Tyr Leu 85 90 95	408
CGT GAA CAC ACA GCA TGG CTT GTA ATG GAA TAT TGT TTA GGA TCT GCT Arg Glu His Thr Ala Trp Leu Val Met Glu Tyr Cys Leu Gly Ser Ala 100 105 110	456
TCG GAT TTA CTA GAA GTT CAT AAA AAG CCA TTA CAA GAA GTG GAA ATA Ser Asp Leu Leu Glu Val His Lys Lys Pro Leu Gln Glu Val Glu Ile 115 120 125	504
GCA GCA ATT ACA CAT GGT GCT CTC CAG GGA TTA GCT TAT TTA CAT TCT Ala Ala Ile Thr His Gly Ala Leu Gln Gly Leu Ala Tyr Leu His Ser 130 135 140	552
CAT ACC ATG ATC CAT AGA GAT ATC AAA GCA GGA AAT ATC CTT CTG ACA His Thr Met Ile His Arg Asp Ile Lys Ala Gly Asn Ile Leu Leu Thr 145 150 155 160	600
GAA CCA GGC CAA GTG AAA CTT GCT GAC TTT GGA TCT GCT TCC ATG GCC Glu Pro Gly Gln Val Lys Leu Ala Asp Phe Gly Ser Ala Ser Met Ala 165 170 175	648
TCC CCT GCC AAT TCT TTT GTG GGA ACA CCA TAT TGG ATG GCC CCA GAA Ser Pro Ala Asn Ser Phe Val Gly Thr Pro Tyr Trp Met Ala Pro Glu 180 185 190	696

GTA ATT TTA GCC ATG GAT GAA GGA CAA TAT GAT GGC AAA GTT GAT GTA	744
Val Ile Leu Ala Met Asp Glu Gly Gln Tyr Asp Gly Lys Val Asp Val	
195 200 205	
TGG TCT CTT GGA ATA ACA TGT ATT GAA TTA GCC GAG AGG AAG CCT CCT	792
Trp Ser Leu Gly Ile Thr Cys Ile Glu Leu Ala Glu Arg Lys Pro Pro	
210 215 220	
TTA TTT AAT ATG AAT GCA ATG AGT GCC TTA TAT CAC ATA GCC CAA AAT	840
Leu Phe Asn Met Asn Ala Met Ser Ala Leu Tyr His Ile Ala Gln Asn	
225 230 235 240	
GAA TCC CCT ACA CTA CAG TCT AAT GAA TGG TCT GAT TAT TTT CGA AAC	888
Glu Ser Pro Thr Leu Gln Ser Asn Glu Trp Ser Asp Tyr Phe Arg Asn	
245 250 255	
TTT GTA GAT TCT TGC CTC CAG AAA ATC CCT CAA GAT CGC CCT ACA TCA	936
Phe Val Asp Ser Cys Leu Gln Lys Ile Pro Gln Asp Arg Pro Thr Ser	
260 265 270	
GAG GAA CTT TTA AAG CAC ATG TTT GTT CTT CGA GAG CGC CCT GAA ACA	984
Glu Glu Leu Leu Lys His Met Phe Val Leu Arg Glu Arg Pro Glu Thr	
275 280 285	
GTG TTA ATA GAT CTT ATT CAA AGG ACA AAG GAT GCA GTA AGA GAG CTG	1032
Val Leu Ile Asp Leu Ile Gln Arg Thr Lys Asp Ala Val Arg Glu Leu	
290 295 300	
GAC AAT CTA CAA TAT CGA AAG ATG AAG AAA CTC CTT TTC CAG GAG GCA	1080
Asp Asn Leu Gln Tyr Arg Lys Met Lys Lys Leu Leu Phe Gln Glu Ala	
305 310 315 320	
CAT AAT GGA CCA GCA GTA GAA GCA CAG GAA GAA GAG GAG GAG CAA GAT	1128
His Asn Gly Pro Ala Val Glu Ala Gln Glu Glu Glu Glu Glu Gln Asp	
325 330 335	
CAT GGT GGT GGC CGG ACA GGA ACA GTA AAT AGT GTT GGA AGC AAT CAG	1176
His Gly Gly Gly Arg Thr Gly Thr Val Asn Ser Val Gly Ser Asn Gln	
340 345 350	
TCT ATC CCC AGT ATG TCT ATC AGT GCC AGT AGC CAA AGC AGC AGT GTT	1224
Ser Ile Pro Ser Met Ser Ile Ser Ala Ser Ser Gln Ser Ser Ser Val	
355 360 365	
AAT AGT CTT CCA GAT GCA TCG GAT GAC AAG AGT GAG CTA GAC ATG ATG	1272
Asn Ser Leu Pro Asp Ala Ser Asp Asp Lys Ser Glu Leu Asp Met Met	
370 375 380	
GAG GGA GAC CAT ACA GTG ATG TCT AAC AGT TCT GTC ATC CAC TTA AAA	1320
Glu Gly Asp His Thr Val Met Ser Asn Ser Ser Val Ile His Leu Lys	
385 390 395 400	
CCT GAG GAG GAA AAT TAC CAA GAA GAA GGA GAT CCT AGA ACA AGA GCA	1368
Pro Glu Glu Glu Asn Tyr Gln Glu Glu Gly Asp Pro Arg Thr Arg Ala	
405 410 415	

TCA GCT CCA CAG TCT CCA CCT CAA GTG TCT CGT CAC AAA TCA CAT TAT	1416
Ser Ala Pro Gln Ser Pro Pro Gln Val Ser Arg His Lys Ser His Tyr	
420 425 430	
CGT AAT AGA GAA CAC TTT GCA ACT ATA CGA ACA GCA TCA CTG GTT ACA	1464
Arg Asn Arg Glu His Phe Ala Thr Ile Arg Thr Ala Ser Leu Val Thr	
435 440 445	
AGA CAG ATG CAA GAA CAT GAG CAG GAC TCT GAA CTT AGA GAA CAG ATG	1512
Arg Gln Met Gln Glu His Glu Gln Asp Ser Glu Leu Arg Glu Gln Met	
450 455 460	
TCT GGT TAT AAG CGG ATG AGG CGA CAG CAT CAG AAG CAG CTG ATG ACT	1560
Ser Gly Tyr Lys Arg Met Arg Arg Gln His Gln Lys Gln Leu Met Thr	
465 470 475 480	
CTG GAA AAT AAA CTG AAG GCA GAA ATG GAC GAA CAT CGG CTC AGA TTA	1608
Leu Glu Asn Lys Leu Lys Ala Glu Met Asp Glu His Arg Leu Arg Leu	
485 490 495	
GAC AAA GAT CTT GAA ACT CAG CGC AAC AAT TTC GCT GCA GAA ATG GAG	1656
Asp Lys Asp Leu Glu Thr Gln Arg Asn Asn Phe Ala Ala Glu Met Glu	
500 505 510	
AAA CTT ATT AAG AAA CAC CAA GCT TCT ATG GAA AAA GAG GCT AAA GTG	1704
Lys Leu Ile Lys Lys His Gln Ala Ser Met Glu Lys Glu Ala Lys Val	
515 520 525	
ATG GCC AAC GAG GAG AAA AAA TTC CAA CAA CAC ATT CAG GCT CAA CAG	1752
Met Ala Asn Glu Glu Lys Lys Phe Gln Gln His Ile Gln Ala Gln Gln	
530 535 540	
AAG AAA GAA CTG AAT AGC TTT TTG GAG TCT CAA AAA AGA GAA TAT AAA	1800
Lys Lys Glu Leu Asn Ser Phe Leu Glu Ser Gln Lys Arg Glu Tyr Lys	
545 550 555 560	
CTT CGA AAA GAG CAG CTT AAG GAG GAG CTG AAT GAA AAC CAG AGC ACA	1848
Leu Arg Lys Glu Gln Leu Lys Glu Glu Leu Asn Glu Asn Gln Ser Thr	
565 570 575	
CCT AAA AAA GAA AAG CAG GAA TGG CTT TCA AAG CAG AAG GAG AAT ATT	1896
Pro Lys Lys Glu Lys Gln Glu Trp Leu Ser Lys Gln Lys Glu Asn Ile	
580 585 590	
CAA CAT TTT CAG GCA GAA GAA GAA GCT AAT CTT CTT CGA CGT CAA AGG	1944
Gln His Phe Gln Ala Glu Glu Glu Ala Asn Leu Leu Arg Arg Gln Arg	
595 600 605	
CAG TAT CTA GAG CTA GAA TGT CGT CGC TTC AAA AGA AGA ATG TTA CTT	1992
Gln Tyr Leu Glu Leu Glu Cys Arg Arg Phe Lys Arg Arg Met Leu Leu	
610 615 620	
GGT CGG CAT AAC TTG GAA CAG GAC CTT GTC AGG GAG GAG TTA AAC AAA	2040
Gly Arg His Asn Leu Glu Gln Asp Leu Val Arg Glu Glu Leu Asn Lys	

