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DNA SEQUENCE CODING FOR A BMP RECEPTOR

TECHNICAL FIELD

The present invention relates to the field of bone formation and development. Specifically, the present invention relates to a bone morphogenetic protein receptor and a DNA sequence coding for said receptor.

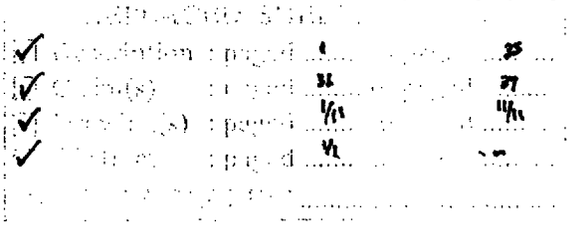
BACKGROUND

Humans and other warm-blooded animals can be afflicted by a number of bone-related disorders. Such disorders range from bone fractures, to debilitating diseases such as osteoporosis. While in healthy individuals, bone growth generally proceeds normally and fractures heal without the need for pharmacological intervention, in certain instances bones may become weakened or may fail to heal properly. For example, healing may proceed slowly in the elderly and in patients undergoing treatment with corticosteroids (e.g., transplant patients). Osteoporosis is a condition in which bone hard tissue is lost disproportionately to the development of new hard tissue. Osteoporosis can generally be defined as the reduction in the quantity of bone, or the atrophy of skeletal tissue: marrow and bone spaces become larger, fibrous binding decreases, and compact bone becomes fragile. Another bone related disorder is osteoarthritis, which is a disorder of the movable joints characterized by deterioration and abrasion of articular cartilage, as well as by formation of new bone at the joint surface.

While a variety of treatments are available for such bone-related disorders, none of the treatments provide optimum results. One of the difficulties facing individuals who treat bone-related disorders is a lack of complete understanding of bone metabolism and of the bone-related disorders. A key to such understanding is identifying and characterizing each of the components involved in bone growth. Bone morphogenetic proteins (BMPs) have been demonstrated to play a role in bone formation and development (Wozney, J.M., *Molec. Reproduct. and Develop.*, *32:* 160-167 (1992)).

Furthermore, the role of BMPs may not be limited to their role in bone. The finding that the BMPs are found at significant concentrations in other tissues such as brain and kidney (Wall, N.A., Blessing, M., Wright, C.V.E., and Hogan, B.L.M., *J. Cell Biol.*, *120:* 493-502 (1993); Özkaynak, E., Schnegelsberg, P.N.J., Jin, D.F., Clifford, G.M., Warren, F.D., Drier, E.A., and Oppermann, H., *J. Biol. Chem.*, *267:* 25220-25227 (1992); Lyons, K.M., Jones, C.M., and Hogan, B.L.M., *Trends in Genetics*, *7:* 408-412 (1991)) suggests that they may play additional roles in

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development and differentiation. In support of this, BMPs have recently been found to promote nerve cell differentiation (Basler, K., Edlund, T., Jessell, T.M., and Yamada, T., *Cell, 73:* 687-702 (1993); Paralkar, V.M., Weeks, B.S., Yu, Y.M., Kleinman, H.K., and Reddi, A.H., *J. Cell Biol.*, 119: 1721-1728 (1992)).

A BMP initiates its biological effect on cells by binding to a specific BMP receptor expressed on the plasma membrane of a BMP-responsive cell. A receptor is a protein, usually spanning the cell membrane, which binds to a ligand from outside the cell, and as a result of that binding sends a signal to the inside of the cell which alters cellular function. In this case, the ligand is the protein BMP, and the signal induces the differentiation of the cell to produce cartilage and bone.

Because of the ability of a BMP receptor to specifically bind BMPs, purified BMP receptor compositions will be useful in diagnostic assays for BMPs, as well as in raising antibodies to the BMP receptor for use in diagnosis and therapy. In addition, purified BMP receptor compositions may be used directly in therapy to bind or scavenge BMPs, thereby providing a means for regulating the bone formation and development activities of BMPs. In order to study the structural and biological characteristics of BMP receptors and the role played by BMPs in the responses of various cell populations to BMPs during bone growth/formation stimulation, or to use a BMP receptor effectively in therapy, diagnosis, or assay, purified compositions of BMP receptor are needed. Such compositions, however, are obtainable in practical yields only by cloning and expressing genes encoding the receptors using recombinant DNA technology. Efforts to purify BMP receptors for use in biochemical analysis or to clone and express mammalian genes encoding BMP receptors have been impeded by lack of a suitable source of receptor protein or mRNA. Prior to the present invention, no cell lines were known to express high levels of BMP receptor constitutively and continuously, which precluded purification of the receptor for protein sequencing or construction of genetic libraries for direct expression cloning. Availability of the BMP receptor sequence will make it possible to generate cell lines with high levels of recombinant BMP receptor for biochemical analysis and use in screening experiments.

Based on the foregoing, there is a need for a BMP receptor DNA sequence and an isolated BMP receptor protein encoded by this sequence.

Objects of the Present Invention

It is an object of the present invention to provide an isolated BMP receptor kinase protein.

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It is also an object of the present invention to provide a DNA sequence encoding a BMP receptor kinase protein.

It is also an object of the present invention to provide a recombinant expression vector encoding a BMP receptor kinase protein.

It is also an object of the present invention to provide a host cell comprising a recombinant expression vector encoding a BMP receptor kinase protein.

It is also an object of the present invention to provide a method for producing a BMP receptor kinase protein.

It is also an object of the present invention to provide a method for identifying compounds capable of binding to a BMP receptor kinase protein.

It is also an object of the present invention to provide a method for determining the amount of a compound capable of binding a BMP receptor kinase protein in a sample.

It is also an object of the present invention to provide antibodies specific for the BMP receptor protein and a method for producing them.

SUMMARY

The present invention relates to an isolated BMP receptor kinase protein or soluble fragment thereof, a DNA sequence coding for said BMP receptor kinase protein or said soluble fragment thereof, a recombinant expression vector comprising said DNA sequence, a host cell comprising said recombinant expression vector, a method of expressing said BMP receptor kinase protein or soluble fragment thereof, a method for identifying compounds capable of binding to said receptor kinase protein, a method for determining the amount of such compounds in a sample, and antibodies to the said BMP receptor kinase protein or soluble fragment thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the DNA sequence of the degenerate oligonucleotide primers used in the PCR amplification of BRK-1. The nucleotide bases adenine, thymine, cytosine, guanine, and inosine are represented by the single letter codes A, T, C, G, and I, respectively. ACT1A and ACT1B refer to the set of degenerate 3' PCR primers. ACT2A and ACT2B refer to the set of degenerate 5' PCR primers.

Figure 2 is an alignment of protein sequences comparing the kinase domains of t-BRK-1 and BRK-1 with other members of the TGF-ß receptor family. DAF1, the Daf-1 receptor kinase from *C. elegans* (Georgi,. L.L., Albert, P.S., and Riddle, D.L.. *Cell, 61*: 635-645 (1990)); MACT, mouse activin receptor type II (Mathews. L.S. and Vale, W.W., *Cell, 65*: 973-982 (1991)); RTGFBR2. rat TGF-ß

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receptor type II (Tsuchida, K., Lewis, K.A., Mathews, L.S., and Vale, W.W. *Biochem. Biophys. Res. Commun.*, *191:* 790-795 (1993)); MACTR1, mouse activin receptor type I (Ebner, R., Chen, R.-H., Shum, L., Lawler, S., Zioncheck, T.F., Lee, A., Lopez, A.R., and Derynck, R., *Science*, *260:* 1344-1348 (1993)); R3, R2, and R4, type I receptors from rat, ligand unknown (He, W.W., Gustafson, M.L., Hirobe, S., and Donahoe, P.K., *Develop. Dynamics*, *196:* 133-142 (1993)). The bracket indicates the predicted kinase termination region for kinases with complete kinase domains.

Figure 3 shows the construct pJT4-J159F, used to express BRK-1 in mammalian cells. CMV, cytomegalovirus early promoter/enhancer; R, the "R" element from the long terminal repeat of human T-cell leukemia virus-1; SP, an intron splice site from the SV40 virus; T3, promoter from the T3 bacteriophage; T7, promoter region from the T7 bacteriophage; poly A, region from the SV40 virus directing polyadenylation of the message; SV40 ORI, origin of replication from the SV40 virus; Amp, ampicillin resistance gene for selection in *E. coli*.

Figure 4 shows the construct pJT6-J159T, used to express t-BRK-1 in mammalian cells. Abbreviations are the same as those in Figure 3.

Figure 5 shows binding of [^{125}I]-BMP-4 to COS-7 cells transfected with the cDNA for BRK-1, using the construct pJT4-J159F. The concentration of [^{125}I]-BMP-4 is 100 pM.

Figure 6 shows crosslinking of radiolabelled BMPs to COS-7 cells transfected with the cDNA for BRK-1. Figure 6A, crosslinking of [^{125}I]-BMP-4 to BRK-1. Lanes on the left, COS-7 cells transfected with the cDNA for BRK-1, using the construct pJT4-J159F; crosslinking in the absence (-) or presence (+) of 10 nM unlabeled BMP-2. Lanes on the right, mock transfected COS-7 cells: crosslinking in the absence (-) or presence (+) of 10 nM unlabeled BMP-2. Figure 6B, crosslinking of [^{125}I]-DR-BMP-2 to BRK-1. Lanes on the left, COS-7 cells transfected with the cDNA for BRK-1, using the construct pJT4-J159F; crosslinking in the absence (-) or presence (+) of 10 nM unlabeled BMP-2.

Figure 7 shows immunoprecipitation of BRK-1 expressed in COS-7 cells and crosslinked to [^{125}I]-BMP-4. Lanes designated "+", COS-7 cells transfected with the cDNA for BRK-1, using the construct pJT4-J159F. Lanes designated "-". mock-transfected COS-7 cells. After transfection, cells were crosslinked to [^{125}I]-BMP-4, then subjected to immunoprecipitation using the indicated antisera. Lanes on the left, antiserum 1351, specific for the extracellular domain (ECD); lanes on the right, antisera 1378, 1379, and 1380, all specific for the kinase domain.

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DESCRIPTION

The present invention answers the need for an isolated BMP receptor protein by providing an isolated BMP receptor kinase protein, a DNA sequence coding for said protein, a recombinant expression vector comprising said DNA sequence, a host cell comprising said recombinant expression vector, a method of expressing said BMP receptor kinase protein, and antibodies to said BMP receptor kinase protein.

As used herein, "BMP receptor kinase protein-1" or "BRK-1" means a protein having the amino acid sequence SEQ ID NO:4, as well as proteins having amino acid sequences substantially similar to SEQ ID NO:4 and which are biologically active in that they are capable of binding BMP-2 and/or BMP-4, or transducing a biological signal initiated by a BMP-2 or BMP-4 molecule binding to a cell, or crossreacting with anti-BRK-1 antibodies raised against BRK-1.

As used herein, "truncated BMP receptor kinase protein" or "t-BRK-1" means a protein having amino acid sequence SEQ ID NO:2.

As used herein, "substantially similar" when used to define either amino acid or nucleic acid sequences, means that a particular subject sequence, for example, a sequence altered by mutagenesis, varies from a reference sequence by one or more substitutions, deletions, or additions, the net effect of which is to retain biological activity of the BRK-1 protein. Alternatively, nucleic acid subunits and analogs are "substantially similar" to the specific DNA sequence disclosed herein if the DNA sequences, as a result of degeneracy in the genetic code, encode an amino acid sequence substantially similar to the reference amino acid sequence.

As used herein, "biologically active" means that a particular molecule shares sufficient amino acid sequence similarity with the embodiments of the present invention disclosed herein to be capable of binding detectable quantities of BMP-2 or BMP-4, or transmitting a BMP-2 or BMP-4 stimulus to a cell, for example, as a component of a hybrid receptor construct. Preferably, biologically active BRK-1 or t-BRK-1 within the scope of the present invention is capable of binding [^{125}I]-BMP-4 with nanomolar or subnanomolar affinity (K_d approximately equal to 10^{-9}M). Preferably, the affinity is from about $1x10^{-10}$M to about $1x9^{-9}$M, more preferably about $5x10^{-10}$ M, as per the saturation binding analysis method disclosed in Example 10, below.

