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(54) Titre : SEQUENCE D'ADN CODANT POUR UN RECEPTEUR DE LA PROTEINE MORPHOGENETIQUE OSSEUSE
 (54) Title: DNA SEQUENCE CODING FOR A BMP RECEPTOR

(57) **Abrégé/Abstract:**

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 BMP receptor kinase protein or soluble fragment thereof, a method for determining the amount of such compounds in a sample, and antibodies to said BMP receptor kinase protein.



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(21) International Application Number: PCT/US94/13534 (22) International Filing Date: 23 November 1994 (23.11.94) (30) Priority Data: 08/158,735 24 November 1993 (24.11.93) US (71) Applicant: THE PROCTER & GAMBLE COMPANY [US/US]; One Procter & Gamble Plaza, Cincinnati, OH 45202 (US). (72) Inventors: COOK, Jonathan, Shaun; 9415 Waxwing Drive, Cincinnati, OH 45241 (US). CORREA, Paul, Elliott; 4245 Miami Trails Lane, Cincinnati, OH 45252 (US). KOENIG, Beth, Broe; 6832 Edmar Court, Cincinnati, OH 45239 (US). ROSENBAUM, Jan, Susan; 7781 Bennington Drive, Cincinnati, OH 45241 (US). TING, Jerry; 7249 Rolling Meadows Drive, West Chester, OH 45069 (US). (74) Agents: REED, T., David, et al.; The Procter & Gamble Company, 5299 Spring Grove Avenue, Cincinnati, OH 45217 (US).	<div style="text-align: right; font-size: 2em; margin-bottom: 10px;">2173102</div> (81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, JP, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, NO, NZ, PL, RO, RU, SI, SK, TJ, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 27 July 1995 (27.07.95) <div style="text-align: right; font-size: 2em; margin-top: 20px;">2173102</div>	
(54) Title: DNA SEQUENCE CODING FOR A BMP RECEPTOR (57) Abstract 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 BMP receptor kinase protein or soluble fragment thereof, a method for determining the amount of such compounds in a sample, and antibodies to said BMP receptor kinase protein.		

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

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.

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- β 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- β

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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 [¹²⁵I]-BMP-4 to COS-7 cells transfected with the cDNA for BRK-1, using the construct pJT4-J159F. The concentration of [¹²⁵I]-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 [¹²⁵I]-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 [¹²⁵I]-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 [¹²⁵I]-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 [¹²⁵I]-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.

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 [¹²⁵I]-BMP-4 with nanomolar or subnanomolar affinity (K_D approximately equal to $10^{-9}M$). Preferably, the affinity is from about $1 \times 10^{-10}M$ to about $1 \times 10^{-9}M$, more preferably about $5 \times 10^{-10}M$, 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

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.

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

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

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

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

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

from Stratagene, La Jolla, CA; this kit contains components necessary for generating cDNA from RNA, including reverse transcriptase from Moloney 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 μ l final volume using the "GENE-AMP™" kit (Perkin-Elmer, Norwalk, CT; a kit containing components necessary for amplification of DNA using the polymerase 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 μ l 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 a α -³²P)-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 Na₂HPO₄, and 1 mM EDTA (ethylenediaminetetraacetic acid)), 1X Denhardt's (0.02% bovine serum

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-β 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

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 ³²P 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

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

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- β -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 "FRENCHTM" 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 DeltaPakTM C4 column, 300 Å, 7.8mm x 30cm, 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 hemocyanin, 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.

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 EcoRV 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™"

electroporation system (BioRad, Hercules, CA). The purified DNA plasmid (10 μ g) is added to the cuvette, and the cells subjected to electroporation at 4.0 kV/cm, with a capacitance of 25 μ Fd. 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 μ g) is taken up in 5 μ l of 30% acetonitrile, 0.1% trifluoroacetic acid (TFA) plus an additional 5 μ l of 1.5 M sodium phosphate, pH 7.4. Carrier free [125 I] (1 mCi, 4-10 μ l) is added, together with 2 μ l of a chloramine T solution (100 μ g/ml). An additional 2 μ l 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 μ l of 50 mM N-acetyl tyrosine, 100 μ l of 60 mM potassium iodide, and 100 μ l of 11M urea in 1 M acetic acid. After a 3.5 minute incubation, unreacted iodine is removed on a PD-10TM 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.