625					630					635					640	
AGG	CAG	ACT	CAG	AAG	GAC	TTA	GAA	CAT	GCA	ATG	TTA	CTG	CGA	CAG	CAT	2088
Arg	Gln	Thr	Gln	Lys	Asp	Leu	Glu	His	Ala	Met	Leu	Leu	Arg	Gln	His	
				645					650					655		
GAA	TCC	ATG	CAA	GAA	CTG	GAG	TTT	CGC	CAC	CTC	AAC	ACT	ATT	CAG	AAG	2136
Glu	Ser	Met	Gln	Glu	Leu	Glu	Phe	Arg	His	Leu	Asn	Thr	Ile	Gln	Lys	
			660					665					670			
ATG	CGC	TGT	GAG	TTG	ATC	AGA	CTG	CAA	CAT	CAA	ACT	GAG	CTT	ACT	AAC	2184
Met	Arg	Cys	Glu	Leu	Ile	Arg	Leu	Gln	His	Gln	Thr	Glu	Leu	Thr	Asn	
		675					680					685				
CAG	CTG	GAA	TAC	AAT	AAG	AGA	AGG	GAA	CGG	GAA	CTA	AGA	CGG	AAA	CAT	2232
Gln	Leu	Glu	Tyr	Asn	Lys	Arg	Arg	Glu	Arg	Glu	Leu	Arg	Arg	Lys	His	
	690					695					700					
GTC	ATG	GAA	GTT	CGA	CAG	CAG	CCT	AAG	AGT	TTG	AAG	TCT	AAA	GAA	CTC	2280
Val	Met	Glu	Val	Arg	Gln	Gln	Pro	Lys	Ser	Leu	Lys	Ser	Lys	Glu	Leu	
705					710					715				720		
CAA	ATA	AAA	AAG	CAG	TTT	CAG	GAT	ACC	TGC	AAA	ATT	CAA	ACC	AGA	CAG	2328
Gln	Ile	Lys	Lys	Gln	Phe	Gln	Asp	Thr	Cys	Lys	Ile	Gln	Thr	Arg	Gln	
			725						730					735		
TAC	AAA	GCA	TTA	AGG	AAT	CAC	CTA	CTG	GAG	ACT	ACA	CCA	AAG	AGT	GAG	2376
Tyr	Lys	Ala	Leu	Arg	Asn	His	Leu	Leu	Glu	Thr	Thr	Pro	Lys	Ser	Glu	
			740					745					750			
CAC	AAA	GCT	GTT	CTG	AAA	AGA	CTC	AAG	GAG	GAA	CAG	ACT	CGG	AAG	TTA	2424
His	Lys	Ala	Val	Leu	Lys	Arg	Leu	Lys	Glu	Glu	Gln	Thr	Arg	Lys	Leu	
		755					760					765				
GCC	ATC	TTG	GCT	GAG	CAG	TAT	GAT	CAT	AGC	ATT	AAT	GAA	ATG	CTC	TCC	2472
Ala	Ile	Leu	Ala	Glu	Gln	Tyr	Asp	His	Ser	Ile	Asn	Glu	Met	Leu	Ser	
	770					775					780					
ACA	CAA	GCT	CTG	CGT	TTG	GAT	GAA	GCA	CAG	GAA	GCA	GAA	TGC	CAG	GTT	2520
Thr	Gln	Ala	Leu	Arg	Leu	Asp	Glu	Ala	Gln	Glu	Ala	Glu	Cys	Gln	Val	
785					790					795				800		
TTG	AAG	ATG	CAG	CTA	CAG	CAG	GAA	CTG	GAG	CTG	TTG	AAT	GCA	TAT	CAG	2568
Leu	Lys	Met	Gln	Leu	Gln	Gln	Glu	Leu	Glu	Leu	Leu	Asn	Ala	Tyr	Gln	
			805						810					815		
AGC	AAA	ATC	AAG	ATG	CAG	GCT	GAG	GCC	CAA	CAT	GAT	CGA	GAG	CTT	CGA	2616
Ser	Lys	Ile	Lys	Met	Gln	Ala	Glu	Ala	Gln	His	Asp	Arg	Glu	Leu	Arg	
			820					825					830			
GAG	CTG	GAA	CAA	AGG	GTC	TCC	CTT	CGG	AGA	GCA	CTC	TTA	GAA	CAG	AAG	2664
Glu	Leu	Glu	Gln	Arg	Val	Ser	Leu	Arg	Arg	Ala	Leu	Leu	Glu	Gln	Lys	
		835					840					845				
ATT	GAA	GAA	GAG	ATG	TTG	GCT	TTG	CAG	AAT	GAA	CGC	ACA	GAA	CGA	ATA	2712

Ile	Glu	Glu	Glu	Met	Leu	Ala	Leu	Gln	Asn	Glu	Arg	Thr	Glu	Arg	Ile	
850						855					860					
CGT	AGC	CTG	CTC	GAG	CGC	CAG	GCC	AGA	GAA	ATT	GAA	GCT	TTT	GAC	TCT	2760
Arg	Ser	Leu	Leu	Glu	Arg	Gln	Ala	Arg	Glu	Ile	Glu	Ala	Phe	Asp	Ser	
865					870				875						880	
GAA	AGC	ATG	AGA	TTA	GGT	TTT	AGT	AAC	ATG	GTC	CTT	TCT	AAT	CTC	TCC	2808
Glu	Ser	Met	Arg	Leu	Gly	Phe	Ser	Asn	Met	Val	Leu	Ser	Asn	Leu	Ser	
				885					890					895		
CCT	GAG	GCA	TTC	AGC	CAC	AGC	TAC	CCA	GGA	GCT	TCT	AGC	TGG	TCT	CAC	2856
Pro	Glu	Ala	Phe	Ser	His	Ser	Tyr	Pro	Gly	Ala	Ser	Ser	Trp	Ser	His	
			900					905					910			
AAT	CCT	ACT	GGG	GGT	TCA	GGA	CCT	CAC	TGG	GGT	CAT	CCC	ATG	GGT	GGC	2904
Asn	Pro	Thr	Gly	Gly	Ser	Gly	Pro	His	Trp	Gly	His	Pro	Met	Gly	Gly	
		915					920					925				
ACA	CCA	CAA	GCT	TGG	GGT	CAT	CCG	ATG	CAA	GGC	GGA	CCC	CAA	CCA	TGG	2952
Thr	Pro	Gln	Ala	Trp	Gly	His	Pro	Met	Gln	Gly	Gly	Pro	Gln	Pro	Trp	
		930				935					940					
GGT	CAC	CCC	TCA	GGG	CCA	ATG	CAA	GGG	GTA	CCT	CGA	GGT	AGC	AGT	ATA	3000
Gly	His	Pro	Ser	Gly	Pro	Met	Gln	Gly	Val	Pro	Arg	Gly	Ser	Ser	Ile	
945					950				955						960	
GGA	GTC	CGC	AAT	AGC	CCC	CAG	GCT	CTG	AGG	CGG	ACA	GCT	TCT	GGG	GGA	3048
Gly	Val	Arg	Asn	Ser	Pro	Gln	Ala	Leu	Arg	Arg	Thr	Ala	Ser	Gly	Gly	
				965					970					975		
CGG	ACG	GAA	CAG	GGC	ATG	AGC	AGA	AGC	ACG	AGT	GTC	ACT	TCA	CAA	ATA	3096
Arg	Thr	Glu	Gln	Gly	Met	Ser	Arg	Ser	Thr	Ser	Val	Thr	Ser	Gln	Ile	
			980					985					990			
TCC	AAT	GGG	TCA	CAC	ATG	TCT	TAC	ACA	TAATAATTGA	AAGTGGCAAT						3143
Ser	Asn	Gly	Ser	His	Met	Ser	Tyr	Thr								
		995					1000									
TCCGCTGGAG	CTGTCTGCCA	AAAGAACTG	CCTACAGACA	TCAGCACAGC	AGCCTCCTCA											3203
CTTGGGTACT	ACCGGGTGA	AGCTGTGCAT	ATGGTATATT	TTATTCGTCT	TTGTAAAGCG											3263
TTATGTTTTG	TGTTTACTAA	TTGGGATGTC	ATAGTATTTG	GCTGCCGGG												3312