As used herein, "soluble fragment" refers to an amino acid sequence corresponding to the extracellular region of BRK-1 or t-BRK-1. Soluble fragments include truncated proteins wherein regions of the receptor molecule not required

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for BMP binding have been deleted. Examples of such soluble fragments of the present invention include, but are not limited to, polypeptides having the amino acid sequences substantially similar to SEQ ID NO:6, amino acid residues 1-152 depicted in SEQ ID NO:2, amino acid residues 1-152 depicted in SEQ ID NO:4; or polypeptides encoded by nucleic acid residues substantially similar to SEQ ID NO:5, residues 291-746 depicted in SEQ ID NO:1, or residues 11-466 depicted in SEQ ID NO:3.

As used herein, "digit-removed BMP-2" and "DR-BMP-2" refer to a fragment of BMP-2 protein wherein the amino terminus of mature BMP-2 has been removed by mild trypsin digestion.

As used herein, "isolated", in reference to the receptor protein of the present invention or DNA sequences encoding said protein, means that the protein or DNA sequence is removed from the complex cellular milieu in which it naturally occurs, and said protein is expressible from said DNA sequence in a cell that does not naturally express it when operably linked to the appropriate regulatory sequences.

As used herein, "operably linked" refers to a condition in which portions of a linear DNA sequence are capable of influencing the activity of other portions of the same linear DNA sequence. For example, DNA for a signal peptide (secretory leader) is operably linked to DNA for a polypeptide if it is expressed as a precursor which participates in the secretion of the polypeptide; a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation. Generally, operably linked means contiguous and, in the case of secretory leaders, contiguous in reading frame.

As used herein, "ATCC" means American Type Culture Collection, Rockville, Maryland.

As used herein, "bone morphogenetic protein 2" or "BMP-2" means a peptide encoded by a DNA sequence contained in ATCC No. 40345 (see ATCC/NIH REPOSITORY CATALOGUE OF HUMAN AND MOUSE DNA PROBES AND LIBRARIES, sixth Edition, 1992, p. 57, hereinafter "ATCC/NIH REPOSITORY CATALOGUE"). Isolation of BMP is disclosed in US Patent No. 5,013,649, Wang, Wozney and Rosen, issued May 7, 1991, US Patent No. 5,166,058, Wang, Wozney and Rosen, issued November 24, 1992; and US Patent No. 5,168,050, Hammonds and Mason, issued December 1, 1992; each of which is incorporated herein by reference.

As used herein, "bone morphogenetic protein 4" or "BMP-4" means a

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peptide encoded by a DNA sequence contained in ATCC No. 40342 (see ATCC/NIH REPOSITORY CATALOGUE). Isolation of BMP-4 is disclosed in US Patent No. 5,013,649. Wang, Wozney and Rosen, issued May 7, 1991, incorporated herein by reference.

As used herein, "DNA sequence" refers to a DNA polymer, in the form of a separate fragment or as a component of a larger DNA construct, which has been derived from DNA isolated at least once in substantially pure form, i.e., free of contaminating endogenous materials and in a quantity or concentration enabling identification, manipulation, and recovery of the sequence and its component nucleotide sequences by standard biochemical methods, for example, using a cloning vector. Such sequences are preferably provided in the form of an open reading frame uninterrupted by internal nontranslated sequences (introns) which are typically present in eukaryotic genes. Genomic DNA containing the relevant sequences could also be used. Sequences of non-translated DNA may be present 5' or 3' from the open reading frame, where the same do not interfere with manipulation or expression of the coding regions. DNA sequences encoding the proteins provided by this invention can be assembled from cDNA fragments and short oligonucleotide linkers, or from a series of oligonucleotides, to provide a synthetic gene which is capable of being expressed in a recombinant transcriptional unit.

As used herein, "recombinant" means that a protein is derived from a DNA sequence which has been manipulated *in vitro* and introduced into a host organism.

As used herein, "microbial" refers to recombinant proteins made in bacterial or fungal (e.g., yeast) expression systems.

As used herein, "recombinant expression vector" refers to a DNA construct used to express DNA which encodes BRK-1 or t-BRK-1 and which includes a transcriptional subunit comprising an assembly of 1) genetic elements having a regulatory role in gene expression, for example, promoters and enhancers, 2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and 3) appropriate transcription and translation initiation and termination sequences. Structural elements intended for use in eukaryotic expression systems (e.g. yeast, insect or mammalian cells) preferably include a signal sequence at the N-terminus of the protein enabling transport to the membrane or extracellular secretion of a translated protein by a host cell. Alternatively, where recombinant protein is expressed without a signal sequence, for expression inside the cell, it may include an N-terminal methionine residue. This residue may

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optionally be subsequently cleaved from the expressed recombinant protein to provide a final product. Using methodology well known in the art, recombinant expression vectors of the present invention can be constructed. Possible vectors for use in the present invention include, but are not limited to: for mammalian cells, pJT4 (discussed further below), pcDNA-1 (Invitrogen, San Diego, Ca) and pSV-SPORT 1 (Gibco-BRL, Gaithersburg, MD); for insect cells, pBlueBac III or pBlueBacHis baculovirus vectors (Invitrogen, San Diego, CA); and for bacterial cells, pET-3 (Novagen, Madison, WI). The DNA sequence coding for BRK-1 or t-BRK-1 can be present in the vector operably linked to regulatory elements. In one embodiment of the present invention, mammalian host cells are preferably transfected with the plasmid construct pJT4-J159F, thereby resulting in expression of BRK-1. In another embodiment of the present invention, mammalian host cells are preferably transfected with the plasmid construct pJT6-J159T, thereby resulting in expression of t-BRK-1. Transfection with the recombinant molecules can be effected using methods well known in the art.

As used herein, "host cell" means a cell comprising a recombinant expression vector of the present invention. Host cells may be stably transfected or transiently transfected within a recombinant expression plasmid or infected by a recombinant virus vector. The host cells include prokaryotic cells, such as *Escherichia coli*, fungal systems such as *Saccharomyces cerevisiae*, permanent cell lines derived from insects such as SF-9 and SF-21, and permanent mammalian cell lines such as Chinese hamster ovary (CHO) and SV40-transformed African green monkey kidney cells (COS).

In one embodiment, the present invention relates to a BMP receptor kinase protein, or soluble fragment thereof. Preferably, the BRK-1 protein has amino acid sequence SEQ ID NO:4. Preferably, the soluble fragment has amino acid sequence SEQ ID. NO:6; preferably the soluble fragment is encoded by nucleic acid sequence SEQ ID NO:5.

In another embodiment, the present invention relates to a DNA sequence coding for the BRK-1 protein. The DNA sequence can be genomic DNA or cDNA. Preferably the DNA sequence is SEQ ID NO:3.

In another embodiment, the present invention relates to a recombinant expression vector comprising a DNA sequence coding for the BRK-1 protein. Preferably the recombinant expression vector is a plasmid having all of the identifying characteristics of the pJT4-J159F plasmid construct contained in ATCC No. 69457.

In another embodiment, the present invention relates to host cells

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comprising the above described recombinant expression vector. Preferably the host cell is a mammalian cell; more preferably a CHO cell or COS cell.

In another embodiment, the present invention relates to a truncated BMP receptor kinase protein, or soluble fragments thereof. Preferably t-BRK-1 has amino acid sequence SEQ ID NO:2. Preferably the soluble fragment of t-BRK-1 has amino acid sequence SEQ ID NO:6; preferably the soluble fragment of t-BRK-1 is encoded by nucleic acid sequence SEQ ID NO:5.

In another embodiment, the present invention relates to a DNA sequence encoding t-BRK-1. Preferably the DNA sequence encoding t-BRK-1 has SEQ ID NO:1.

In another embodiment, the present invention relates to a recombinant expression vector comprising a DNA sequence coding for t-BRK-1. Preferably the recombinant expression vector is a plasmid having all of the identifying characteristics of the pJT6-J159T plasmid construct contained in ATCC No. 69474.

In another embodiment, the present invention relates to a host cell comprising the recombinant expression vector comprising t-BRK-1. Preferably the host cell is a mammalian cell; more preferably a CHO cell or COS cell.

In another embodiment, the present invention relates to a method for producing BRK-1 or t-BRK-1 comprising isolating BRK-1 or t-BRK-1 from the host cells described above.

In another embodiment, the present invention relates to a method for identifying compounds (e.g., BMP (preferably BMP-2 or BMP-4), and other as yet to be discovered compounds) capable of binding to a BMP receptor kinase protein, the method comprising introducing a sample comprising the compounds to the BMP receptor kinase protein and allowing the compounds to bind to the receptor kinase protein. Preferably the receptor kinase protein has amino acid sequence SEQ ID NO:4 (t-BRK-1) or a soluble fragment thereof, or SEQ ID NO:2 (BRK-1) or soluble fragment thereof. Such a method is also useful for determining the amount of BMP or other receptor binding compound present in the sample.

For example, BMP concentration in a sample could be determined by radioreceptor assay, in which unlabeled BMP in the sample competes with labeled tracer BMP for binding to the BRK-1 or t-BRK-1 receptor. As the amount of BMP in the sample increases, it reduces the amount of labeled BMP which is able to bind to BRK-1 or t-BRK-1. Comparison with a standard curve prepared with known concentrations of unlabeled BMP allows accurate quantitation of BMP

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concentration in the sample. Labeling of tracer BMP is preferably done by iodination with [^{125}I]NaI. BRK-1 or t-BRK-1 can be expressed in the outer membrane of a stable cell line, or supplied as a soluble fragment, or as a soluble fragment covalently attached to a solid support. To perform the assay, unlabeled BMP from the sample and labeled tracer BMP compete for binding to the receptor until equilibrium is reached. The receptor-BMP complex is then isolated from free ligand, for example by washing (in the case of an adherent cell line), rapid filtration or centrifugation (in the case of a nonadherent cell line or receptor bound to a solid support), or precipitation of the receptor-ligand complex with antibodies, polyethylene glycol, or other precipitating agent followed by filtration or centrifugation (in the case of a soluble receptor). The amount of labeled BMP in the complex is then quantitated, typically by gamma counting, and compared to known standards. These methods have been described in the literature using other receptors (Williams, M., *Med. Res. Rev.*, 11:147-184 (1991); Higuchi, M. and Aggarwal, B.B., *Anal. Biochem.*, 204:53-58 (1992); Cain, M.J., R.K, Garlick and P.M. Sweetman, *J. Cardiovasc. Pharm.*, 17:S150-S151 (1991); each of which are incorporated herein by reference), and could readily be adapted to the BRK-1 receptor/BMP system.

The same technique would also be applied in high-throughput screens to identify compounds capable of binding to BRK-1 or t-BRK-1. In such a method, the higher affinity of the compound for BRK-1 or t-BRK-1 (or soluble fragment thereof), the more efficiently it will compete with the tracer for binding to the receptor, and the lower the counts in the receptor-ligand complex. In this case, one would compare a series of compounds at the same concentration range to see which competed for receptor binding with the highest affinity.

In another embodiment, the present invention relates to antibodies specific for BRK-1 or t-BRK-1, and a method for producing the same.

Preferably, for expression of the BRK-1 or t-BRK-1 in systems where the protein product is to be secreted, as in a mammalian cell, the first 23 amino acids of SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:6 constitute a signal sequence that directs the protein product to the secretion apparatus of the cell. Subsequently, the protein product will be incorporated into the membrane if a transmembrane domain is present (as in t-BRK-1 (SEQ ID NO:2) or BRK-1 (SEQ ID NO:4)), or secreted if no transmembrane domain is present (as in a soluble form of t-BRK-1 or BRK-1 (SEQ ID NO:6)). However, the amino acids constituting the signal sequence are generally removed by proteolysis during post-translational processing, so that the mature, processed protein is predicted to start at amino

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acid Gln 24.

For expression systems where the product is accumulated intracellularly, as in bacteria (e.g., *E. coli*), the amino acids constituting the signal sequence would preferably be omitted, and an extra methionine would preferably be added to the N-terminus to serve as a start codon.