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 μ g) is solubilized in 500 μ l 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 μ m, 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.

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 MgSO₄, 1.2 mM CaCl₂, 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 [¹²⁵I]-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 MgSO₄, 1.2 mM CaCl₂, 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 [¹²⁵I]-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 quantitative 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 [¹²⁵I]-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 5×10^{-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

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 [¹²⁵I]-BMP-4 or [¹²⁵I]-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% β-mercaptoethanol, 2% SDS, 0.1% bromophenol blue, 10% glycerol), vortexed for 30-45 min at 4°C, boiled for 5 minutes, and centrifuged (13,000 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 [¹²⁵I]-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.

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 [¹²⁵I]-DRBMP-2 as the ligand. A similar labeled band is observed. As with [¹²⁵I]-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 [¹²⁵I]-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 1×10^7 cell/dish. They are then crosslinked to [¹²⁵I]-BMP-4 as described in this example, except that the incubation with [¹²⁵I]-BMP-4 and unlabeled ligand is carried out in a total of 4 ml, instead of 500 μ l, 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 Na₂EDTA, 0.5% Nonidet P-40TM, 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 μ l of "PANSORBINTM" (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 μ l 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 BeckmanTM 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 μ l of reducing sample buffer. Solubilized proteins are subjected to electrophoresis in SDS polyacrylamide gels and autoradiography as

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.

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.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Cook, Jonathan S.
Correa, Paul E.
Koenig, Beth B.
Rosenbaum, Jan S.
Ting, Jerry
- (ii) TITLE OF INVENTION: Isolation of Growth Factor Receptor cDNA
pJ159
- (iii) NUMBER OF SEQUENCES: 6
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: The Procter & Gamble Company
 - (B) STREET: 11810 East Miami River Road
 - (C) CITY: Cincinnati
 - (D) STATE: Ohio
 - (E) COUNTRY: U.S.A.
 - (F) ZIP: 45239-8707
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Corstanje, Brahm J.
 - (B) REGISTRATION NUMBER: 34,804
 - (C) REFERENCE/DOCKET NUMBER: 5088
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 - (A) TELEPHONE: (513) 627-2858
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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 CGGCCACCC CCACGCCCCG CCCGGGAGGG      60
ACGGGGGGGAG AGAGAGCGCG GCGACGGGTA TCTGGGTCAA AGCTGTTTCGG AGAAATTGGA      120
ACTACAGTTT TATCTAGCCA CATCTCTGAG AATTCTGAAG AAAGCAGCAG GTGAAAGTCA      180

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TTGCCAAGTG	ATTTTGTCT	GTAAGGAAGC	CTCCCTCATT	CACTTACACC	AGTGAGACAG												240
CAGGACCAGT	CATTCAAAGG	GCCGTGTACA	GGACGCGTGC	GAATCAGACA	ATG	ACT											296
						Met	Thr										
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

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	

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

(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

- (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

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					

AGACTGCAAG AACTTCTTCA CCCAAGGAAT GGGTGGGATT AGCATGGAAT AGGATGTTGA 1696
 CTTGGTTTCC AGACTCCTTC CTCTACATCT TCACAGGCTG CTAACAGTAA ACCTTACCGC 1756
 ACTCTACAGA ATACAAGATT GGAAGTTGGA 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 AATTTTCATAA AAGCCATCCA CCTTGTAGCT 2296
 GTAGTAACTT CTCCACTGAC TTTATTTTTA GCATAATAGT TGTGAAGGCC AACTCCATG 2356
 TAAAGTGTC 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

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

34

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:

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			

CLAIMS

1. An isolated DNA sequence coding for an isolated BMP receptor kinase protein having an amino acid sequence SEQ ID NO: 4 or soluble fragment having amino acid sequence SEQ ID NO: 6.
2. The DNA sequence of Claim 1, wherein the DNA sequence is SEQ ID NO: 3.
3. An isolated truncated BMP receptor kinase protein having amino acid sequence SEQ ID NO: 2 or soluble fragment having amino acid sequence SEQ ID NO: 6..
4. An isolated DNA sequence coding for the truncated BMP receptor kinase protein of Claim 3.
5. The DNA sequence of Claim 4, wherein the DNA sequence is SEQ ID NO: 1.
6. A DNA sequence coding for the soluble fragment of Claim 3.
7. The DNA sequence of Claim 6, wherein the DNA sequence is SEQ ID NO: 5.
8. A recombinant expression vector comprising the DNA sequence of Claim 1.
9. A recombinant expression vector comprising the DNA sequence of Claim 2.
10. The recombinant expression vector of Claim 9, wherein the vector is a plasmid having all of the identifying characteristics of pJT4-J159F contained in ATCC No. 69457.
11. A recombinant expression vector comprising the DNA sequence of Claim 4.

12. A recombinant expression vector comprising the DNA sequence of Claim 5.
13. The recombinant expression vector of Claim 12, wherein the vector is a plasmid having all of the identifying characteristics of pJT6-J159T contained in ATCC No. 69474.
14. A host cell comprising the recombinant expression vector of Claim 8.
15. A host cell comprising the recombinant expression vector of Claim 9.
16. A mammalian host cell comprising the recombinant expression vector of Claim 10.
17. The mammalian host cell of Claim 16, wherein the cell is a Chinese hamster ovary cell.
18. The mammalian host cell of Claim 16, wherein the cell is a COS cell.
19. A host cell comprising the recombinant expression vector of Claim 11.
20. A host cell comprising the recombinant expression vector of Claim 12.
21. A mammalian host cell comprising the recombinant expression vector of Claim 13.
22. The mammalian host cell of Claim 21, wherein the cell is a Chinese hamster ovary cell.
23. The mammalian host cell of Claim 21, wherein the cell is a COS cell.
24. A method for producing BMP receptor kinase protein comprising culturing the host cell of Claim 14 in a manner allowing expression of the BMP receptor kinase protein and isolation of the BMP receptor kinase protein.

25. A method for producing truncated BMP receptor kinase protein comprising culturing the host cell of Claim 20 in a manner allowing expression of the truncated BMP receptor kinase protein and isolation of the BMP receptor kinase protein.
26. 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, wherein the BMP receptor kinase protein has an amino acid sequence SEQ ID NO: 2 or soluble fragment having amino acid sequence SEQ ID NO:6.

Fig. 1

PRIMERS ACT 1A & ACT 1B:

Antisense primers derived from sequence in Kinase Domain VIB:

	A	A	TA	A	T
Act1A:	5'	-GCG	GAA	TTC	TT
		AT	TCI	C	TG
		G	G	G	CTI
					A
					I
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					C
					GG
					G
					GG
					T
					CTI
					A
					I
					G
					C
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DAF1	QIRLTGRVSGRFGNVSRGDYRG	EAVAVKVFNALDEPAFHKETEIEFETRMRLRHPNVLRYIGSDRV	65
MACT	PLQLLEVKARGRFGCVWKAQLLN	EYVAVKIFPIQDKQSWQNEYEVYSLPGMKHENILQFIGAEKR	65
RTGFBR2	PIELDTLVGKGRFAEVYKAKLKQNTSEQFETVAVKIFPYEYSSWKTEKDFSDINLKHENILQFLTAEER		71
MACTR1	QITLLECVGKGRYGEVWRGSWQ	ENVAVKIFSSRDEKSWFRETELYNTVMLRHENILGFIASDMT	65
R3	QVALVECVGKGRYGEVWRGSWHG	ESVAVKIFSSRDEQSWFRETEIYNTVLLRHDNILGFIASDMT	65
R2	TIVLQEIIGKGRFGEVWRGRWRG	GDVAVKIFSSREERSWFREAEIYQTVMLRHENILGFIAADNK	65
R4	TIVI.QESIGKGRFGEVWRGKWRG	EEVAVKIFSSREERSWFREAEIYQTVMLRHENILGFIAADNK	65
BRK-1	QIQMVRQVGKGRYGEVWMGKWRG	EKVAVKVFVFTTEEASWFRETEIYQTVLMRHENILGFIAADIK	65
T-BRK-1	QIQMVRQVGKGRYGEVWMGKWRG	EKVAVKVFVFTTEEASWFRETEIYQTVLMRHENILGFIAADIK	65