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1001 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

Met Pro Ser Thr Asn Arg Ala Gly Ser Leu Lys Asp Pro Glu Ile Ala
 1 5 10 15
 Glu Leu Phe Phe Lys Glu Asp Pro Glu Lys Leu Phe Thr Asp Leu Arg
 20 25 30
 Glu Ile Gly His Gly Ser Phe Gly Ala Val Tyr Phe Ala Arg Asp Val
 35 40 45
 Arg Thr Asn Glu Val Val Ala Ile Lys Lys Met Ser Tyr Ser Gly Lys
 50 55 60
 Gln Ser Thr Glu Lys Trp Gln Asp Ile Ile Lys Glu Val Lys Phe Leu
 65 70 75 80
 Gln Arg Ile Lys His Pro Asn Ser Ile Glu Tyr Lys Gly Cys Tyr Leu
 85 90 95
 Arg Glu His Thr Ala Trp Leu Val Met Glu Tyr Cys Leu Gly Ser Ala
 100 105 110
 Ser Asp Leu Leu Glu Val His Lys Lys Pro Leu Gln Glu Val Glu Ile
 115 120 125
 Ala Ala Ile Thr His Gly Ala Leu Gln Gly Leu Ala Tyr Leu His Ser
 130 135 140
 His Thr Met Ile His Arg Asp Ile Lys Ala Gly Asn Ile Leu Leu Thr
 145 150 155 160
 Glu Pro Gly Gln Val Lys Leu Ala Asp Phe Gly Ser Ala Ser Met Ala
 165 170 175
 Ser Pro Ala Asn Ser Phe Val Gly Thr Pro Tyr Trp Met Ala Pro Glu
 180 185 190
 Val Ile Leu Ala Met Asp Glu Gly Gln Tyr Asp Gly Lys Val Asp Val
 195 200 205
 Trp Ser Leu Gly Ile Thr Cys Ile Glu Leu Ala Glu Arg Lys Pro Pro
 210 215 220
 Leu Phe Asn Met Asn Ala Met Ser Ala Leu Tyr His Ile Ala Gln Asn
 225 230 235 240
 Glu Ser Pro Thr Leu Gln Ser Asn Glu Trp Ser Asp Tyr Phe Arg Asn
 245 250 255
 Phe Val Asp Ser Cys Leu Gln Lys Ile Pro Gln Asp Arg Pro Thr Ser
 260 265 270
 Glu Glu Leu Leu Lys His Met Phe Val Leu Arg Glu Arg Pro Glu Thr
 275 280 285

Val Leu Ile Asp Leu Ile Gln Arg Thr Lys Asp Ala Val Arg Glu Leu
 290 295 300

Asp Asn Leu Gln Tyr Arg Lys Met Lys Lys Leu Leu Phe Gln Glu Ala
 305 310 315 320

His Asn Gly Pro Ala Val Glu Ala Gln Glu Glu Glu Glu Glu Gln Asp
 325 330 335

His Gly Gly Gly Arg Thr Gly Thr Val Asn Ser Val Gly Ser Asn Gln
 340 345 350

Ser Ile Pro Ser Met Ser Ile Ser Ala Ser Ser Gln Ser Ser Ser Val
 355 360 365

Asn Ser Leu Pro Asp Ala Ser Asp Asp Lys Ser Glu Leu Asp Met Met
 370 375 380

Glu Gly Asp His Thr Val Met Ser Asn Ser Ser Val Ile His Leu Lys
 385 390 395 400

Pro Glu Glu Glu Asn Tyr Gln Glu Glu Gly Asp Pro Arg Thr Arg Ala
 405 410 415

Ser Ala Pro Gln Ser Pro Pro Gln Val Ser Arg His Lys Ser His Tyr
 420 425 430

Arg Asn Arg Glu His Phe Ala Thr Ile Arg Thr Ala Ser Leu Val Thr
 435 440 445

Arg Gln Met Gln Glu His Glu Gln Asp Ser Glu Leu Arg Glu Gln Met
 450 455 460

Ser Gly Tyr Lys Arg Met Arg Arg Gln His Gln Lys Gln Leu Met Thr
 465 470 475 480

Leu Glu Asn Lys Leu Lys Ala Glu Met Asp Glu His Arg Leu Arg Leu
 485 490 495

Asp Lys Asp Leu Glu Thr Gln Arg Asn Asn Phe Ala Ala Glu Met Glu
 500 505 510

Lys Leu Ile Lys Lys His Gln Ala Ser Met Glu Lys Glu Ala Lys Val
 515 520 525

Met Ala Asn Glu Glu Lys Lys Phe Gln Gln His Ile Gln Ala Gln Gln
 530 535 540

Lys Lys Glu Leu Asn Ser Phe Leu Glu Ser Gln Lys Arg Glu Tyr Lys
 545 550 555 560

Leu Arg Lys Glu Gln Leu Lys Glu Glu Leu Asn Glu Asn Gln Ser Thr
 565 570 575

Pro Lys Lys Glu Lys Gln Glu Trp Leu Ser Lys Gln Lys Glu Asn Ile

580										585					590						
Gln	His	Phe	Gln	Ala	Glu	Glu	Glu	Ala	Asn	Leu	Leu	Arg	Arg	Gln	Arg						
		595						600						605							
Gln	Tyr	Leu	Glu	Leu	Glu	Cys	Arg	Arg	Phe	Lys	Arg	Arg	Met	Leu	Leu						
	610					615					620										
Gly	Arg	His	Asn	Leu	Glu	Gln	Asp	Leu	Val	Arg	Glu	Glu	Leu	Asn	Lys						
625					630					635					640						
Arg	Gln	Thr	Gln	Lys	Asp	Leu	Glu	His	Ala	Met	Leu	Leu	Arg	Gln	His						
				645					650					655							
Glu	Ser	Met	Gln	Glu	Leu	Glu	Phe	Arg	His	Leu	Asn	Thr	Ile	Gln	Lys						
			660					665					670								
Met	Arg	Cys	Glu	Leu	Ile	Arg	Leu	Gln	His	Gln	Thr	Glu	Leu	Thr	Asn						
		675					680					685									
Gln	Leu	Glu	Tyr	Asn	Lys	Arg	Arg	Glu	Arg	Glu	Leu	Arg	Arg	Lys	His						
	690					695					700										
Val	Met	Glu	Val	Arg	Gln	Gln	Pro	Lys	Ser	Leu	Lys	Ser	Lys	Glu	Leu						
705					710					715					720						
Gln	Ile	Lys	Lys	Gln	Phe	Gln	Asp	Thr	Cys	Lys	Ile	Gln	Thr	Arg	Gln						
				725					730					735							
Tyr	Lys	Ala	Leu	Arg	Asn	His	Leu	Leu	Glu	Thr	Thr	Pro	Lys	Ser	Glu						
			740					745					750								
His	Lys	Ala	Val	Leu	Lys	Arg	Leu	Lys	Glu	Glu	Gln	Thr	Arg	Lys	Leu						
		755					760					765									
Ala	Ile	Leu	Ala	Glu	Gln	Tyr	Asp	His	Ser	Ile	Asn	Glu	Met	Leu	Ser						
	770					775					780										
Thr	Gln	Ala	Leu	Arg	Leu	Asp	Glu	Ala	Gln	Glu	Ala	Glu	Cys	Gln	Val						
785					790					795					800						
Leu	Lys	Met	Gln	Leu	Gln	Gln	Glu	Leu	Glu	Leu	Leu	Asn	Ala	Tyr	Gln						
			805					810						815							
Ser	Lys	Ile	Lys	Met	Gln	Ala	Glu	Ala	Gln	His	Asp	Arg	Glu	Leu	Arg						
			820					825					830								
Glu	Leu	Glu	Gln	Arg	Val	Ser	Leu	Arg	Arg	Ala	Leu	Leu	Glu	Gln	Lys						
	835						840					845									
Ile	Glu	Glu	Glu	Met	Leu	Ala	Leu	Gln	Asn	Glu	Arg	Thr	Glu	Arg	Ile						
	850					855					860										
Arg	Ser	Leu	Leu	Glu	Arg	Gln	Ala	Arg	Glu	Ile	Glu	Ala	Phe	Asp	Ser						
865					870					875					880						