This invention is useful for determining whether a ligand, such as a known or putative drug, is capable of binding to and/or activating the BRK-1 receptor encoded by the DNA molecules of the present invention. Transfection of said DNA sequence into the cell systems described herein provides an assay system for the ability of ligands to bind to and/or activate the receptor encoded by the isolated DNA molecule. Recombinant cell lines, such as those described herein, are useful as living cell cultures for competitive binding assays between known or candidate drugs and ligands which bind to the receptor and which are labeled by radioactive, spectroscopic or other reagents. Membrane preparations containing the receptor isolated from transfected cells are also useful for competitive binding assays. Soluble receptors derived from the ligand binding domain of the receptor can also be employed in high throughput screening of drug candidates. Functional assays of intracellular signaling can act as assays for binding affinity and efficacy in the activation of receptor function. In addition, the recombinant cell lines may be modified to include a reporter gene operably linked to a response element such that a signal sent by the receptor turns on the reporter gene. Such a system is especially useful in high throughput screens directed at identification of receptor agonists. These recombinant cell lines constitute "drug discovery systems", useful for the identification of natural or synthetic compounds with potential for drug development. Such identified compounds could be further modified or used directly as therapeutic compounds to activate or inhibit the natural functions of the receptor encoded by the isolated DNA molecule.

Stable cell lines expressing high numbers of BRK-1 or a soluble form thereof are also useful as a source of protein for receptor purification. The purified receptor or its soluble form can then be used for high-throughput screening assays for the purposes described above. The purified receptor or its soluble form can also be used for determination of the structure of the BMP: BRK-1 complex, using X-ray crystallography or NMR techniques, which could then be used in rational design of BMP agonists or antagonists.

The nucleotide sequences disclosed herein, SEQ ID NO:1 and SEQ ID NO:3, represent the sequence of t-BRK-1 and BRK-1, respectively, isolated from mouse NIH3T3 cells. These sequences could be readily used to obtain the cDNA

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for BRK-1 from other species, such as human. These cDNA sequences can also be readily used to isolate the genomic DNA for BRK-1. This would permit analysis of the regulatory elements controlling receptor gene expression, which may offer new opportunities for therapeutic intervention. The nucleotide sequences are also useful to determine the distribution of the BRK-1 in normal tissues and in disease states, which allows an assessment of its physiological role *in vivo*.

For purposes of illustrating a preferred embodiment of the present invention, the following non-limiting examples are discussed in detail.

Example 1

Isolation of the BRK-1 PCR fragment

PCR primers are designed based on an alignment of the protein sequences for the activin (Mathews, L.S. and Vale, W.W., *Cell 65*: 973-982 (1991)) and Daf-1 (Georgi, L.L., Albert, P.S., and Riddle, D.L., *Cell 61*: 635-645 (1990)) receptor kinases, in comparison to the kinase domain sequences of the highly related cytosolic *raf* protein kinases (Nishida, Y., Hata, M., Toshikazu, A., Ryo, H., Yamagata, M., Shimizu, K.; and Nishizuka, *EMBO J.* 7:775-781 (1988); Bonner, T.L., Oppermann, H., Seeburg, P., Kerby, S.B., Gunnell, M.A., Young, A.C., and Rapp, U.R., *Nucleic Acids Res. 14*: 1009-1015 (1986)). This alignment shows that the activin and Daf-1 receptor kinases contain a unique insert in kinase domain VI that is not present in the *raf* kinases. Hence, primers are designed to generate fragments that will include this insert and increase the probability of cloning a receptor kinase that is highly related to the activin and Daf-1 receptors. The sense primer is designed as a degenerate oligonucleotide primer that would encode the protein sequence E A/Y V A V K V/I F, found in kinase domain II of the activin and Daf-1 receptors. ACT2A and ACT2B are the names assigned to refer to this set of degenerate 5' PCR primers, which are illustrated in Figure 1. The antisense primer is designed as a degenerate oligonucleotide primer pool that would encode the antisense strand corresponding to protein sequence K P A M/I A/S H R D I K, found in domain VIB of the activin and Daf-1 receptors. ACT1A and ACT1B are the names assigned to refer to this set of degenerate 3' PCR primers, which are illustrated in Figure 1.

Total RNA is isolated from mouse NIH3T3 cells (ATCC CRL 1648) using "RNAZOL" (Tel-Test, Friendswood, TX; a solution for rapid isolation of RNA, containing guanidinium thiocyanate, phenol, and β-mercaptoethanol). Poly A+ RNA is then prepared by chromatography on oligo(dT) cellulose chromatography (Pharmacia LKB, Piscataway, NJ). Single stranded DNA is generated from 200 ng polyadenylated mRNA using reverse transcriptase (first strand synthesis kit

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from. Stratagene. La Jolla. CA: this kit contains components necessary for generating cDNA from RNA, including reverse transcriptase from Maloney murine leukemia virus, primers, nucleotides and buffers). A portion of this material (20%) is then amplified by the polymerase chain reaction (hereinafter PCR) using 50 pmol of the 5' primers ACT2A and ACT2B shown in Figure 1, and 250 pmol of each of the 3' primers ACT1A and ACT1B shown in Figure 1. The reaction is run in a 100 µl final volume using the "GENE-AMP" kit (Perkin-Elmer, Norwalk, CT: a kit containing components necessary for amplification of DNA using the polymerese chain reaction, including "AMPLITAQ", a recombinant form of the DNA polymerase from *Thermus aquaticus* (Perkin-Elmer, Norwalk, CT). nucleotides, and buffers) using a Perkin-Elmer thermal cycler. Standard PCR reaction conditions are used: melting at 94° for 2 min, followed by 35 cycles of melting (94°, 30 sec), annealing (55°, 30 sec), and extension (72° C, 30 sec). After the completion of this first PCR reaction, a 10 µl aliquot of the reaction is removed and subjected to another 35 cycles of amplification with fresh reagents. Products of this secondary PCR are then ligated into the vector pCR 1000 (Invitrogen, San Diego, CA) for clonal selection and sequence analysis.

By this method, a PCR fragment of approximately 300 bp is isolated, whose DNA sequence shows a strong homology to the genes for the Daf-1 receptor (Georgi,. L.L., Albert, P.S., and Riddle, D.L., *Cell*, *61*: 635-645 (1990)) and mouse activin Type II receptor cDNAs (Mathews, L.S. and Vale, W.W., *Cell*, *65*: 973-982 (1991)).

Example 2

Isolation of t-BRK-1 DNA

With the PCR fragment in hand, it is next necessary to screen a cDNA library with this fragment in order to isolate a full-length receptor clone.

The PCR fragment is excised, purified by gel electrophoresis, and labeled with (α-^{32}P)-dCTP, using a random priming method using a "PRIME-IT" Random Primer Labeling Kit (Stratagene, La Jolla, CA; a kit containing components necessary for random primer labeling of cDNA, including exonuclease deficient Klenow polymerase, random 9-mer primers, and buffers). The labeled probe is then used to screen a cDNA library prepared from mouse NIH3T3 cells in the vector "λ ZAP II" (Stratagene, La Jolla, CA; a lambda cloning vector which accepts inserts up to 10 kb in length and permits automatic excision of inserts in the "pBLUESCRIPT SK(-)" plasmid). Hybridization is performed for 24-48 hours at 42°C in 5X SSPE (1X SSPE = 0.15 M NaCl, 10 mM Na_2HPO_4, and 1 mM EDTA (ethylenediaminetetraacetic acid)), 1X Denhardt's (0.02% bovine serum

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albumin. 0.02% polyvinylpyrrolidone. 0.02% Ficoll). 100 µg/ml salmon testis DNA. 50% formamide. Membranes are washed first at 42°C and subsequently at 55°C in 0.1X SSPE. and 0.2% sodium dodecyl sulfate (SDS), and the positive plaques identified by autoradiography at -70°C on "KODAK X-OMAT AR" film (Kodak. Rochester, NY: scientific imaging film) with intensifying screens. Dilution and screening of positive plaques yields an isolated pure phage, which is designated J159#7.

The cloning site in the "λ ZAP II" vector contains the sequence of the plasmid "pBLUESCRIPT SK(-)" (Stratagene, La Jolla, CA; a 2.96 kb colony-producing phagemid derived from pUC19). This plasmid sequence, containing the cloned insert, is excised from the purified lambda phage J159#7, using R408 helper phage (Stratagene, La Jolla, CA). This yields the t-BRK-1 cDNA subcloned into "pBLUESCRIPT SK(-)". The resulting plasmid, which we designate pBLUESCRIPT-J159T, is suitable for sequence analysis.

Example 3

t-BRK-1 Sequence Analysis

The isolated plasmid pBLUESCRIPT-J159T containing the t-BRK-1 cDNA is then sequenced on both strands, using either the "SEQUENASE" Ver. 2.0 kit (U.S. Biochemicals, Cleveland, OH; a kit containing components for manual DNA sequencing using the dideoxy terminator method, including "SEQUENASE" (a modified form of T7 DNA polymerase deficient in exonuclease activity, U.S. Biochemicals, Cleveland, OH), nucleotide mixes for labeling and extension, dideoxy nucleotide terminators, pyrophosphatase and buffers) or the "TAQ DYE DEOXY" Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City CA: a kit containing components for automated DNA sequencing using the dideoxy terminator method, including "AMPLITAQ", nucleotide mix, dye-labeled dideoxy nucleotide terminators, and buffers) with a Model 373A DNA Sequencer (Applied Biosystems, Foster City, CA). The complete DNA sequence (SEQ ID NO:1) shows an open reading frame of 1500 base pairs following an initiator ATG.

Comparison of this sequence with the sequence of that of known receptor kinases of the TGF-ß family shows a strong homology, but indicates that the kinase domain of t-BRK-1 is considerably shorter than that of related receptor kinases (Fig. 2). Mutational studies on the *src* kinase have yielded information that specifies the minimal amino acid residues that are necessary at the C terminus of the kinase domain in order for the kinase to function as an active enzyme. Deletions of amino acids upstream of this area yield inactive kinases, presumably because the kinase structure is destabilized (Yaciuk, P. and

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Shalloway, D., *Molec. and Cell. Biol.*, *6*: 2807-2819 (1986)). It is therefore generally agreed by those skilled in the art that the end of the kinase domain occurs at a hydrophobic residue located 10 residues downstream of the invariant arginine in kinase domain XI for tyrosine kinases, or 12-18 amino acids downstream of this invariant arginine for serine/threonine kinases, respectively (Hanks, S.K. and Quinn, A.M., *Meth. Enzymol.*, *200*: 38-62 (1991)). This region is indicated by the square bracket in Figure 2. The stop codon in t- BRK-1 is located 20 amino acids prior to the start of the region that specifies the end of the kinase domain, and t-BRK-1 is therefore believed to be a truncated receptor clone. Further analysis of the sequence reveals a putative intact intron-exon junction at position 1763, indicating the possibility that the messenger RNA which served as the template for this cDNA has undergone incomplete post-transcriptional splicing of the RNA, such that a portion of an intron was included in the sequence.

At the N-terminal end of the protein, a eukaryotic signal sequence is identified, with a predicted cleavage site between amino acids 23 and 24 (see SEQ ID NO:2). Thus, after post-translational processing, the mature protein is expected to begin at amino acid Gln24. A region of high hydrophobicity at amino acids 153-176 indicates the presence of a transmembrane region which divides the protein into an extracellular ligand binding domain and an intracellular kinase domain.

Example 4

Isolation of BRK-1 DNA

In order to isolate a BRK-1 cDNA that does not include a premature termination in the kinase domain, another cDNA library with a broader representation of mRNA is prepared from NIH3T3 poly A+ RNA (Stratagene, La Jolla, CA). This library, constructed in "UNI-ZAP XR" (Stratagene, La Jolla, CA: a lambda cloning vector which allows construction of unidirectional cDNA libraries) uses both a poly dT sequence and a random primer for the synthesis of the cDNA library. This library is then screened with the J159 PCR fragment, labeled with ^{32}P by the random priming method ("PRIME-IT" Random Primer Labeling Kit). Screening and isolation of clones is carried out as described above (Example 2). Several additional clones are obtained and subjected to sequence analysis.