Fig. 2A

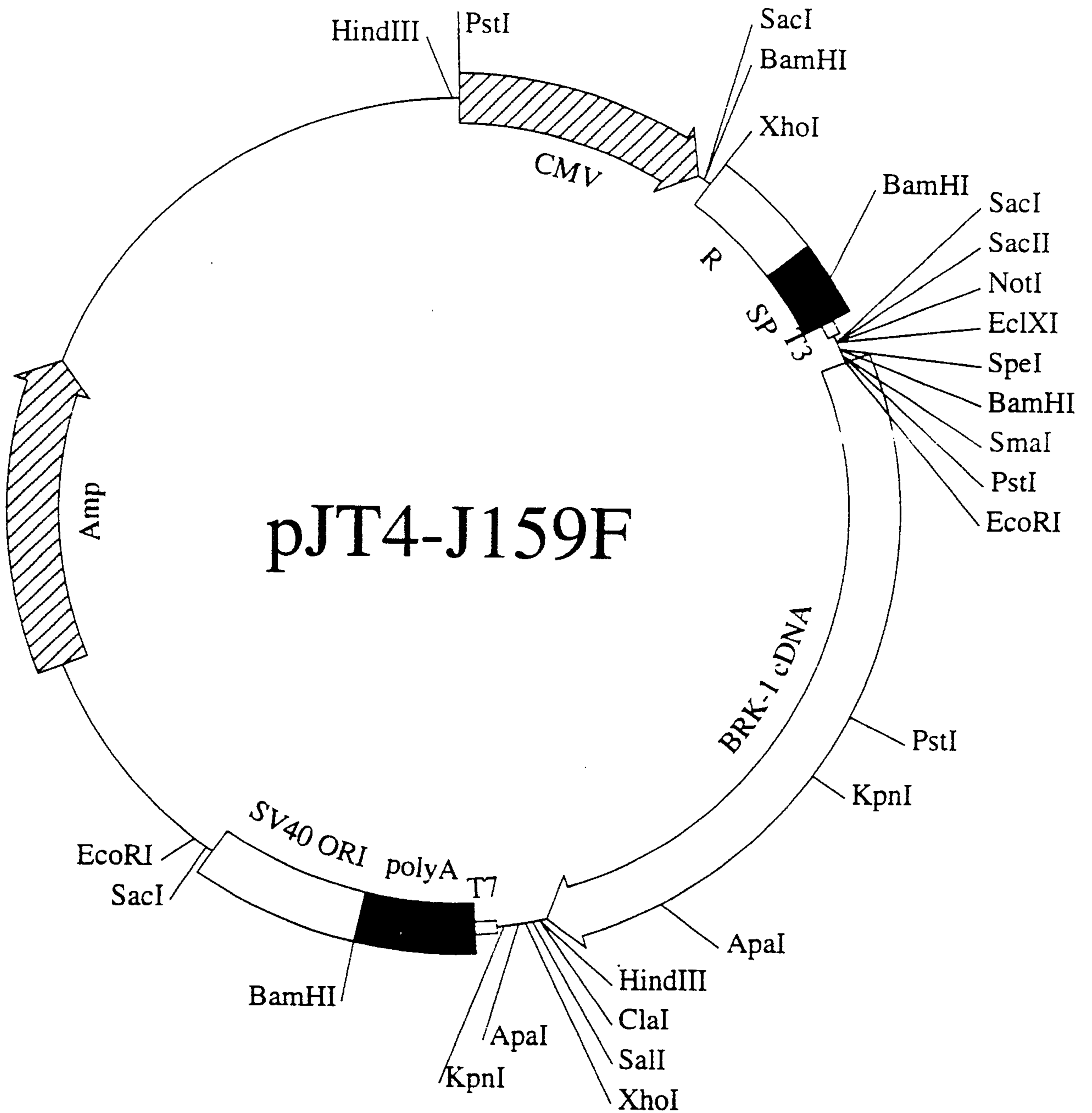


Fig. 3