Glu	Ser	Met	Arg	Leu	Gly	Phe	Ser	Asn	Met	Val	Leu	Ser	Asn	Leu	Ser	
				885				890				895				
Pro	Glu	Ala	Phe	Ser	His	Ser	Tyr	Pro	Gly	Ala	Ser	Ser	Trp	Ser	His	
				900				905				910				
Asn	Pro	Thr	Gly	Gly	Ser	Gly	Pro	His	Trp	Gly	His	Pro	Met	Gly	Gly	
				915				920				925				
Thr	Pro	Gln	Ala	Trp	Gly	His	Pro	Met	Gln	Gly	Gly	Pro	Gln	Pro	Trp	
				930				935				940				
Gly	His	Pro	Ser	Gly	Pro	Met	Gln	Gly	Val	Pro	Arg	Gly	Ser	Ser	Ile	
945				950				955				960				
Gly	Val	Arg	Asn	Ser	Pro	Gln	Ala	Leu	Arg	Arg	Thr	Ala	Ser	Gly	Gly	
				965				970				975				
Arg	Thr	Glu	Gln	Gly	Met	Ser	Arg	Ser	Thr	Ser	Val	Thr	Ser	Gln	Ile	
				980				985				990				
Ser	Asn	Gly	Ser	His	Met	Ser	Tyr	Thr								
995				1000												

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4296 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 193..3171

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AGGGGAGGCT	TCCCGGGCCC	GCCCCTCAGG	AAGGGCGAAA	GCTGAGGAAG	AGGTGGCGAG		60									
GGGGAAGGTC	TCCTTGCCCC	TCTCCCCGCT	TGTCAGAGCA	ACTGGAGTAC	CCCAGGCGGA		120									
AGCGGAGGCG	CTGGGGCACC	ATAGTGACCC	CTACCAGGCA	AGATCCCAAT	TTCAGGGCCC		180									
CCAGGGGGCCA	TC	ATG	CCA	GCT	GGG	GGC	CGG	GCC	GGG	AGC	CTG	AAG	GAC		228	
		Met	Pro	Ala	Gly	Gly	Arg	Ala	Gly	Ser	Leu	Lys	Asp			
		1					5						10			
CCT	GAT	GTA	GCT	GAG	CTC	TTC	TTC	AAA	GAT	GAC	CCT	GAG	AAG	CTT	TTC	276
Pro	Asp	Val	Ala	Glu	Leu	Phe	Phe	Lys	Asp	Asp	Pro	Glu	Lys	Leu	Phe	
		15						20					25			
TCT	GAC	CTC	CGG	GAA	ATT	GGC	CAT	GGC	AGT	TTT	GGA	GCT	GTG	TAC	TTT	324
Ser	Asp	Leu	Arg	Glu	Ile	Gly	His	Gly	Ser	Phe	Gly	Ala	Val	Tyr	Phe	

30	35	40	
GCC CGG GAT GTC CGG AAC AGT GAG GTG GTG GCC ATC AAG AAG ATG TCC			372
Ala Arg Asp Val Arg Asn Ser Glu Val Val Ala Ile Lys Lys Met Ser			
45	50	55	60
TAT AGT GGG AAG CAA TCA AAT GAG AAA TGG CAG GAT ATC ATC AAG GAG			420
Tyr Ser Gly Lys Gln Ser Asn Glu Lys Trp Gln Asp Ile Ile Lys Glu			
	65	70	75
GTG CGG TTC TTA CAG AAG CTA CGG CAT CCT AAT ACC ATT CAG TAC CGG			468
Val Arg Phe Leu Gln Lys Leu Arg His Pro Asn Thr Ile Gln Tyr Arg			
	80	85	90
GGC TGT TAC CTG AGG GAG CAC ACA GCT TGG CTG GTG ATG GAG TAT TGC			516
Gly Cys Tyr Leu Arg Glu His Thr Ala Trp Leu Val Met Glu Tyr Cys			
	95	100	105
CTG GGT TCA GCT TCT GAT CTT CTC GAA GTG CAC AAG AAG CCG CTG CAG			564
Leu Gly Ser Ala Ser Asp Leu Leu Glu Val His Lys Lys Pro Leu Gln			
	110	115	120
GAG GTA GAG ATT GCA GCT GTG ACC CAT GGT GCG CTT CAG GGC CTG GCC			612
Glu Val Glu Ile Ala Ala Val Thr His Gly Ala Leu Gln Gly Leu Ala			
	125	130	135
140			
TAT CTA CAT TCA CAC AAC ATG ATC CAT AGA GAT GTG AAG GCT GGG AAC			660
Tyr Leu His Ser His Asn Met Ile His Arg Asp Val Lys Ala Gly Asn			
	145	150	155
ATC TTG CTG TCA GAA CCA GGC TTG GTG AAA CTG GGG GAC TTT GGC TCC			708
Ile Leu Leu Ser Glu Pro Gly Leu Val Lys Leu Gly Asp Phe Gly Ser			
	160	165	170
GCA TCC ATC ATG GCA CCT GCC AAC TCA TTT GTG GGC ACT CCA TAC TGG			756
Ala Ser Ile Met Ala Pro Ala Asn Ser Phe Val Gly Thr Pro Tyr Trp			
	175	180	185
ATG GCT CCA GAG GTG ATC CTA GCC ATG GAT GAG GGA CAA TAT GAT GGC			804
Met Ala Pro Glu Val Ile Leu Ala Met Asp Glu Gly Gln Tyr Asp Gly			
	190	195	200
AAA GTG GAT GTC TGG TCC TTG GGG ATA ACC TGT ATT GAG CTA GCG GAG			852
Lys Val Asp Val Trp Ser Leu Gly Ile Thr Cys Ile Glu Leu Ala Glu			
	205	210	215
220			
CGG AAG CCA CCA CTG TTT AAC ATG AAT GCA ATG AGT GCC TTA TAC CAC			900
Arg Lys Pro Pro Leu Phe Asn Met Asn Ala Met Ser Ala Leu Tyr His			
	225	230	235
ATT GCA CAG AAT GAA TCC CCT GCT CTC CAG TCA GGA CAC TGG TCT GAG			948
Ile Ala Gln Asn Glu Ser Pro Ala Leu Gln Ser Gly His Trp Ser Glu			
	240	245	250
TAC TTC CGG AAT TTT GTT GAC TCC TGT CTT CAG AAA ATC CCT CAA GAC			996