Example 5

BRK-1 Sequence Analysis

Sequence analysis of the clones is carried out using the "TAQ DYE DEOXY" Terminator Cycle Sequencing Kit and the Applied Biosystems Model

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373A DNA Sequencer (Applied BioSystems, Foster City, CA). Comparison of the clones to the sequence of t-BRK-1 and to other receptor kinases (Figure 2) indicates that one clone (SEQ ID NO:3) contains the full length coding sequence, with no intron present, and the complete kinase domain. The plasmid from this clone is designated pBLUESCRIPT-J159F. The open reading frame is 1596 base pairs encoding a protein with 532 amino acids, with a predicted molecular mass of about 60,059. This cDNA is designated as BRK-1.

The DNA sequence of BRK-1 is identical to that of t-BRK-1 over nucleotides 1-1483 in SEQ ID NO: 3 (nucleotides 281-1763 in SEQ ID NO:1), and hence identical in amino acid sequence over amino acids 1-491 in SEQ ID NO: 4 and SEQ ID NO:2. Thus, the N-terminal signal sequence and the transmembrane domain observed in t-BRK-1 and described in Example 3 are identically present in full length BRK-1. The entire ligand binding domain is also identical. The nucleotide sequence for t-BRK-1 (SEQ ID NO:1) contains a 90 base pair insert (nucleotides 1764-1853) which is absent in the nucleotide sequence of BRK-1 (SEQ ID NO:3), which may represent a portion of an incompletely spliced intron. After this point, nucleotides 1854-2035 of the t-BRK-1 sequence are identical to nucleotides 1484-1665 of the BRK-1 sequence. Hence, removal of the 90 base pair insert in t-BRK-1 yields a coding sequence identical to that of BRK-1.

Example 6

Generation of Antibodies to BRK-1

In order to demonstrate expression of the BRK-1 receptor, to demonstrate ligand binding, and to identify other proteins which may complex with BRK-1, the availability of antibodies specific for the receptor is highly useful. Polyclonal antisera are accordingly produced in rabbits for this purpose, using two antigens.

First, the mature extracellular ligand binding domain, comprising amino acids 24-152 of SEQ ID NO:2 (or amino acids 24-152 of SEQ ID NO:4), is expressed in *E. coli* using the "QIA EXPRESS" bacterial expression system (Qiagen, Chatsworth, CA; a kit for high-level expression of proteins in *E. coli*, which incorporates into the protein an affinity tag of six histidines to allow rapid purification of the recombinant protein by metal chelate chromatography; the kit includes pQE-12 expression vector, a plasmid encoding the *lac* repressor, *E. coli* host strains and metal chelate resin). A portion of the nucleotide sequence, comprising nucleotides 360-746 in SEQ ID NO: 1 (or nucleotides 80 to 466 in SEQ ID NO:3), is amplified by the polymerase chain reaction using primers which incorporate Bgl II sites at the 5' and 3' ends. Specifically, the primer for the 5' end is CCATAGATCTCAGAATCTAGATAGT, and for the 3' end is

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GGTAAGATCTTCGGATCCTGCCATC. The amplified insert is inserted into the PQE12 vector (Qiagen, Chatsworth, CA), which directs the expression of the insert with six histidines at the C terminal end of the protein. After transformation of *E. coli* strain JM101 with this construct, the transformed strain is grown in LB broth supplemented at mid-log phase with isopropyl thio-ß-galactoside (IPTG), which induces expression of the protein driven by the *lac* promoter. Three hours after addition of IPTG, cells are harvested by centrifugation and lysed in a "FRENCH" pressure cell (SLM-Aminco, Urbana, IL; a dispersion unit for disintegrating bacteria under high pressure using a hydraulic press) using two passes at 16,000 psi. The extracellular domain is purified by chromatography on a nickel metal chelate column, according to the manufacturer's instructions. Further purification is attained by chromatography on a preparative C4 reverse phase column (Waters DeltaPak C4 column, 300 Å, 7.8 mm x 30 cm, Millipore, Milford, MA), using a linear gradient of 0.05% TFA (trifluoroacetic acid) in water to 0.05% TFA in 80% acetonitrile, over 90 minutes at a flow rate of 2.8 ml/min. Peak fractions eluting at 38% acetonitrile are pooled, dried under vacuum, and used to immunize three New Zealand White rabbits (Hazleton Washington, Vienna, VA). Antisera are evaluated by Western blots for their ability to detect the purified *E. coli* antigen. The antiserum with the highest titer is designated 1353.

A second antigen, intended to recognize the intracellular kinase domain, is generated from a peptide having an identical amino acid sequence to amino acids 398-420 of SEQ ID NO: 4 (or amino acids 398-420 of SEQ ID NO:2), with the addition of a cysteine at the C terminus to permit conjugation of the peptide; i.e., indicated by the single letter amino acid abbreviations, LNTRVGTKRYMAPEVLDESLNKNC. Comparison of the amino acid sequence of the kinase domain of BRK-1 with the kinase domain of the Raf protein suggests that this region of BRK-1 corresponds to a region of the Raf kinase which was used to make highly specific antibodies (Kolch, W., Weissinger, E., Mischak, H., Troppmair, J., Showalter, S.D., Lloyd, P., Heidecker, G., and Rapp, U.R. *Oncogene 5:* 713-720 (1990)). This peptide is conjugated by standard methods to keyhole limpet hemocynanin, and used to immunize three New Zealand White rabbits (Hazleton Washington, Vienna, VA). The resulting antisera are evaluated for their ability to recognize the original peptide coated on plastic, using an antibody capture ELISA (enzyme-linked immunosorbent assay). The antisera are designated 1378, 1379, and 1380.

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Example 7

Expression of BRK-1

In order to identify the function of BRK-1, it is necessary to express the protein and test to see whether it binds a specific ligand. This is preferably done in a mammalian cell line, since this maximizes the chance of expressing a correctly processed protein in the cell membrane. To this end, the BRK-1 cDNA is subcloned into the expression vector pJT4 to generate the plasmid pJT4-J159F. The BRK-1 insert from pBLUESCRIPT-J159F is digested with the restriction endonuclease Alf III, generating a linearized plasmid with a single overhang. The overhanging end is filled in using DNA Polymerase I Klenow fragment, generating a blunt end. The linearized plasmid is then digested with Not I, liberating the insert from the plasmid. The pJT4 expression vector is digested with Not I and EcoRV, and ligated to the insert. The blunt end generated by the Klenow reaction is compatible with the EcoRV site, which is also a blunt end; ligation eliminates the Eco RV site. The resulting construct is shown in Figure 3.

The pJT4 vector, optimized for transient expression in COS cells, includes the cytomegalovirus early promoter and enhancer, which gives very efficient transcription of message; an "R" element from the long terminal repeat of the human T-cell leukemia virus-1, which has been shown to increase expression levels further (Takebe, Y., M. Seiki, J.-I. Fujisawa, P. Hoy, K. Yokota, M. Yoshida and N. Arai, *Mol. Cell. Biol.*, 8:466-472 (1988)); an intron splice site from SV40, which is believed to enhance message stability; a multiple cloning site; a polyadenylation signal derived from SV40, which directs the addition of a poly A tail to the message, as is required for most eukaryotic mRNA; and the SV40 origin of replication, which permits the replication of the plasmid to extremely high copy number in cells which contain the SV40 large T antigen, such as COS cells. In addition, for manipulation and amplification of the vector in bacteria, the vector contains an *E. coli* origin of replication and an ampicillin resistance gene.

Transient expression of BRK-1 using pJT4-J159F is carried out in COS-7 cells (ATCC CRL 1651) using electroporation. Cells are grown to confluence in DME (Dulbecco's Modified Eagle) high glucose media supplemented with 5% fetal bovine serum (Hyclone, Logan, Utah), nonessential amino acids (GIBCO, Gaithersburg, MD), and glutamine, then trypsinized to release cells from the plate. The detached cells are pelleted in a tabletop centrifuge, then resuspended in fresh media at a concentration of 6.25×10^6 cells/ml. The cell suspension (5×10^6 cells, 0.8 ml) is transferred to the cuvette from a BioRad "GENE PULSER"

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electroporation system (BioRad. Hercules. CA). The purified DNA plasmid (10 µg) is added to the cuvette. and the cells subjected to electoporation at 4.0 kV/cm. with a capacitance of 25 µFd. Cells are then plated and allowed to recover Fresh media is supplied after 24 hr. At 48 hr, cells are ready to be tested for binding of BMP-4.

Example 8

Expression of t-BRK-1

For expression of t-BRK-1, the cDNA insert from pBLUESCRIPT-J159T is excised using the restriction endonucleases Not I and Xho I, and subcloned into the Not I and Sal I sites of the expression vector pJT6, generating the construct pJT6-J159T shown in Figure 4. The vector pJT6 is identical to pJT4, described in Example 6, except for the opposite orientation of the multiple cloning site, and the presence of a spacer DNA between the Pst I and Bam H1 sites of the multiple cloning region.

Transient expression of t-BRK-1 in COS cells using the pJT6-J159T construct is carried out exactly as described above for BRK-1 (Example 7).

Example 9

Preparation of radiolabeled BMP ligands

The preferred radioligand in all these studies is BMP-4. BMP-4 is labeled with ^{125}I by the chloramine-T method (Frolik, C.A., Wakefield, L.M., Smith, D.M., and Sporn, M.B. *J. Biol. Chem. 259:* 10995-11000 (1984)). BMP-4 (2 µg) is taken up in 5 µl of 30% acetonitrile, 0.1% trifluoracetic acid (TFA) plus an additional 5 µl of 1.5 M sodium phosphate, pH 7.4. Carrier free [^{125}I] (1 mCi, 4-10 µl) is added, together with 2 µl of a chloramine T solution (100 µg/ml). An additional 2 µl of the chloramine T solution is added at 2.0 min and at 3.5 min. After 4.5 minutes, the reaction is stopped by the addition of 10 µl of 50 mM N-acetyl tyrosine, 100 µl of 60 mM potassium iodide, and 100 µl of 11M urea in 1 M acetic acid. After a 3.5 minute incubation, unreacted iodine is removed on a PD-10 gel filtration column (Pharmacia, Piscataway, NJ) run in 4 mM HCl, 75 mM NaCl, 1 mg/ml bovine serum albumin (BSA). The resulting labeled protein is >95% precipitable by trichloroacetic acid, indicating that all [^{125}I] is protein bound, and has a typical specific activity of 3000-8000 Ci/mmol.

BMP-2 can be radiolabeled in the same manner and used as a radioligand. However, use of BMP-2 results in very high nonspecific binding, presumably because of binding of BMP-2 to extracellular matrix proteins. Such nonspecific binding can be significantly reduced. and hence the usefulness of BMP-2 as a radioligand significantly improved, by removal of amino terminus of the protein.

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presumably because this removes the region responsible for binding to the extracellular matrix. Removal of the amino terminus from BMP-2 can be accomplished by partial proteolysis with trypsin (Wozney, J.M., *Mol. Rep. Dev.*, 32:160-167 (1992)). This yields a derivative designated "digit-removed BMP-2", or DR-BMP-2. Preparation and purification of DR-BMP-2 is carried out as follows.

BMP-2 (100-250 µg) is solubilized in 500 µl of 4 M urea, 0.1 M NaCl, 0.05 M Tris-HCl (pH 8.2). Trypsin (Sequencing Grade; Boehringer Mannheim, Indianapolis, IN) is added to a trypsin/BMP-2 ratio of 1/50 (w/w), and the digestion mixture is incubated at 37°C for 2 hr. Digestion is stopped by the addition of phenylmethylsulfonylfluoride (PMSF) to a final concentration of 1 mM, and the mixture is frozen and stored at -20°C until purification.

DR-BMP-2 is purified from the digestion mixture by reversed phase HPLC (high performance liquid chromatography) on a Waters Delta-Pak C4 column (5 µm, 300 A, 3.9 X 150 mm; Millipore Corp., Milford, MA). The entire digestion mixture is injected directly onto the column, and DR-BMP-2 is eluted using a linear gradient from 0.05% TFA to 0.05% TFA, 60% acetonitrile over 80 min. at a flow rate of 0.7 ml/min. The majority of DR-BMP-2 elutes as a well-defined peak at about 36% acetonitrile, as monitored by absorbance at 214 nm and after Coomassie blue staining of an SDS-polyacrylamide gel. PMSF, PMSF-inactivated trypsin, and any remaining intact BMP-2 are separated from DR-BMP-2 under these chromatographic conditions. Purified DR-BMP-2 is aliquoted, taken to dryness under vacuum, and stored at -20°C.