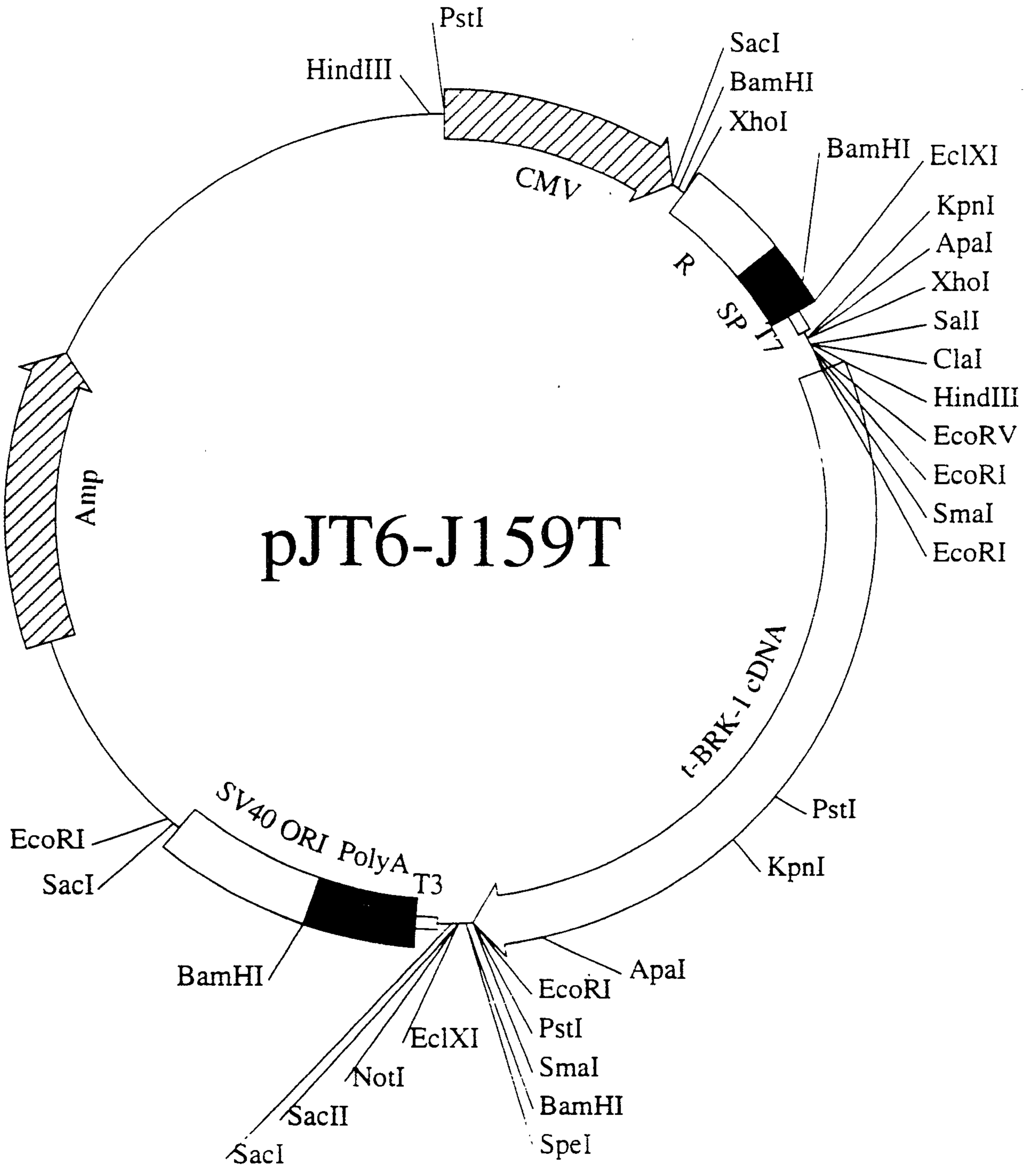


Fig. 4

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