Tyr	Phe	Arg	Asn	Phe	Val	Asp	Ser	Cys	Leu	Gln	Lys	Ile	Pro	Gln	Asp	
		255					260					265				
AGA	CCA	ACC	TCA	GAG	GTT	CTT	TTG	AAG	CAC	CGC	TTT	GTG	CTC	CGG	GAG	1044
Arg	Pro	Thr	Ser	Glu	Val	Leu	Leu	Lys	His	Arg	Phe	Val	Leu	Arg	Glu	
		270				275					280					
CGG	CCA	CCC	ACA	GTC	ATC	ATG	GAC	CTA	ATT	CAG	AGG	ACC	AAG	GAT	GCT	1092
Arg	Pro	Pro	Thr	Val	Ile	Met	Asp	Leu	Ile	Gln	Arg	Thr	Lys	Asp	Ala	
285					290					295					300	
GTA	CGG	GAA	CTA	GAT	AAC	CTG	CAG	TAC	CGA	AAG	ATG	AAG	AAG	ATA	CTA	1140
Val	Arg	Glu	Leu	Asp	Asn	Leu	Gln	Tyr	Arg	Lys	Met	Lys	Lys	Ile	Leu	
				305				310						315		
TTC	CAA	GAG	GCA	CCC	AAT	GGC	CCT	GGT	GCT	GAG	GCC	CCA	GAG	GAA	GAG	1188
Phe	Gln	Glu	Ala	Pro	Asn	Gly	Pro	Gly	Ala	Glu	Ala	Pro	Glu	Glu	Glu	
			320					325					330			
GAG	GAA	GCA	GAA	CCT	TAC	ATG	CAC	CGA	GCA	GGG	ACA	CTG	ACC	AGT	CTA	1236
Glu	Glu	Ala	Glu	Pro	Tyr	Met	His	Arg	Ala	Gly	Thr	Leu	Thr	Ser	Leu	
		335					340					345				
GAG	AGT	AGC	CAT	TCA	GTG	CCC	AGC	ATG	TCC	ATC	AGC	GCC	TCC	AGC	CAA	1284
Glu	Ser	Ser	His	Ser	Val	Pro	Ser	Met	Ser	Ile	Ser	Ala	Ser	Ser	Gln	
	350					355					360					
AGC	AGC	TCA	GTC	AAC	AGC	CTA	GCA	GAT	GCC	TCA	GAT	AAT	GAA	GAA	GAG	1332
Ser	Ser	Ser	Val	Asn	Ser	Leu	Ala	Asp	Ala	Ser	Asp	Asn	Glu	Glu	Glu	
365					370				375						380	
GAG	GAG	GAG	GAA	GAG	GAA	GAA	GAA	GAG	GAG	GAG	GAA	GAA	GAA	GGC	CCT	1380
Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Gly	Pro	
				385				390						395		
GAA	TCC	CGA	GAG	ATG	GCC	ATG	ATG	CAG	GAG	GGG	GAG	CAT	ACA	GTC	ACT	1428
Glu	Ser	Arg	Glu	Met	Ala	Met	Met	Gln	Glu	Gly	Glu	His	Thr	Val	Thr	
			400					405					410			
TCC	CAC	AGC	TCC	ATC	ATC	CAC	CGG	CTG	CCG	GGC	TCA	GAC	AAC	CTA	TAT	1476
Ser	His	Ser	Ser	Ile	Ile	His	Arg	Leu	Pro	Gly	Ser	Asp	Asn	Leu	Tyr	
		415					420					425				
GAT	GAT	CCC	TAC	CAG	CCA	GAG	ATG	ACC	CCA	GGT	CCA	CTC	CAA	CCA	CCT	1524
Asp	Asp	Pro	Tyr	Gln	Pro	Glu	Met	Thr	Pro	Gly	Pro	Leu	Gln	Pro	Pro	
		430				435					440					
GCA	GCC	CCT	CCC	ACC	TCC	ACC	TCC	TCC	TCT	TCT	GCT	CGC	CGC	AGA	GCT	1572
Ala	Ala	Pro	Pro	Thr	Ser	Thr	Ser	Ser	Ser	Ser	Ala	Arg	Arg	Arg	Ala	
445					450				455						460	
TAT	TGC	CGC	AAC	CGA	GAC	CAC	TTT	GCC	ACC	ATC	CGT	ACT	GCC	TCC	CTG	1620
Tyr	Cys	Arg	Asn	Arg	Asp	His	Phe	Ala	Thr	Ile	Arg	Thr	Ala	Ser	Leu	
			465					470						475		

GTC AGC CGT CAG ATC CAG GAG CAT GAG CAG GAC TCG GCC CTG CGG GAG	1668
Val Ser Arg Gln Ile Gln Glu His Glu Gln Asp Ser Ala Leu Arg Glu	
480 485 490	
CAA CTA AGT GGC TAC AAG CGG ATG CGG CGT CAG CAC CAG AAG CAA CTG	1716
Gln Leu Ser Gly Tyr Lys Arg Met Arg Arg Gln His Gln Lys Gln Leu	
495 500 505	
CTG GCC CTG GAG TCC CGT CTG AGG GGT GAA CGT GAG GAG CAC AGT GGG	1764
Leu Ala Leu Glu Ser Arg Leu Arg Gly Glu Arg Glu Glu His Ser Gly	
510 515 520	
CGG TTG CAG CGT GAA CTC GAG GCA CAG CGG GCT GGC TTT GGG ACT GAG	1812
Arg Leu Gln Arg Glu Leu Glu Ala Gln Arg Ala Gly Phe Gly Thr Glu	
525 530 535 540	
GCT GAG AAG CTG GCC CGG AGG CAC CAG GCC ATT GGT GAG AAG GAA GCA	1860
Ala Glu Lys Leu Ala Arg Arg His Gln Ala Ile Gly Glu Lys Glu Ala	
545 550 555	
CGA GCT GCT CAG GCT GAG GAG CGG AAG TTC CAG CAG CAC ATC TTG GGG	1908
Arg Ala Ala Gln Ala Glu Glu Arg Lys Phe Gln Gln His Ile Leu Gly	
560 565 570	
CAG CAG AAG AAG GAA CTG GCT GCC CTG CTG GAG GCA CAG AAG CGA ACC	1956
Gln Gln Lys Lys Glu Leu Ala Ala Leu Leu Glu Ala Gln Lys Arg Thr	
575 580 585	
TAT AAG CTT CGG AAG GAG CAG TTG AAA GAG GAG CTC CAG GAG AAC CCT	2004
Tyr Lys Leu Arg Lys Glu Gln Leu Lys Glu Glu Leu Gln Glu Asn Pro	
590 595 600	
AGC ACA CCC AAA CGA GAG AAG GCT GAG TGG CTG TTG AGG CAG AAA GAG	2052
Ser Thr Pro Lys Arg Glu Lys Ala Glu Trp Leu Leu Arg Gln Lys Glu	
605 610 615 620	
CAG TTG CAA CAG TGC CAG GCA GAG GAG GAG GCA GGG CTA CTG CGG AGG	2100
Gln Leu Gln Gln Cys Gln Ala Glu Glu Glu Ala Gly Leu Leu Arg Arg	
625 630 635	
CAA CGC CAG TAC TTT GAG CTT CAG TGT CGC CAA TAC AAG CGC AAG ATG	2148
Gln Arg Gln Tyr Phe Glu Leu Gln Cys Arg Gln Tyr Lys Arg Lys Met	
640 645 650	
CTA CTG GCT CGG CAC AGC CTA GAC CAG GAC CTG CTT CGA GAG GAC TTG	2196
Leu Leu Ala Arg His Ser Leu Asp Gln Asp Leu Leu Arg Glu Asp Leu	
655 660 665	
AAT AAG AAA CAG ACA CAG AAG GAC TTG GAG TGT GCT CTG CTG TTA CGG	2244
Asn Lys Lys Gln Thr Gln Lys Asp Leu Glu Cys Ala Leu Leu Leu Arg	
670 675 680	
CAG CAT GAG GCT ACC CGA GAG CTG GAG CTA CGA CAG CTC CAG GCT GTC	2292
Gln His Glu Ala Thr Arg Glu Leu Glu Leu Arg Gln Leu Gln Ala Val	
685 690 695 700	