Analysis by SDS-polyacrylamide gel electrophoresis shows that the molecular weight of DR-BMP-2 is decreased by approximately 2000 daltons compared to BMP-2 under nonreduced conditions, and by about 1000 daltons under reducing conditions. Amino-terminal protein sequencing demonstrates that roughly 70% of DR-BMP-2 begins at Lys290, while the remaining 30% begins at Leu292. Results from amino acid analysis are entirely consistent with the sequencing results and suggest that the COOH-terminus of the protein is unaffected by trypsin treatment. Radiolabeling of DR-BMP-2 is carried out exactly as described for BMP-4.

Example 10

BMP Binding to BRK-1

Binding of DR-BMP-2 and BMP-4 to BRK-1 can be demonstrated by three separate methods: whole cell binding of radiolabeled BMP; covalent crosslinking of radiolabeled BMP to the receptor; and immunoprecipitation of the receptor crosslinked to labeled ligand. These three methods are described in detail below.

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For whole cell binding experiments, COS-7 cells are transfected with pJT4-J159F as described in example 7. After electroporation, cells are seeded in 12 well plates at 670,000 cells/well. Media is changed after 24 hr, and binding experiments are carried out at 48 hr. At that time, cells are washed once with binding buffer (50 mM Hepes buffer, pH 7.4, 128 mM NaCl, 5 mM KCl, 5 mM $MgSO_4$, 1.2 mM $CaCl_2$, 2 mg/ml BSA), then equilibrated in the same buffer at 4°C for 30 min with gentle shaking. The buffer is then aspirated, and to each well is added 500 µl of binding buffer (4°C), containing [^{125}I]-BMP-4 tracer (100 pM), as well as varying concentrations of unlabeled BMP-2, BMP-4, or other unlabeled ligand, depending on the assay. For determination of nonspecific binding, BMP-2 is added to the binding buffer at a final concentration of 10 nM. To prevent degradation of ligand during the incubation, a protease inhibitor cocktail is also added, to give a final concentration of 10 µg/ml leupeptin, 10 µg/ml antipain, 50 µg/ml aprotinin, 100 µg/ml benzamidine, 100 µg/ml soybean trypsin inhibitor, 10 µg/ml bestatin, 10 µg/ml pepstatin, and 300 µM PMSF. The cells are incubated for 4 hr at 4°C with gently shaking. At the end of the incubation period, the buffer is aspirated, and the cells are rinsed 4 times with 1 ml washing buffer (50 mM HEPES, pH 7.4, 128 mM NaCl, 5 mM KCl, 5 mM $MgSO_4$, 1.2 mM $CaCl_2$, 0.5 mg/ml BSA). After the final wash is aspirated, 750 µl of solubilization buffer (10 mM TrisCl, pH 7.4, 1 mM EDTA, 1% (v/v) Triton X-100) is added to each well and incubated at room temperature for 15 min. The solubilized cells are then transferred to fresh tubes and counted in a Packard Model 5005 "COBRA" Gamma Counter (Packard Instrument Co., Downers Grove, IL).

Results of such an experiment are shown in Figure 5. Specific binding of [^{125}I]-BMP-4 to COS-7 cells transfected with the cDNA to BRK-1 (using the construct pJT4-J159F) is three times higher than binding to mock-transfected COS-7 cells.

In order to obtain a more quanitative characterization of binding to BRK-1, a saturation binding analysis is performed on COS-7 cells transfected with the cDNA for BRK-1, using the construct pJT4-J159F. Binding of [^{125}I]-BMP-4 is examined over a concentration range of 10-1000 pM, with nonspecific binding determined with 10 nM unlabeled BMP-4. Binding data was analyzed using LIGAND software (Version 3.0; Elsevier-Biosoft, Cambridge, UK) to obtain an affinity (K_d) for BRK-1 of 5 x 10^{-10} M, well within the physiological range expected for a BMP receptor.

A second method of demonstrating binding of BMPs to BRK-1 is to crosslink the radiolabeled ligand to the BRK-1 receptor. In this method, the

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bifunctional crosslinking reagent disuccinimidyl suberate (DSS) (Pierce Chemical. Rockford, IL) is used to covalently crosslink bound radiolabeled ligand to its receptor by reaction with free amino groups on lysine residues in the two proteins. Following the crosslinking, cellular proteins are separated by gel electrophoresis. and radioactive bands visualized. The labeled bands represent the receptor selectively "tagged" with the radiolabeled ligand. In this procedure, cells are transfected with pJT4-J159F as described in example 7, then seeded into 12 well plates at 670,000 cells/well. Media is changed after 24 hr. At 48 hr after electroporation, the cells are washed, equilibrated, and incubated with [^{125}I]-BMP-4 or [^{125}I]-DRBMP-2 and competing unlabeled ligands as described in this example for whole cell binding studies. After completion of the 4 hour incubation with ligand, the cells are washed three times at 4°C with 2 ml of binding buffer having the same composition as described above, except that no BSA is added. To each well is then added 1 ml of fresh BSA-free binding buffer, followed by freshly prepared DSS to a final concentration of 135 µM. After swirling gently to mix the DSS. the plates are incubated for exactly 15 minutes at 4°C with gentle shaking. At this point the media is aspirated and the cells washed with 3 ml detachment buffer (10 mM Tris, 0.25 M sucrose, 1 mM EDTA, 0.3 mM PMSF, pH 7.4). An additional 0.75 ml detachment buffer is added to each well; the cells are scraped off into the buffer and transferred to fresh microcentrifuge tubes. Each well is then rinsed with an additional 0.5 ml detachment buffer, which is added to the corresponding tube. The samples are centrifuged (13,000 x g, 15 min) and the supernatant discarded. The pellets are taken up in 20 µL reducing sample buffer (125 mM TrisCl, pH 6.8, 1% ß-mercaptoethanol, 2% SDS, 0.1% bromphenol blue, 10% glycerol), vortexed for 30-45 min at 4°C, boiled for 5 minutes, and centrifuged (13,0000 x g, 5 min). The supernatants are loaded onto 7.5% SDS-polyacrylamide gels and subjected to electrophoresis. The gels are stained in 0.12% Coomassie Blue, 5% methanol, 7.5% acetic acid, destained in 5% methanol, 7.5% acetic acid, then dried between sheets of cellophane. Radioactivity on the dried gel is visualized and quantitated on a PhosphorImager (Molecular Devices, Sunnyvale, CA), or subjected to autoradiography using "KODAK X-OMAT AR" film (Kodak, Rochester, NY).

Result of such experiments are shown in Figure 6. Figure 6A shows crosslinking of [^{125}I]-BMP-4 to COS-7 cells transfected with BRK-1 using the construct pJT4-J159F. In lane 1, note the presence of a labeled band at molecular weight 76,800 which corresponds to BRK-1 covalently crosslinked to BMP-4. This band is greatly reduced by the addition of 10 nM BMP-2 to the cells

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during incubation and is absent in mock-transfected COS cells. This indicates specific binding of BMP-4 to BRK-1. Since the labeled band represents the receptor covalently crosslinked to BMP-4 (monomer molecular weight 16,400), the molecular weight of the receptor can be estimated at 60,400, which is consistent with the amino acid sequence of BRK-1. Figure 6B shows the same experiment using [^{125}I]-DRBMP-2 as the ligand. A similar labeled band is observed. As with [^{125}I]-BMP-4, the labeled band is greatly reduced by the addition of 10 nM BMP-2 to the cells and is absent in mock transfected cells. Taken together, these data indicate that both BMP-2 and BMP-4 are specifically bound by BRK-1.

In a third demonstration of BMP binding to BRK-1, COS cells transfected with the cDNA for BRK-1 are first crosslinked to [^{125}I]-BMP-4, then subjected to immunoprecipitation with antibodies specific for BRK-1. In this procedure, COS-7 cells are transfected with pJT-J159F as described in Example 7 and plated into 100 mm dishes seeded at 1 x 10^7 cell/dish. They are then crosslinked to [^{125}I]-BMP-4 as described in this example, except that the incubation with [^{125}I]-BMP-4 and unlabeled ligand is carried out in a total of 4 ml, instead of 500 µl, and all other volumes are increased accordingly. Following the crosslinking, cells are washed three times with ice-cold PBS [phosphate buffered saline], then lysed with 4 ml of RIP buffer (20 mM TrisCl, pH 8.0, 100 mM NaCl, 1 mM Na_2EDTA, 0.5% Nonidet P-40, 0.5% sodium deoxycholate, 10 mM sodium iodide, and 1% bovine serum albumin). The lysate is centrifuged in a Beckman GPR tabletop centrifuge at 3500 rpm (3000 x g) for 10 min. The supernatant is transferred to a fresh tube and made 0.1% in SDS. To remove any existing antibody present in the lysate, 200 µl of "PANSORBIN" (Calbiochem, La Jolla, CA; a 10% solution of *Staphylococcus aureus)* is added. After a 30 minute incubation at 4°C, the lysate is centrifuged as before, and the supernatant again transferred to a fresh tube, split into aliquots as required.

The primary antibody--1353, for the extracellular domain; or 1378, 1379, or 1380, for the kinase domain--is then added to the tube at a final dilution of 1:100, and incubated for 2 hr on ice. To precipitate the complex of antigen: primary antibody, 50 µl of "PANSORBIN" is then added and incubated 30 min on ice. The complex is pelleted at 3500 rpm (3000 x g) for 10 min in a Beckman GPR centrifuge (Beckman Instruments, Fullerton, CA) and the supernatant discarded. The pellet is washed three times in RIP buffer containing 0.1% SDS, and once in TNEN buffer (20 mM Tris, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5% NP-40). The pellet is resuspended in 25 µl of reducing sample buffer. Solubilized proteins are subjected to electrophoresis is SDS polyacrylamide gels and autoradiography as

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described above for crosslinking experiments.

Results of such an experiment are shown in Figure 7. Antibodies to both the extracellular domain (lane 1) and the intracellular domain (lanes 3-5) precipitate a band of molecular weight of approximately 81.000. Subtracting the monomer weight of BMP-4 (16,400), the molecular weight of the receptor is estimated at 64,000, similar to the result obtained in the crosslinking experiments described above. All three antisera to the intracellular domain precipitate the same protein. This characteristic band is absent in mock-transfected cells (lanes 2 and 6). This experiment demonstrates that the crosslinked labeled band observed in crosslinking experiments, such as those shown in Figure 6, is immunologically related to BRK-1, because it is precipitated by four separate antibodies specific for BRK-1.

Deposit of t-BRK-1 and BRK-1

E. coli transformed with pJT6-J159T (SEQ ID NO:1 subcloned into expression vector pJT6) was deposited with the ATCC on October 20, 1993, and assigned ATCC Designation No. 69474.

E. coli transformed with pBLUESCRIPT-J159T (SEQ ID NO:1 subcloned into expression vector "pBLUESCRIPT SK(-)") was deposited with the ATCC on October 7, 1993, and assigned ATCC Designation No. 69458.

E. coli transformed with pJT4-J159F (SEQ ID NO:3 subcloned into expression vector pJT4) was deposited with the ATCC on October 7, 1993, and assigned ATCC Designation No. 69457.

As is recognized in the art, there are occasionally errors in DNA and amino acid sequencing methods. As a result, the sequences encoded in the deposited material are incorporated herein by reference and controlling in the event of an error in any of the sequences found in the written description of the present invention. It is further noted that one of ordinary skill in the art reproducing Applicants' work from the written disclosure can discover any sequencing errors using routine skill. The deposit of ATCC No. 69457 and ATCC No. 69474 is not to be considered as an admission that the deposited material is essential to the practice of the present invention.

All publications mentioned hereinabove are hereby incorporated in their entirety by reference.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to one skilled in the art and are to be included in the spirit and purview of this application and scope of the appended claims.