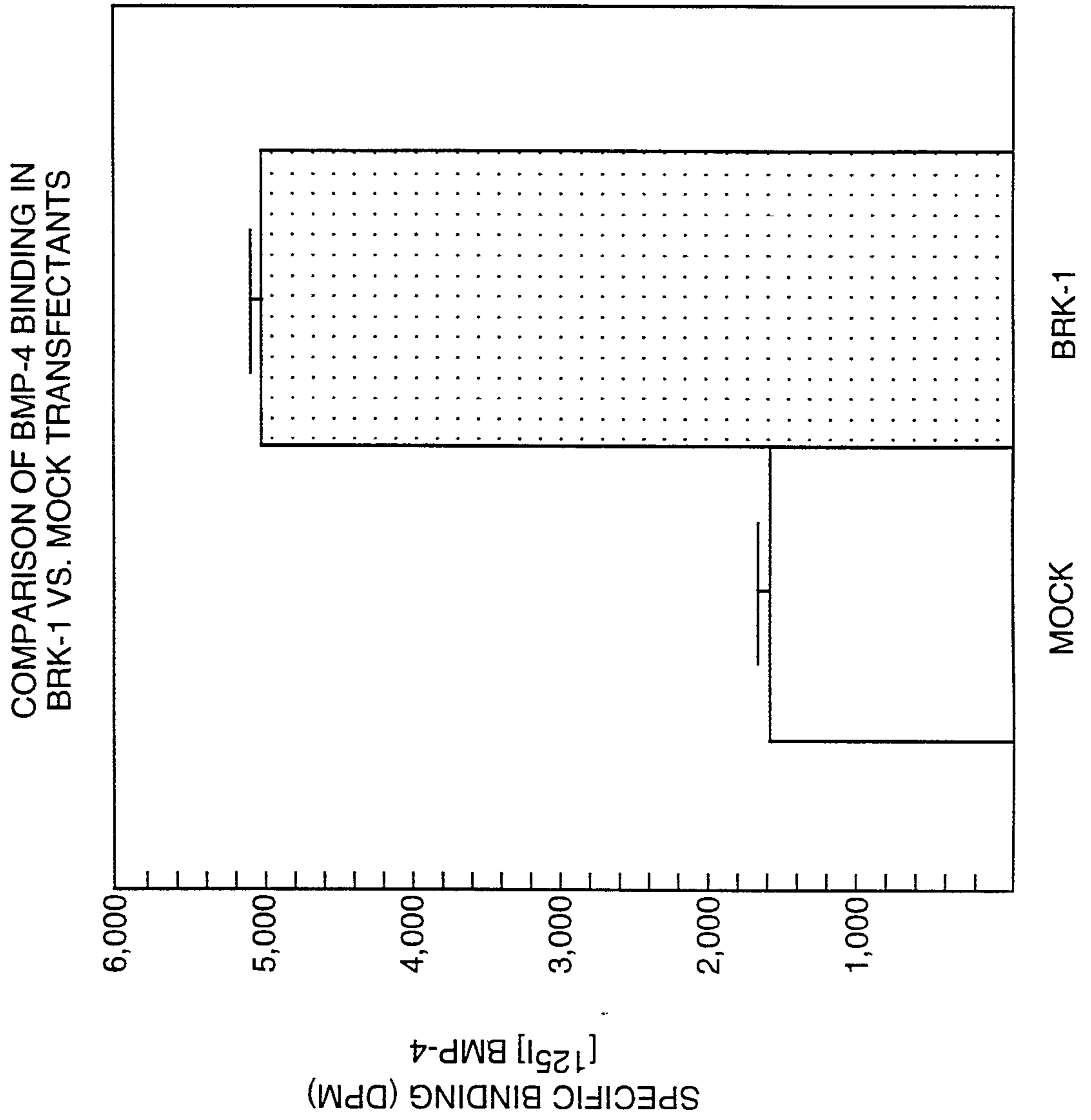