CAG CGC ACA CGT GCT GAA CTC ACC CGC CTT CAG CAC CAG ACA GAG CTA	2340
Gln Arg Thr Arg Ala Glu Leu Thr Arg Leu Gln His Gln Thr Glu Leu	
705 710 715	
GGC AAC CAG TTG GAG TAC AAC AAG CGA CGG GAG CAA GAG TTG CGG CAG	2388
Gly Asn Gln Leu Glu Tyr Asn Lys Arg Arg Glu Gln Glu Leu Arg Gln	
720 725 730	
AAG CAC GCG GCC CAG GTT CGC CAG CAG CCC AAG AGC CTC AAA GTA CGT	2436
Lys His Ala Ala Gln Val Arg Gln Gln Pro Lys Ser Leu Lys Val Arg	
735 740 745	
GCA GGC CAG CTA CCC ATG GGC CTC CCT GCT ACC GGG GCT CTG GGA CCA	2484
Ala Gly Gln Leu Pro Met Gly Leu Pro Ala Thr Gly Ala Leu Gly Pro	
750 755 760	
CTC AGC ACA GGC ACC CTT AGT GAA GAG CAG CCC TGC TCA TCT GGC CAG	2532
Leu Ser Thr Gly Thr Leu Ser Glu Glu Gln Pro Cys Ser Ser Gly Gln	
765 770 775 780	
GAG GCA ATC CTG GGC CAA AGG ATG CTG GGA GAG GAG GAG GAA GCA GTG	2580
Glu Ala Ile Leu Gly Gln Arg Met Leu Gly Glu Glu Glu Glu Ala Val	
785 790 795	
CCA GAG AGA ATG ATT CTG GGA AAG GAA GGG ACT ACT TTG GAG CCA GAG	2628
Pro Glu Arg Met Ile Leu Gly Lys Glu Gly Thr Thr Leu Glu Pro Glu	
800 805 810	
GAG CAG AGG ATT CTG GGG GAA GAA ATG GGA ACC TTT AGT TCC AGC CCA	2676
Glu Gln Arg Ile Leu Gly Glu Glu Met Gly Thr Phe Ser Ser Ser Pro	
815 820 825	
CAA AAA CAT AGG AGT CTG GTT AAT GAG GAA GAT TGG GAT ATA TCT AAA	2724
Gln Lys His Arg Ser Leu Val Asn Glu Glu Asp Trp Asp Ile Ser Lys	
830 835 840	
GAA ATG AAG GAG AGT AGA GTC CCA TCC CTG GCA TCC CAG GAG AGA AAT	2772
Glu Met Lys Glu Ser Arg Val Pro Ser Leu Ala Ser Gln Glu Arg Asn	
845 850 855 860	
ATT ATT GGC CAG GAA GAG GCT GGG GCA TGG AAT CTG TGG GAG AAG GAG	2820
Ile Ile Gly Gln Glu Glu Ala Gly Ala Trp Asn Leu Trp Glu Lys Glu	
865 870 875	
CAT GGA AAC CTT GTG GAT ATG GAG TTC AAG CTT GGC TGG GTC CAG GGT	2868
His Gly Asn Leu Val Asp Met Glu Phe Lys Leu Gly Trp Val Gln Gly	
880 885 890	
CCA GTT CTG ACT CCA GTG CCT GAG GAG GAA GAG GAG GAG GAA GAG GAG	2916
Pro Val Leu Thr Pro Val Pro Glu Glu Glu Glu Glu Glu Glu Glu	
895 900 905	
GGA GGG GCT CCA ATT GGA ACC CCC AGG GAC CCT GGA GAT GGC TGT CCT	2964
Gly Gly Ala Pro Ile Gly Thr Pro Arg Asp Pro Gly Asp Gly Cys Pro	

910	915	920	
TCC CCA GAT ATC CCC CCA GAG CCA CCT CCA TCA CAT CTG AGA CAG TAC			3012
Ser Pro Asp Ile Pro Pro Glu Pro Pro Pro Ser His Leu Arg Gln Tyr			
925	930	935	940
CCT GCT AGC CAG CTT CCT GGA TTC TTG TCT CAT GGC CTC CTG ACT GGC			3060
Pro Ala Ser Gln Leu Pro Gly Phe Leu Ser His Gly Leu Leu Thr Gly			
945	950		955
CTC TCC TTT GCA GTG GGG TCC TCC TCT GGC CTC TTG CCC CTA CTA CTT			3108
Leu Ser Phe Ala Val Gly Ser Ser Ser Gly Leu Leu Pro Leu Leu Leu			
960	965		970
CTG CTG CTA CTC CCA TTG CTG GCA CCC AGG TGG AGG TGG CTT GCA GGC			3156
Leu Leu Leu Leu Pro Leu Leu Ala Pro Arg Trp Arg Trp Leu Ala Gly			
975	980		985
AGC ACT GCT GGC CCT T GAGGTAGGAC TAGTGGGCCT GGGGGCTTCA			3202
Ser Thr Ala Gly Pro			
990			
TACCTGTTCC TTTGTACAGC TCTACACCTG CCACCCAGTC TGTTCCTTACT CCTGGCTCAG			3262
GGCACTGCAC TGGGGGCTGT CCTTAGCCTG AGCTGGCGCA GAGGCCTTAT GGGTGTGCCT			3322
CTGGGCCTTG GGGCTGCCTG GCTCCTAGCT TGGCCCAGCC TGGCTTTACC TCTGGCAGCT			3382
ATGGCGGCTG GGGGCAAATG GGTACGGCAG CAAGGCCCCC AGATGCGTCG GGGCATCTCT			3442
CGACTCTGGT TGCGGGTTCT GCTACGCCTG TCACCCATGG TCTTTCGGGC CCTACAGGGC			3502
TGTGCGGCTG TGGGAGACCG GGGGCTGTTT GCCCTGTACC CTAAGACCAA TAAGAATGGT			3562
TTCCGAAGTC GACTGCCTGT CCCTTGCCCC CGTCAGGGAA ATCCTCGCAC TACACAGCAC			3622
CCACTAGCTC TGTTAGCAAG AGTTTGGGCT CTGTGCAAGG GCTGGAAGT GCGCCTAGCA			3682
CGGGCTAGCC ATAGATTAGC TTCTTGTTTG CCCCCCTGGG CTGTTTCATAT ACTAGCTAGC			3742
TGGGGCCTGC TTAAGGGTGA AAGGCCAGT CGGATCCCTC GGCTGCTACC GCGAAGCCAA			3802
CGCCGTCTTG GGCTCTCAGC TTCCCGACAG CTACCACCAG GGAAGTGTAGC TGGGCGGAGA			3862
TCTCAGACCC GCAGGGCCCT GCCTCCCTGG AGGTAACCAG TTCTAACCCT CCACCCAAAT			3922
TTAGGGCATT GAGCACTTTA TCTCCCATGA CTCAGTAAAG TCTCTCCAGT CCCTTGGCCT			3982
CTCCTCCCTT TCTGACCTTT CTTCCTCAGT ATGTTTCCCC AGGTCCAATC CCAGCCCCAG			4042
ATGTAGATTT CTAGACAGGC AGCCTCCTCT ACTGTGGAGT CCAGAATGAC ACTCTGTGT			4102
TTTCCCCAGT CCCCTAAGTT ATTGCTGTCC CCTGCTGTGT GTGTGCTCAT CCTCACCCTC			4162
ATCGGCTCAG GCCTGGGGCC AGGGGTGGCA GGGAGGGAAG TCATGGGGGT TTTCCCTCTT			4222

TGATTTTGT TTTCTGTCTC CCTTCCAACC TGTCCCCTTC CCCTCCACCA AAAGAGAAAA 4282
 AAAAAAAAAA AAAA 4296

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 993 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Pro	Ala	Gly	Gly	Arg	Ala	Gly	Ser	Leu	Lys	Asp	Pro	Asp	Val	Ala	1	5	10	15
Glu	Leu	Phe	Phe	Lys	Asp	Asp	Pro	Glu	Lys	Leu	Phe	Ser	Asp	Leu	Arg	20	25	30	
Glu	Ile	Gly	His	Gly	Ser	Phe	Gly	Ala	Val	Tyr	Phe	Ala	Arg	Asp	Val	35	40	45	
Arg	Asn	Ser	Glu	Val	Val	Ala	Ile	Lys	Lys	Met	Ser	Tyr	Ser	Gly	Lys	50	55	60	
Gln	Ser	Asn	Glu	Lys	Trp	Gln	Asp	Ile	Ile	Lys	Glu	Val	Arg	Phe	Leu	65	70	75	80
Gln	Lys	Leu	Arg	His	Pro	Asn	Thr	Ile	Gln	Tyr	Arg	Gly	Cys	Tyr	Leu	85	90	95	
Arg	Glu	His	Thr	Ala	Trp	Leu	Val	Met	Glu	Tyr	Cys	Leu	Gly	Ser	Ala	100	105	110	
Ser	Asp	Leu	Leu	Glu	Val	His	Lys	Lys	Pro	Leu	Gln	Glu	Val	Glu	Ile	115	120	125	
Ala	Ala	Val	Thr	His	Gly	Ala	Leu	Gln	Gly	Leu	Ala	Tyr	Leu	His	Ser	130	135	140	
His	Asn	Met	Ile	His	Arg	Asp	Val	Lys	Ala	Gly	Asn	Ile	Leu	Leu	Ser	145	150	155	160
Glu	Pro	Gly	Leu	Val	Lys	Leu	Gly	Asp	Phe	Gly	Ser	Ala	Ser	Ile	Met	165	170	175	
Ala	Pro	Ala	Asn	Ser	Phe	Val	Gly	Thr	Pro	Tyr	Trp	Met	Ala	Pro	Glu	180	185	190	
Val	Ile	Leu	Ala	Met	Asp	Glu	Gly	Gln	Tyr	Asp	Gly	Lys	Val	Asp	Val	195	200	205	