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2056 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 291..1793

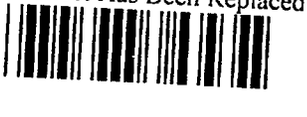
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CGAATTCCTC GCGCCGTGGG AGGGGCGGCC CGGCCCACCC CCACGCCCCG CCCGGGAGGG 60

ACGGGGGGAG AGAGAGCGCG GCGACGGGTA TCTGGGTCAA AGCTGTTCGG AGAAATTGGA 120

ACTACAGTTT TATCTAGCCA CATCTCTGAG AATTCTGAAG AAAGCAGCAG GTGAAAGTCA 180

This Sheet Has Been Replaced



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TTGCCAAGTG ATTTTGTTCT GTAAGGAAGC CTCCCTCATT CACTTACACC AGTGAGACAG      241

CAGGACCAGT CATTCAAAGG GCCGTGTACA GGACGCGTGC GAATCAGACA ATG ACT         296
                                                           Met Thr
                                                             1

CAG CTA TAC ACT TAC ATC AGA TTA CTG GGA GCC TGT CTG TTC ATC ATT         344
Gln Leu Tyr Thr Tyr Ile Arg Leu Leu Gly Ala Cys Leu Phe Ile Ile
          5                  10                  15

TCT CAT GTT CAA GGG CAG AAT CTA GAT AGT ATG CTC CAT GGC ACT GGT         392
Ser His Val Gln Gly Gln Asn Leu Asp Ser Met Leu His Gly Thr Gly
     20                  25                  30

ATG AAA TCA GAC TTG GAC CAG AAG AAG CCA GAA AAT GGA GTG ACT TTA         440
Met Lys Ser Asp Leu Asp Gln Lys Lys Pro Glu Asn Gly Val Thr Leu
 35                  40                  45                  50

GCA CCA GAG GAT ACC TTG CCT TTC TTA AAG TGC TAT TGC TCA GGA CAC         488
Ala Pro Glu Asp Thr Leu Pro Phe Leu Lys Cys Tyr Cys Ser Gly His
                55                  60                  65

TGC CCA GAT GAT GCT ATT AAT AAC ACA TGC ATA ACT AAT GGC CAT TGC         536
Cys Pro Asp Asp Ala Ile Asn Asn Thr Cys Ile Thr Asn Gly His Cys
            70                  75                  80

TTT GCC ATT ATA GAA GAA GAT GAT CAG GGA GAA ACC ACA TTA ACT TCT         584
Phe Ala Ile Ile Glu Glu Asp Asp Gln Gly Glu Thr Thr Leu Thr Ser
          85                  90                  95

GGG TGT ATG AAG TAT GAA GGC TCT GAT TTT CAA TGC AAG GAT TCA CCG         632
Gly Cys Met Lys Tyr Glu Gly Ser Asp Phe Gln Cys Lys Asp Ser Pro
     100                 105                 110

AAA GCC CAG CTA CGC AGG ACA ATA GAA TGT TGT CGG ACC AAT TTG TGC         680
Lys Ala Gln Leu Arg Arg Thr Ile Glu Cys Cys Arg Thr Asn Leu Cys
115                 120                 125                 130

AAC CAG TAT TTG CAG CCT ACA CTG CCC CCT GTT GTT ATA GGT CCG TTC         728
Asn Gln Tyr Leu Gln Pro Thr Leu Pro Pro Val Val Ile Gly Pro Phe
                135                 140                 145

TTT GAT GGC AGC ATC CGA TGG CTG GTT GTG CTC ATT TCC ATG GCT GTC         776
Phe Asp Gly Ser Ile Arg Trp Leu Val Val Leu Ile Ser Met Ala Val
            150                 155                 160

TGT ATA GTT GCT ATG ATC ATC TTC TCC AGC TGC TTT TGC TAT AAG CAT         824
Cys Ile Val Ala Met Ile Ile Phe Ser Ser Cys Phe Cys Tyr Lys His
          165                 170                 175

TAT TGT AAG AGT ATC TCA AGC AGG GGT CGT TAC AAC CGT GAT TTG GAA         872
Tyr Cys Lys Ser Ile Ser Ser Arg Gly Arg Tyr Asn Arg Asp Leu Glu
     180                 185                 190

CAG GAT GAA GCA TTT ATT CCA GTA GGA GAA TCA TTG AAA GAC CTG ATT         920
Gln Asp Glu Ala Phe Ile Pro Val Gly Glu Ser Leu Lys Asp Leu Ile
195                 200                 205                 210

GAC CAG TCC CAA AGC TCT GGG AGT GGA TCT GGA TTG CCT TTA TTG GTT         968
Asp Gln Ser Gln Ser Ser Gly Ser Gly Ser Gly Leu Pro Leu Leu Val
                215                 220                 225

CAG CGA ACT ATT GCC AAA CAG ATT CAG ATG GTT CGG CAG GTT GGT AAA        1016
Gln Arg Thr Ile Ala Lys Gln Ile Gln Met Val Arg Gln Val Gly Lys
            230                 235                 240

GGC CGC TAT GGA GAA GTA TGG ATG GGT AAA TGG CGT GGT GAA AAA GTG        1064
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This Sheet Has Been Replaced



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Gly Arg Tyr Gly Glu Val Trp Met Gly Lys Trp Arg Gly Glu Lys Val
        245                 250                 255

GCT GTC AAA GTG TTT TTT ACC ACT GAA GAA GCT AGC TGG TTT AGA GAA      1112
Ala Val Lys Val Phe Phe Thr Thr Glu Glu Ala Ser Trp Phe Arg Glu
    260                 265                 270

ACA GAA ATC TAC CAG ACG GTG TTA ATG CGT CAT GAA AAT ATA CTT GGT      1160
Thr Glu Ile Tyr Gln Thr Val Leu Met Arg His Glu Asn Ile Leu Gly
275                 280                 285                 290

TTT ATA GCT GCA GAC ATT AAA GGC ACT GGT TCC TGG ACT CAG CTG TAT      1208
Phe Ile Ala Ala Asp Ile Lys Gly Thr Gly Ser Trp Thr Gln Leu Tyr
                295                 300                 305

TTG ATT ACT GAT TAC CAT GAA AAT GGA TCT CTC TAT GAC TTC CTG AAA      1256
Leu Ile Thr Asp Tyr His Glu Asn Gly Ser Leu Tyr Asp Phe Leu Lys
            310                 315                 320

TGT GCC ACA CTA GAC ACC AGA GCC CTA CTC AAG TTA GCT TAT TCT GCT      1304
Cys Ala Thr Leu Asp Thr Arg Ala Leu Leu Lys Leu Ala Tyr Ser Ala
        325                 330                 335

GCT TGT GGT CTG TGC CAC CTC CAC ACA GAA ATT TAT GGT ACC CAA GGG      1352
Ala Cys Gly Leu Cys His Leu His Thr Glu Ile Tyr Gly Thr Gln Gly
    340                 345                 350

AAG CCT GCA ATT GCT CAT CGA GAC CTG AAG AGC AAA AAC ATC CTT ATT      1400
Lys Pro Ala Ile Ala His Arg Asp Leu Lys Ser Lys Asn Ile Leu Ile
355                 360                 365                 370

AAG AAA AAT GGA AGT TGC TGT ATT GCT GAC CTG GGC CTA GCT GTT AAA      1448
Lys Lys Asn Gly Ser Cys Cys Ile Ala Asp Leu Gly Leu Ala Val Lys
                375                 380                 385

TTC AAC AGT GAT ACA AAT GAA GTT GAC ATA CCC TTG AAT ACC AGG GTG      1496
Phe Asn Ser Asp Thr Asn Glu Val Asp Ile Pro Leu Asn Thr Arg Val
            390                 395                 400

GGC ACC AAG CGG TAC ATG GCT CCA GAA GTG CTG GAT GAA AGC CTG AAT      1544
Gly Thr Lys Arg Tyr Met Ala Pro Glu Val Leu Asp Glu Ser Leu Asn
        405                 410                 415

AAA AAC CAT TTC CAG CCC TAC ATC ATG GCT GAC ATC TAT AGC TTT GGT      1592
Lys Asn His Phe Gln Pro Tyr Ile Met Ala Asp Ile Tyr Ser Phe Gly
    420                 425                 430

TTG ATC ATT TGG GAA ATG GCT CGT CGT TGT ATT ACA GGA GGA ATC GTG      1640
Leu Ile Ile Trp Glu Met Ala Arg Arg Cys Ile Thr Gly Gly Ile Val
435                 440                 445                 450

GAG GAA TAT CAA TTA CCA TAT TAC AAC ATG GTG CCC AGT GAC CCA TCC      1688
Glu Glu Tyr Gln Leu Pro Tyr Tyr Asn Met Val Pro Ser Asp Pro Ser
                455                 460                 465

TAT GAG GAC ATG CGT GAG GTT GTG TGT GTG AAA CGC TTG CGG CCA ATC      1736
Tyr Glu Asp Met Arg Glu Val Val Cys Val Lys Arg Leu Arg Pro Ile
            470                 475                 480

GTG TCT AAC CGC TGG AAC AGC GAT GAA GTA AGT TGG AGC CAA GTC CCT      1784
Val Ser Asn Arg Trp Asn Ser Asp Glu Val Ser Trp Ser Gln Val Pro
        485                 490                 495

GTA AAG TGATGAGTGA GTGCCGAGTT ACTCTGTGCT CACCACACTC TGTTTGCATT      1840
Val Lys
    500
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This Sheet Has Been Replaced



TATTTCTCTT TAGTGTCTTC GAGCAGTTTT GAAGCTAATG TCAGAATGTT GGGCCCATAA 1901

TCCAGCCTCC AGACTCACAG CTTTGAGAAT CAAGAAGACA CTTGCAAAAA TGGTTGAATC 1960

CCAGGATGTA AAGATTTGAC AATTAAACAA TTTTGAGGGA GAATTTAGAC TGCAAGAACT 2020

TCTTCACCCA AGGAAGGAAT TCCTGCAGGC CCGGGG 2056

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 500 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

```
Met Thr Gln Leu Tyr Thr Tyr Ile Arg Leu Leu Gly Ala Cys Leu Phe
 1               5                  10                  15

Ile Ile Ser His Val Gln Gly Gln Asn Leu Asp Ser Met Leu His Gly
            20                  25                  30

Thr Gly Met Lys Ser Asp Leu Asp Gln Lys Lys Pro Glu Asn Gly Val
        35                  40                  45

Thr Leu Ala Pro Glu Asp Thr Leu Pro Phe Leu Lys Cys Tyr Cys Ser
    50                  55                  60

Gly His Cys Pro Asp Asp Ala Ile Asn Asn Thr Cys Ile Thr Asn Gly
65                  70                  75                  80

His Cys Phe Ala Ile Ile Glu Glu Asp Asp Gln Gly Glu Thr Thr Leu
                85                  90                  95

Thr Ser Gly Cys Met Lys Tyr Glu Gly Ser Asp Phe Gln Cys Lys Asp
            100                 105                 110

Ser Pro Lys Ala Gln Leu Arg Arg Thr Ile Glu Cys Cys Arg Thr Asn
        115                 120                 125

Leu Cys Asn Gln Tyr Leu Gln Pro Thr Leu Pro Pro Val Val Ile Gly
    130                 135                 140

Pro Phe Phe Asp Gly Ser Ile Arg Trp Leu Val Val Leu Ile Ser Met
145                 150                 155                 160

Ala Val Cys Ile Val Ala Met Ile Ile Phe Ser Ser Cys Phe Cys Tyr
                165                 170                 175

Lys His Tyr Cys Lys Ser Ile Ser Ser Arg Gly Arg Tyr Asn Arg Asp
            180                 185                 190

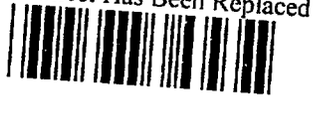
Leu Glu Gln Asp Glu Ala Phe Ile Pro Val Gly Glu Ser Leu Lys Asp
        195                 200                 205

Leu Ile Asp Gln Ser Gln Ser Ser Gly Ser Gly Ser Gly Leu Pro Leu
    210                 215                 220

Leu Val Gln Arg Thr Ile Ala Lys Gln Ile Gln Met Val Arg Gln Val
225                 230                 235                 240

Gly Lys Gly Arg Tyr Gly Glu Val Trp Met Gly Lys Trp Arg Gly Glu
                245                 250                 255
```

This Sheet Has Been Replaced



```
Lys Val Ala Val Lys Val Phe Phe Thr Thr Glu Glu Ala Ser Trp Phe
            260                 265                 270

Arg Glu Thr Glu Ile Tyr Gln Thr Val Leu Met Arg His Glu Asn Ile
        275                 280                 285

Leu Gly Phe Ile Ala Ala Asp Ile Lys Gly Thr Gly Ser Trp Thr Gln
    290                 295                 300

Leu Tyr Leu Ile Thr Asp Tyr His Glu Asn Gly Ser Leu Tyr Asp Phe
305                 310                 315                 320

Leu Lys Cys Ala Thr Leu Asp Thr Arg Ala Leu Leu Lys Leu Ala Tyr
                325                 330                 335

Ser Ala Ala Cys Gly Leu Cys His Leu His Thr Glu Ile Tyr Gly Thr
            340                 345                 350

Gln Gly Lys Pro Ala Ile Ala His Arg Asp Leu Lys Ser Lys Asn Ile
        355                 360                 365

Leu Ile Lys Lys Asn Gly Ser Cys Cys Ile Ala Asp Leu Gly Leu Ala
    370                 375                 380

Val Lys Phe Asn Ser Asp Thr Asn Glu Val Asp Ile Pro Leu Asn Thr
385                 390                 395                 400

Arg Val Gly Thr Lys Arg Tyr Met Ala Pro Glu Val Leu Asp Glu Ser
                405                 410                 415

Leu Asn Lys Asn His Phe Gln Pro Tyr Ile Met Ala Asp Ile Tyr Ser
            420                 425                 430

Phe Gly Leu Ile Ile Trp Glu Met Ala Arg Arg Cys Ile Thr Gly Gly
        435                 440                 445

Ile Val Glu Glu Tyr Gln Leu Pro Tyr Tyr Asn Met Val Pro Ser Asp
    450                 455                 460

Pro Ser Tyr Glu Asp Met Arg Glu Val Val Cys Val Lys Arg Leu Arg
465                 470                 475                 480

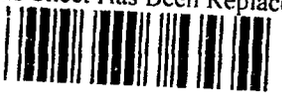
Pro Ile Val Ser Asn Arg Trp Asn Ser Asp Glu Val Ser Trp Ser Gln
                485                 490                 495

Val Pro Val Lys
            500
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2402 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

This Sheet Has Been Replaced



(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: join(11..1609)

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

GAATCAGACA ATG ACT CAG CTA TAC ACT TAC ATC AGA TTA CTG GGA GCC 49

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                Met Thr Gln Leu Tyr Thr Tyr Ile Arg Leu Leu Gly Ala
                 1               5                  10

TGT CTG TTC ATC ATT TCT CAT GTT CAA GGG CAG AAT CTA GAT AGT ATG          97
Cys Leu Phe Ile Ile Ser His Val Gln Gly Gln Asn Leu Asp Ser Met
     15                  20                  25

CTC CAT GGC ACT GGT ATG AAA TCA GAC TTG GAC CAG AAG AAG CCA GAA         145
Leu His Gly Thr Gly Met Lys Ser Asp Leu Asp Gln Lys Lys Pro Glu
 30                  35                  40                  45

AAT GGA GTG ACT TTA GCA CCA GAG GAT ACC TTG CCT TTC TTA AAG TGC         193
Asn Gly Val Thr Leu Ala Pro Glu Asp Thr Leu Pro Phe Leu Lys Cys
                 50                  55                  60

TAT TGC TCA GGA CAC TGC CCA GAT GAT GCT ATT AAT AAC ACA TGC ATA         241
Tyr Cys Ser Gly His Cys Pro Asp Asp Ala Ile Asn Asn Thr Cys Ile
             65                  70                  75

ACT AAT GGC CAT TGC TTT GCC ATT ATA GAA GAA GAT GAT CAG GGA GAA         289
Thr Asn Gly His Cys Phe Ala Ile Ile Glu Glu Asp Asp Gln Gly Glu
         80                  85                  90

ACC ACA TTA ACT TCT GGG TGT ATG AAG TAT GAA GGC TCT GAT TTT CAA         337
Thr Thr Leu Thr Ser Gly Cys Met Lys Tyr Glu Gly Ser Asp Phe Gln
     95                  100                 105

TGC AAG GAT TCA CCG AAA GCC CAG CTA CGC AGG ACA ATA GAA TGT TGT         385
Cys Lys Asp Ser Pro Lys Ala Gln Leu Arg Arg Thr Ile Glu Cys Cys
110                 115                 120                 125

CGG ACC AAT TTG TGC AAC CAG TAT TTG CAG CCT ACA CTG CCC CCT GTT         433
Arg Thr Asn Leu Cys Asn Gln Tyr Leu Gln Pro Thr Leu Pro Pro Val
                130                 135                 140

GTT ATA GGT CCG TTC TTT GAT GGC AGC ATC CGA TGG CTG GTT GTG CTC         481
Val Ile Gly Pro Phe Phe Asp Gly Ser Ile Arg Trp Leu Val Val Leu
            145                 150                 155

ATT TCC ATG GCT GTC TGT ATA GTT GCT ATG ATC ATC TTC TCC AGC TGC         529
Ile Ser Met Ala Val Cys Ile Val Ala Met Ile Ile Phe Ser Ser Cys
        160                 165                 170

TTT TGC TAT AAG CAT TAT TGT AAG AGT ATC TCA AGC AGG GGT CGT TAC         577
Phe Cys Tyr Lys His Tyr Cys Lys Ser Ile Ser Ser Arg Gly Arg Tyr
    175                 180                 185

AAC CGT GAT TTG GAA CAG GAT GAA GCA TTT ATT CCA GTA GGA GAA TCA         625
Asn Arg Asp Leu Glu Gln Asp Glu Ala Phe Ile Pro Val Gly Glu Ser
190                 195                 200                 205

TTG AAA GAC CTG ATT GAC CAG TCC CAA AGC TCT GGG AGT GGA TCT GGA         673
Leu Lys Asp Leu Ile Asp Gln Ser Gln Ser Ser Gly Ser Gly Ser Gly
                210                 215                 220

TTG CCT TTA TTG GTT CAG CGA ACT ATT GCC AAA CAG ATT CAG ATG GTT         721
Leu Pro Leu Leu Val Gln Arg Thr Ile Ala Lys Gln Ile Gln Met Val
            225                 230                 235

CGG CAG GTT GGT AAA GGC CGC TAT GGA GAA GTA TGG ATG GGT AAA TGG         769
Arg Gln Val Gly Lys Gly Arg Tyr Gly Glu Val Trp Met Gly Lys Trp
        240                 245                 250

CGT GGT GAA AAA GTG GCT GTC AAA GTG TTT TTT ACC ACT GAA GAA GCT         817
Arg Gly Glu Lys Val Ala Val Lys Val Phe Phe Thr Thr Glu Glu Ala
    255                 260                 265
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This Sheet Has Been Replaced



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AGC TGG TTT AGA GAA ACA GAA ATC TAC CAG ACG GTG TTA ATG CGT CAT        865
Ser Trp Phe Arg Glu Thr Glu Ile Tyr Gln Thr Val Leu Met Arg His
270                 275                 280                 285

GAA AAT ATA CTT GGT TTT ATA GCT GCA GAC ATT AAA GGC ACT GGT TCC        913
Glu Asn Ile Leu Gly Phe Ile Ala Ala Asp Ile Lys Gly Thr Gly Ser
                290                 295                 300

TGG ACT CAG CTG TAT TTG ATT ACT GAT TAC CAT GAA AAT GGA TCT CTC        961
Trp Thr Gln Leu Tyr Leu Ile Thr Asp Tyr His Glu Asn Gly Ser Leu
            305                 310                 315

TAT GAC TTC CTG AAA TGT GCC ACA CTA GAC ACC AGA GCC CTA CTC AAG       1009
Tyr Asp Phe Leu Lys Cys Ala Thr Leu Asp Thr Arg Ala Leu Leu Lys
        320                 325                 330

TTA GCT TAT TCT GCT GCT TGT GGT CTG TGC CAC CTC CAC ACA GAA ATT       1057
Leu Ala Tyr Ser Ala Ala Cys Gly Leu Cys His Leu His Thr Glu Ile
    335                 340                 345

TAT GGT ACC CAA GGG AAG CCT GCA ATT GCT CAT CGA GAC CTG AAG AGC       1105
Tyr Gly Thr Gln Gly Lys Pro Ala Ile Ala His Arg Asp Leu Lys Ser
350                 355                 360                 365

AAA AAC ATC CTT ATT AAG AAA AAT GGA AGT TGC TGT ATT GCT GAC CTG       1153
Lys Asn Ile Leu Ile Lys Lys Asn Gly Ser Cys Cys Ile Ala Asp Leu
                370                 375                 380

GGC CTA GCT GTT AAA TTC AAC AGT GAT ACA AAT GAA GTT GAC ATA CCC       1201
Gly Leu Ala Val Lys Phe Asn Ser Asp Thr Asn Glu Val Asp Ile Pro
            385                 390                 395

TTG AAT ACC AGG GTG GGC ACC AAG CGG TAC ATG GCT CCA GAA GTG CTG       1249
Leu Asn Thr Arg Val Gly Thr Lys Arg Tyr Met Ala Pro Glu Val Leu
        400                 405                 410

GAT GAA AGC CTG AAT AAA AAC CAT TTC CAG CCC TAC ATC ATG GCT GAC       1297
Asp Glu Ser Leu Asn Lys Asn His Phe Gln Pro Tyr Ile Met Ala Asp
    415                 420                 425

ATC TAT AGC TTT GGT TTG ATC ATT TGG GAA ATG GCT CGT CGT TGT ATT       1345
Ile Tyr Ser Phe Gly Leu Ile Ile Trp Glu Met Ala Arg Arg Cys Ile
430                 435                 440                 445

ACA GGA GGA ATC GTG GAG GAA TAT CAA TTA CCA TAT TAC AAC ATG GTG       1393
Thr Gly Gly Ile Val Glu Glu Tyr Gln Leu Pro Tyr Tyr Asn Met Val
                450                 455                 460

CCC AGT GAC CCA TCC TAT GAG GAC ATG CGT GAG GTT GTG TGT GTG AAA       1441
Pro Ser Asp Pro Ser Tyr Glu Asp Met Arg Glu Val Val Cys Val Lys
            465                 470                 475

CGC TTG CGG CCA ATC GTG TCT AAC CGC TGG AAC AGC GAT GAA TGT CTT       1489
Arg Leu Arg Pro Ile Val Ser Asn Arg Trp Asn Ser Asp Glu Cys Leu
        480                 485                 490

CGA GCA GTT TTG AAG CTA ATG TCA GAA TGT TGG GCC CAT AAT CCA GCC       1537
Arg Ala Val Leu Lys Leu Met Ser Glu Cys Trp Ala His Asn Pro Ala
    495                 500                 505

TCC AGA CTC ACA GCT TTG AGA ATC AAG AAG ACA CTT GCA AAA ATG GTT       1585
Ser Arg Leu Thr Ala Leu Arg Ile Lys Lys Thr Leu Ala Lys Met Val
510                 515                 520                 525

GAA TCC CAG GAT GTA AAG ATT TGACAATTAA ACAATTTTGA GGGAGAATTT          1636
Glu Ser Gln Asp Val Lys Ile
                530
```