Trp Ser Leu Gly Ile Thr Cys Ile Glu Leu Ala Glu Arg Lys Pro Pro
 210 215 220
 Leu Phe Asn Met Asn Ala Met Ser Ala Leu Tyr His Ile Ala Gln Asn
 225 230 235 240
 Glu Ser Pro Ala Leu Gln Ser Gly His Trp Ser Glu Tyr Phe Arg Asn
 245 250 255
 Phe Val Asp Ser Cys Leu Gln Lys Ile Pro Gln Asp Arg Pro Thr Ser
 260 265 270
 Glu Val Leu Leu Lys His Arg Phe Val Leu Arg Glu Arg Pro Pro Thr
 275 280 285
 Val Ile Met Asp Leu Ile Gln Arg Thr Lys Asp Ala Val Arg Glu Leu
 290 295 300
 Asp Asn Leu Gln Tyr Arg Lys Met Lys Lys Ile Leu Phe Gln Glu Ala
 305 310 315 320
 Pro Asn Gly Pro Gly Ala Glu Ala Pro Glu Glu Glu Glu Glu Ala Glu
 325 330 335
 Pro Tyr Met His Arg Ala Gly Thr Leu Thr Ser Leu Glu Ser Ser His
 340 345 350
 Ser Val Pro Ser Met Ser Ile Ser Ala Ser Ser Gln Ser Ser Ser Val
 355 360 365
 Asn Ser Leu Ala Asp Ala Ser Asp Asn Glu Glu Glu Glu Glu Glu
 370 375 380
 Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Gly Pro Glu Ser Arg Glu
 385 390 395 400
 Met Ala Met Met Gln Glu Gly Glu His Thr Val Thr Ser His Ser Ser
 405 410 415
 Ile Ile His Arg Leu Pro Gly Ser Asp Asn Leu Tyr Asp Asp Pro Tyr
 420 425 430
 Gln Pro Glu Met Thr Pro Gly Pro Leu Gln Pro Pro Ala Ala Pro Pro
 435 440 445
 Thr Ser Thr Ser Ser Ser Ser Ala Arg Arg Arg Ala Tyr Cys Arg Asn
 450 455 460
 Arg Asp His Phe Ala Thr Ile Arg Thr Ala Ser Leu Val Ser Arg Gln
 465 470 475 480
 Ile Gln Glu His Glu Gln Asp Ser Ala Leu Arg Glu Gln Leu Ser Gly
 485 490 495
 Tyr Lys Arg Met Arg Arg Gln His Gln Lys Gln Leu Leu Ala Leu Glu

500					505					510						
Ser	Arg	Leu	Arg	Gly	Glu	Arg	Glu	Glu	His	Ser	Gly	Arg	Leu	Gln	Arg	
515					520					525						
Glu	Leu	Glu	Ala	Gln	Arg	Ala	Gly	Phe	Gly	Thr	Glu	Ala	Glu	Lys	Leu	
530					535					540						
Ala	Arg	Arg	His	Gln	Ala	Ile	Gly	Glu	Lys	Glu	Ala	Arg	Ala	Ala	Gln	
545					550					555					560	
Ala	Glu	Glu	Arg	Lys	Phe	Gln	Gln	His	Ile	Leu	Gly	Gln	Gln	Lys	Lys	
565					570					575						
Glu	Leu	Ala	Ala	Leu	Leu	Glu	Ala	Gln	Lys	Arg	Thr	Tyr	Lys	Leu	Arg	
580					585					590						
Lys	Glu	Gln	Leu	Lys	Glu	Glu	Leu	Gln	Glu	Asn	Pro	Ser	Thr	Pro	Lys	
595					600					605						
Arg	Glu	Lys	Ala	Glu	Trp	Leu	Leu	Arg	Gln	Lys	Glu	Gln	Leu	Gln	Gln	
610					615					620						
Cys	Gln	Ala	Glu	Glu	Glu	Ala	Gly	Leu	Leu	Arg	Arg	Gln	Arg	Gln	Tyr	
625					630					635					640	
Phe	Glu	Leu	Gln	Cys	Arg	Gln	Tyr	Lys	Arg	Lys	Met	Leu	Leu	Ala	Arg	
645					650					655						
His	Ser	Leu	Asp	Gln	Asp	Leu	Leu	Arg	Glu	Asp	Leu	Asn	Lys	Lys	Gln	
660					665					670						
Thr	Gln	Lys	Asp	Leu	Glu	Cys	Ala	Leu	Leu	Leu	Arg	Gln	His	Glu	Ala	
675					680					685						
Thr	Arg	Glu	Leu	Glu	Leu	Arg	Gln	Leu	Gln	Ala	Val	Gln	Arg	Thr	Arg	
690					695					700						
Ala	Glu	Leu	Thr	Arg	Leu	Gln	His	Gln	Thr	Glu	Leu	Gly	Asn	Gln	Leu	
705					710					715					720	
Glu	Tyr	Asn	Lys	Arg	Arg	Glu	Gln	Glu	Leu	Arg	Gln	Lys	His	Ala	Ala	
725					730					735						
Gln	Val	Arg	Gln	Gln	Pro	Lys	Ser	Leu	Lys	Val	Arg	Ala	Gly	Gln	Leu	
740					745					750						
Pro	Met	Gly	Leu	Pro	Ala	Thr	Gly	Ala	Leu	Gly	Pro	Leu	Ser	Thr	Gly	
755					760					765						
Thr	Leu	Ser	Glu	Glu	Gln	Pro	Cys	Ser	Ser	Gly	Gln	Glu	Ala	Ile	Leu	
770					775					780						
Gly	Gln	Arg	Met	Leu	Gly	Glu	Glu	Glu	Glu	Ala	Val	Pro	Glu	Arg	Met	
785					790					795					800	

Ile Leu Gly Lys Glu Gly Thr Thr Leu Glu Pro Glu Glu Gln Arg Ile
805 810 815

Leu Gly Glu Glu Met Gly Thr Phe Ser Ser Ser Pro Gln Lys His Arg
820 825 830

Ser Leu Val Asn Glu Glu Asp Trp Asp Ile Ser Lys Glu Met Lys Glu
835 840 845

Ser Arg Val Pro Ser Leu Ala Ser Gln Glu Arg Asn Ile Ile Gly Gln
850 855 860

Glu Glu Ala Gly Ala Trp Asn Leu Trp Glu Lys Glu His Gly Asn Leu
865 870 875 880

Val Asp Met Glu Phe Lys Leu Gly Trp Val Gln Gly Pro Val Leu Thr
885 890 895

Pro Val Pro Glu Glu Glu Glu Glu Glu Glu Glu Glu Gly Gly Ala Pro
900 905 910

Ile Gly Thr Pro Arg Asp Pro Gly Asp Gly Cys Pro Ser Pro Asp Ile
915 920 925

Pro Pro Glu Pro Pro Pro Ser His Leu Arg Gln Tyr Pro Ala Ser Gln
930 935 940

Leu Pro Gly Phe Leu Ser His Gly Leu Leu Thr Gly Leu Ser Phe Ala
945 950 955 960

Val Gly Ser Ser Ser Gly Leu Leu Pro Leu Leu Leu Leu Leu Leu Leu
965 970 975

Pro Leu Leu Ala Pro Arg Trp Arg Trp Leu Ala Gly Ser Thr Ala Gly
980 985 990

Pro

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 414 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ACGANTCACC AGTTGGAAGT TACTCCAAAG AATGAGCACA AAACAATCTT AAAGACACTG	60
AAAGATGAGC AGACAAGAAA ACTTGCCATT TNGGCAGAGC AGTATGAACA GAGTATAAAT	120
GAAATGATGG CCTCTCANGC GTTACGGCTA GATGAGGCTC AAGAAGCAGA ATGCCAGGCC	180

TTGAGGCTAC AGCTCCAGCA GGAAATGGAG CTGCTCAACG CCTACCAGAG CAAAATCAAG	240
ATGCAAACAG AGGCACAACA TGAACGTGAG CTCCAGAAGC TAGAGCAGAG AGTGTCTCTG	300
CGCAGAGCAC ACCTTGAGCA GAAGATTGAA GAGGAGCTGG CTGCCCTTCA GAAGGAACGC	360
AGCGAGAGAA TAAAGAACCT ATTGGAAAGG CAAGAGCGAG AGATTGGAAA CTTT	414

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 314 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GAACAAAGTC ATGCCTTAAT AGTTCTGCTG ATGTTGGCCT TTCCTGAGGT ATTTTCTGCA	60
AGCAGTAATC AACAAATCTC CTAAAGGAGT CTGTCCATTC ATTAGACTGT AACGTTGGGG	120
AGTCATTCTG GGCAATGTGA TATAAGGCAC TCATTGCATT CATGTTGAAA AGGGGCGGCT	180
TCCGTTCCGC CAATTCAATA CAAGTGATGC CAAGTGACCA AATATCAACT TTCCCATCAT	240
ACTGTCCTTC ATCCATAGCT AAGATCACCT CTGGAGCCAT CCAGTAAGGT GTGCCCACGA	300
AGGAGTTGGC CAGG	314

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 370 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ACCAAATTCC CAAATCCCAT TCTGAGGCTC TCCATGTCAA AAGTTTCAAT CTCTCGCTCT	60
TGCCTTTCCA ATAGGTTCTT TATTCTCTCG CTGCGTTCCT TCTGAAGGGC AGCCAGCTCC	120
TCTTCAATCT TCTGCTCAAG GTGTGGTCTG CGCAGAGACA CTCTCTGCTC TAGCTTCTGG	180
AGCTCACGTT CATGTTGTGC CTCTGTTNGN ATCTTGATTT GGNTCTGGTA GGC GTTGAGC	240
AGCTCCATTT CCTGCTGGAG CTGTAGCCTC AAGGCCTGGC ATTCTGCTTC TTGAGCCTCA	300
TCTAGCCGTA ACGCTTGAGA GGCCATCATT TCATTTATAC TCTGTTTATA CTGCTCTGCC	360
AAAATGGCAA	370

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 190 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CAACAGCAGA AAAACTTAAA GGCCATGGAA ATGCAAATTA AAAACAGTT TCAGGACACT	60
TGCAAAGTAC AGACCAAACA GTATAAGCA CTCAAGAATC ACCAGTTGGA AGTTACTCCA	120
AAGAATGAGC ACAAACAAT CTAAAGACA CTGAAAGATG AGCAGACAAG AAAACTTGCC	180
ATTTTGGCAG	190

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 65 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GAGCAGTATG AACAGAGTAT AAATGAAATG ATGGCCTCTC AAGCGTTACG GCTAGATGAG	60
GCTCA	65

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 219 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ACGAGTCCCC CCGAGAGCTA GAGTACAGGC AGCTGCACAC GTTACAGAAG CTACGCATGG	60
ATCTGATCCG TTTACAGCAC CAGACGGAAC TGAAAACCA GCTGGAGTAC AATAAGAGGC	120
GAGAAAGAGA ACTGCACAGA AAGCATGTCA TGGAACCTCG GCAACAGCCA AAAA ACTTAA	180
AGGCCATGGA ANTGCAATTT AAAAAACAGT TCCAGGAAA	219

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 85 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GTGCATATGG TATATTTNAT TCATTTTGT AAAGCGTTCT GTTTTGTGTT TACTAATTGG 60
GATGTCATAG TACTTGGCTG CCGGG 85

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 46 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CTCACTTGGG TACTACAGTG TGGAAGCTGA GTGCATATGG TATATT 46

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 116 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GATATTTGGT CATTGGGTAT CACGTGTATA GAGCTGGCCG AACGTCGTCC ACCATTGTTC 60
AGTATGAATG CAATGTCTGC CCTCTACCAT ATTGCTCAAA ATGATCCTCC AACTCT 116

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 118 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CTGAAAGGCC TGGATTATCT GCACTCAGAG CGCAAGATCC ACCGAGATAT CAAAGCTGCC 60
AACGTGCTGC TCTCGGAGCA GGGTGATGTG AAGATGGCAG ACTTCGGTGT GGCTGGCA 118

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 110 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GACCCAGAGG AACTCTTCAC CAAGCTTGAC CGCATTGGCA AAGGCTCATT TGGGGAGGTG 60
TACAAGGGGA TCGACAACCA CACCAAGGAA GTGGTGGCCA TCAAGATCAT 110

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 134 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TCAGGATTCT GGAGCTCTGG AGTCCATTA GTGGCTATCA GATACAATGC CCTGAGTGGA 60
TTTTTCATTAA GGTAAGGGGG TTCACCTTCC ACCATTTCAA TTGCCATAAT TCCAAGAGAC 120
CAGATATCAA CTTT 134

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 278 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Met Ala Pro Ala Val Leu Gln Lys Pro Gly Val Ile Lys Asp Pro Ser
1 5 10 15

Ile Ala Ala Leu Phe Ser Asn Lys Asp Pro Glu Gln Asp Leu Arg Glu
20 25 30

Ile Gly His Gly Ser Phe Gly Ala Val Tyr Phe Ala Tyr Asp Lys Lys
35 40 45

Asn Glu Gln Thr Val Ala Ile Lys Lys Met Asn Phe Ser Gly Lys Gln
50 55 60

Ala Val Glu Lys Trp Asn Asp Ile Leu Lys Glu Val Ser Phe Leu Asn
65 70 75 80

Thr Val Val His Pro His Ile Val Asp Tyr Lys Ala Cys Phe Leu Lys
85 90 95

Asp Thr Thr Cys Trp Leu Val Met Glu Tyr Cys Ile Gly Ser Ala Ala
100 105 110

Asp Ile Val Asp Val Leu Arg Lys Gly Met Arg Glu Val Glu Ile Ala

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 99/08165

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/54 C12N9/12 C12N1/21 C07K16/40 A61K38/45
 A61K31/70 A61K39/395 C12Q1/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	WO 99 02699 A (CADUS PHARMACEUTICAL CORP) 21 January 1999 (1999-01-21) abstract 96% identity in 758 AA overlap between KDS2 (SEQ ID 4) and TA02 (SEQ ID 4) SEQ ID 1 of W09902699 contains SEQ ID 5,8,9 and 10 of the application --- -/--	7-11,13, 15-17, 38-46



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

2 September 1999

Date of mailing of the international search report

15/09/1999

Name and mailing address of the ISA

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Authorized officer

Lejeune, R

INTERNATIONAL SEARCH REPORT

International Application No

PC1/US 99/08165

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	HUTCHISON M: "Isolation of TA01, a protein kinase that activates MEKs in stress-activated protein kinase cascades." JOURNAL OF BIOLOGICAL CHEMISTRY., vol. 273, no. 44, 30 October 1998 (1998-10-30), pages 28625-28632, XP002114118 AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD., US ISSN: 0021-9258 the whole document	1-6,11, 12, 14-17, 21-26,28
X	--- DATABASE EMBL 'Online! Accession Number AA234623, 6 March 1997 (1997-03-06) HILLIER L ET AL: "zr66h09.r1 Soares NhHMPu S1 Homo sapiens cDNA clone 668417 5' similar to WP:T17E9.1 CE01405" XP002114120 92.7% identity in 521 bp overlap with SEQ ID 1	17
P,X	--- ALLEN K M ET AL: "PAK3 mutation in nonsyndromic X-linked mental retardation" NATURE GENETICS, vol. 20, no. 1, September 1998 (1998-09), pages 25-30, XP002114119 NEW YORK, NY, US ISSN: 1061-4036 figure 1 -& DATABASE EMBL 'Online! accession number AF068864, 23 September 1998 (1998-09-23) ALLEN K M ET AL: "Homo sapiens p21-activated kinase 3 (PAK3) mRNA, complete cds." XP002114121 AF068864 has 100% identity in a 134 bp overlap with SEQ ID 16	38-44
A	--- WO 98 00539 A (CHIRON CORP) 8 January 1998 (1998-01-08) the whole document	1-17
A	--- CREASY C L ET AL: "Cloning and characterization of a member of the MST subfamily of Ste20-like kinases" GENE, vol. 167, no. 1, 1 January 1995 (1995-01-01), page 303-306 XP004043060 ISSN: 0378-1119 -----	1-17

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 99/08165

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 22,23,25,26,31-35, 48 and 49, in so far as in vivo methods are concerned, are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/08165

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9902699 A	21-01-1999	AU 8296698 A	08-02-1999
WO 9800539 A	08-01-1998	AU 4326097 A	21-01-1998