This Sheet Has Been Replaced



AGACTGCAAG AACTTCTTCA CCCAAGGAAT GGGTGGGATT AGCATGGAAT AGGATGTTGA 1696

CTTGGTTTCC AGACTCCTTC CTCTACATCT TCACAGGCTG CTAACAGTAA ACCTTACCGC 1756

ACTCTACAGA ATACAAGATT GGAACTTGGA ACTTGGAACT TCAAACATGT CATTCTTTAT 1816

ATATGGACAG CTGTGTTTTA AATGTGGGGT TTTTGTGTTT TGCTTTCTTT GTTTTGTTTT 1876

GGTTTTGATG CTTTTTTGGT TTTTATGAAC TGCATCAAGA CTCCAATCCT GATAAGAAGT 1936

CTCTGGTCAA CCTCTGGGTA CTCACTATCC TGTCCATAAA GTGGTGCTTT CTGTGAAAGC 1996

CTTAAGAAAA TTAATGAGCT CAGCAGAGAT GGAAAAAGGC ATATTTGGCT TCTACCAGAG 2056

AAAACATCTG TCTGTGTTCT GTCTTTGTAA ACAGCCTATA GATTATGATC TCTTTGGGAT 2116

ACTGCCTGGC TTATGATGGT GCACCATACC TTTGATATAC ATACCAGAAT TCTCTCCTGC 2176

CCTAGGGCTA AGAAGACAAG AATGTAGAGG TTGCACAGGA GGTATTTTGT GACCAGTGGT 2236

TTAAATTGCA ATATCTAGTT GGCAATCGCC AATTTCATAA AAGCCATCCA CCTTGTAGCT 2296

GTAGTAACTT CTCCACTGAC TTTATTTTTA GCATAATAGT TGTGAAGGCC AAACTCCATG 2356

TAAAGTGTCC ATAGACTTGG ACTGTTTTCC CCCAGCTCTG ATTACC 2402

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 532 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

```
Met Thr Gln Leu Tyr Thr Tyr Ile Arg Leu Leu Gly Ala Cys Leu Phe
 1               5                  10                  15

Ile Ile Ser His Val Gln Gly Gln Asn Leu Asp Ser Met Leu His Gly
            20                  25                  30

Thr Gly Met Lys Ser Asp Leu Asp Gln Lys Lys Pro Glu Asn Gly Val
        35                  40                  45

Thr Leu Ala Pro Glu Asp Thr Leu Pro Phe Leu Lys Cys Tyr Cys Ser
    50                  55                  60

Gly His Cys Pro Asp Asp Ala Ile Asn Asn Thr Cys Ile Thr Asn Gly
65                  70                  75                  80

His Cys Phe Ala Ile Ile Glu Glu Asp Asp Gln Gly Glu Thr Thr Leu
                85                  90                  95

Thr Ser Gly Cys Met Lys Tyr Glu Gly Ser Asp Phe Gln Cys Lys Asp
            100                 105                 110

Ser Pro Lys Ala Gln Leu Arg Arg Thr Ile Glu Cys Cys Arg Thr Asn
        115                 120                 125

Leu Cys Asn Gln Tyr Leu Gln Pro Thr Leu Pro Pro Val Val Ile Gly
    130                 135                 140

Pro Phe Phe Asp Gly Ser Ile Arg Trp Leu Val Val Leu Ile Ser Met
```

This Sheet Has Been Replaced



145 150 155 160

Ala Val Cys Ile Val Ala Met Ile Ile Phe Ser Ser Cys Phe Cys Tyr
165 170 175

Lys His Tyr Cys Lys Ser Ile Ser Ser Arg Gly Arg Tyr Asn Arg Asp
180 185 190

Leu Glu Gln Asp Glu Ala Phe Ile Pro Val Gly Glu Ser Leu Lys Asp
195 200 205

Leu Ile Asp Gln Ser Gln Ser Ser Gly Ser Gly Ser Gly Leu Pro Leu
210 215 220

Leu Val Gln Arg Thr Ile Ala Lys Gln Ile Gln Met Val Arg Gln Val
225 230 235 240

Gly Lys Gly Arg Tyr Gly Glu Val Trp Met Gly Lys Trp Arg Gly Glu
245 250 255

Lys Val Ala Val Lys Val Phe Phe Thr Thr Glu Glu Ala Ser Trp Phe
260 265 270

Arg Glu Thr Glu Ile Tyr Gln Thr Val Leu Met Arg His Glu Asn Ile
275 280 285

Leu Gly Phe Ile Ala Ala Asp Ile Lys Gly Thr Gly Ser Trp Thr Gln
290 295 300

Leu Tyr Leu Ile Thr Asp Tyr His Glu Asn Gly Ser Leu Tyr Asp Phe
305 310 315 320

Leu Lys Cys Ala Thr Leu Asp Thr Arg Ala Leu Leu Lys Leu Ala Tyr
325 330 335

Ser Ala Ala Cys Gly Leu Cys His Leu His Thr Glu Ile Tyr Gly Thr
340 345 350

Gln Gly Lys Pro Ala Ile Ala His Arg Asp Leu Lys Ser Lys Asn Ile
355 360 365

Leu Ile Lys Lys Asn Gly Ser Cys Cys Ile Ala Asp Leu Gly Leu Ala
370 375 380

Val Lys Phe Asn Ser Asp Thr Asn Glu Val Asp Ile Pro Leu Asn Thr
385 390 395 400

This Sheet Has Been Replaced



```
Arg Val Gly Thr Lys Arg Tyr Met Ala Pro Glu Val Leu Asp Glu Ser
                405                 410                 415

Leu Asn Lys Asn His Phe Gln Pro Tyr Ile Met Ala Asp Ile Tyr Ser
            420                 425                 430

Phe Gly Leu Ile Ile Trp Glu Met Ala Arg Arg Cys Ile Thr Gly Gly
        435                 440                 445

Ile Val Glu Glu Tyr Gln Leu Pro Tyr Tyr Asn Met Val Pro Ser Asp
    450                 455                 460

Pro Ser Tyr Glu Asp Met Arg Glu Val Val Cys Val Lys Arg Leu Arg
465                 470                 475                 480

Pro Ile Val Ser Asn Arg Trp Asn Ser Asp Glu Cys Leu Arg Ala Val
                485                 490                 495

Leu Lys Leu Met Ser Glu Cys Trp Ala His Asn Pro Ala Ser Arg Leu
            500                 505                 510

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

Asp Val Lys Ile
    530
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 466 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 11..466

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

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GAATCAGACA ATG ACT CAG CTA TAC ACT TAC ATC AGA TTA CTG GGA GCC      49
           Met Thr Gln Leu Tyr Thr Tyr Ile Arg Leu Leu Gly Ala
             1               5                  10

TGT CTG TTC ATC ATT TCT CAT GTT CAA GGG CAG AAT CTA GAT AGT ATG      97
Cys Leu Phe Ile Ile Ser His Val Gln Gly Gln Asn Leu Asp Ser Met
     15                  20                  25

CTC CAT GGC ACT GGT ATG AAA TCA GAC TTG GAC CAG AAG AAG CCA GAA     145
Leu His Gly Thr Gly Met Lys Ser Asp Leu Asp Gln Lys Lys Pro Glu
 30                  35                  40                  45

AAT GGA GTG ACT TTA GCA CCA GAG GAT ACC TTG CCT TTC TTA AAG TGC     193
Asn Gly Val Thr Leu Ala Pro Glu Asp Thr Leu Pro Phe Leu Lys Cys
                 50                  55                  60

TAT TGC TCA GGA CAC TGC CCA GAT GAT GCT ATT AAT AAC ACA TGC ATA     241
Tyr Cys Ser Gly His Cys Pro Asp Asp Ala Ile Asn Asn Thr Cys Ile
             65                  70                  75

ACT AAT GGC CAT TGC TTT GCC ATT ATA GAA GAA GAT GAT CAG GGA GAA     289
Thr Asn Gly His Cys Phe Ala Ile Ile Glu Glu Asp Asp Gln Gly Glu
         80                  85                  90
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ACC ACA TTA ACT TCT GGG TGT ATG AAG TAT GAA GGC TCT GAT TTT CAA      337
Thr Thr Leu Thr Ser Gly Cys Met Lys Tyr Glu Gly Ser Asp Phe Gln
     95                  100                 105

TGC AAG GAT TCA CCG AAA GCC CAG CTA CGC AGG ACA ATA GAA TGT TGT      385
Cys Lys Asp Ser Pro Lys Ala Gln Leu Arg Arg Thr Ile Glu Cys Cys
110                 115                 120                 125

CGG ACC AAT TTG TGC AAC CAG TAT TTG CAG CCT ACA CTG CCC CCT GTT      433
Arg Thr Asn Leu Cys Asn Gln Tyr Leu Gln Pro Thr Leu Pro Pro Val
                130                 135                 140

GTT ATA GGT CCG TTC TTT GAT GGC AGC ATC CGA                          466
Val Ile Gly Pro Phe Phe Asp Gly Ser Ile Arg
            145                 150
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 152 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

```
Met Thr Gln Leu Tyr Thr Tyr Ile Arg Leu Leu Gly Ala Cys Leu Phe
 1               5                  10                  15

Ile Ile Ser His Val Gln Gly Gln Asn Leu Asp Ser Met Leu His Gly
            20                  25                  30

Thr Gly Met Lys Ser Asp Leu Asp Gln Lys Lys Pro Glu Asn Gly Val
        35                  40                  45

Thr Leu Ala Pro Glu Asp Thr Leu Pro Phe Leu Lys Cys Tyr Cys Ser
    50                  55                  60

Gly His Cys Pro Asp Asp Ala Ile Asn Asn Thr Cys Ile Thr Asn Gly
65                  70                  75                  80

His Cys Phe Ala Ile Ile Glu Glu Asp Asp Gln Gly Glu Thr Thr Leu
                85                  90                  95

Thr Ser Gly Cys Met Lys Tyr Glu Gly Ser Asp Phe Gln Cys Lys Asp
            100                 105                 110

Ser Pro Lys Ala Gln Leu Arg Arg Thr Ile Glu Cys Cys Arg Thr Asn
        115                 120                 125

Leu Cys Asn Gln Tyr Leu Gln Pro Thr Leu Pro Pro Val Val Ile Gly
    130                 135                 140

Pro Phe Phe Asp Gly Ser Ile Arg
145                 150
```

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WHAT IS CLAIMED IS:

1. An isolated BMP receptor kinase protein having amino acid sequence SEQ ID NO:4, or soluble fragment thereof.

2. An isolated DNA sequence coding for the BMP receptor kinase protein of Claim 1.

3. The DNA sequence of Claim 2, wherein the DNA sequence is SEQ ID NO:3.

4. An isolated truncated BMP receptor kinase protein having amino acid sequence SEQ ID NO:2, or soluble fragment thereof.

5. An isolated DNA sequence coding for the truncated BMP receptor kinase protein of Claim 4.

6. The DNA sequence of Claim 5, wherein the DNA sequence is SEQ ID NO:1

7. The soluble fragment of Claim 4, wherein the soluble fragment has amino acid sequence SEQ ID NO:6

8. A DNA sequence coding for the soluble fragment of Claim 7.

9. The DNA sequence of Claim 8, wherein the DNA sequence is SEQ ID NO:5.

10. A recombinant expression vector comprising the DNA sequence of Claim 2.

11. A recombinant expression vector comprising the DNA sequence of Claim 3.

12. The recombinant expression vector of Claim 11, wherein the vector is a plasmid having all of the identifying characteristics of pJT4-J159F contained in ATCC No. 69457.

13. A recombinant expression vector comprising the DNA sequence of Claim 5.

14. A recombinant expression vector comprising the DNA sequence of Claim 6.

15. The recombinant expression vector of Claim 14, wherein the vector is a plasmid having all of the identifying characteristics of pJT6-J159T contained in ATCC No. 69474.

16. A host cell comprising the recombinant expression vector of Claim 10.

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17. A host cell comprising the recombinant expression vector of Claim 11.

18. A mammalian host cell comprising the recombinant expression vector of Claim 12.

19. The mammalian host cell of Claim 18, wherein the cell is a Chinese hamster ovary cell.

20. The mammalian host cell of Claim 18, wherein the cell is a COS cell.

21. A host cell comprising the recombinant expression vector of Claim 13.

22. A host cell comprising the recombinant expression vector of Claim 14.

23. A mammalian host cell comprising the recombinant expresstion vector of Claim 15.

24. The mammalian host cell of Claim 22, wherein the cell is a Chinese hamster ovary cell.

25. The mammalian host cell of Claim 22, wherein the cell is a COS cell.

26. A method for producing BMP receptor kinase protein comprising culturing the host cell of Claim 16 in a manner allowing expression of the BMP receptor kinase protein and isolation of the BMP receptor kinase protein.

27. A method for producing truncated BMP receptor kinase protein comprising culturing the host cell of Claim 17 in a manner allowing expression of the truncated BMP receptor kinase protein and isolation of the BMP receptor kinase protein.

28. A method for identifying compounds capable of binding to a BMP receptor kinase protein, the method comprising introducing a sample comprising the compounds to the BMP receptor kinase protein and allowing the compounds to bind to the BMP receptor kinase protein, wherein the BMP receptor kinase protein has amino acid sequence SEQ ID NO:4 or soluble fragment thereof, or SEQ ID NO:2 or soluble fragment thereof.

29. An antibody directed to the BMP receptor kinase protein of Claim 1.

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