Fig. 5

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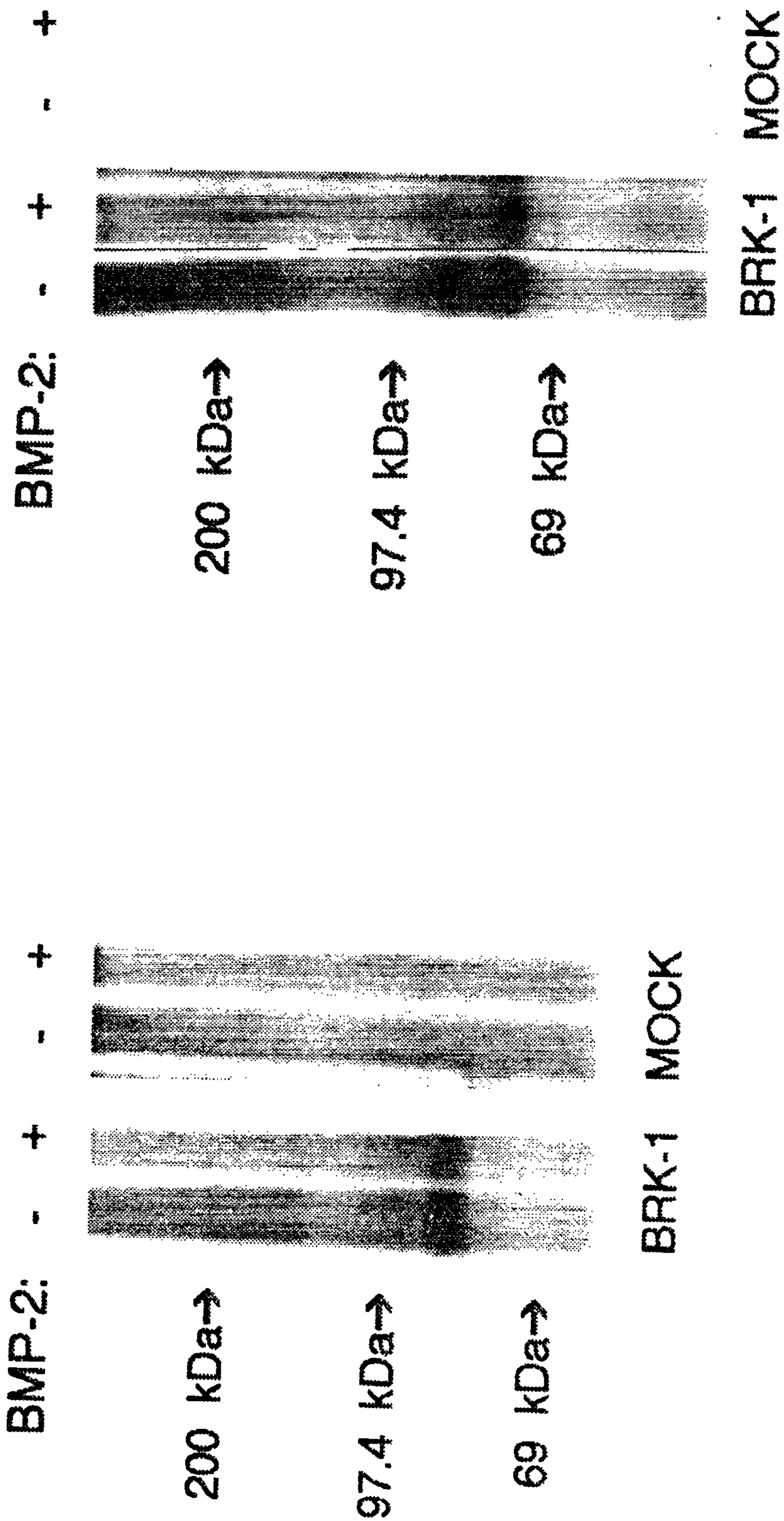


Fig. 6A

Fig. 6B

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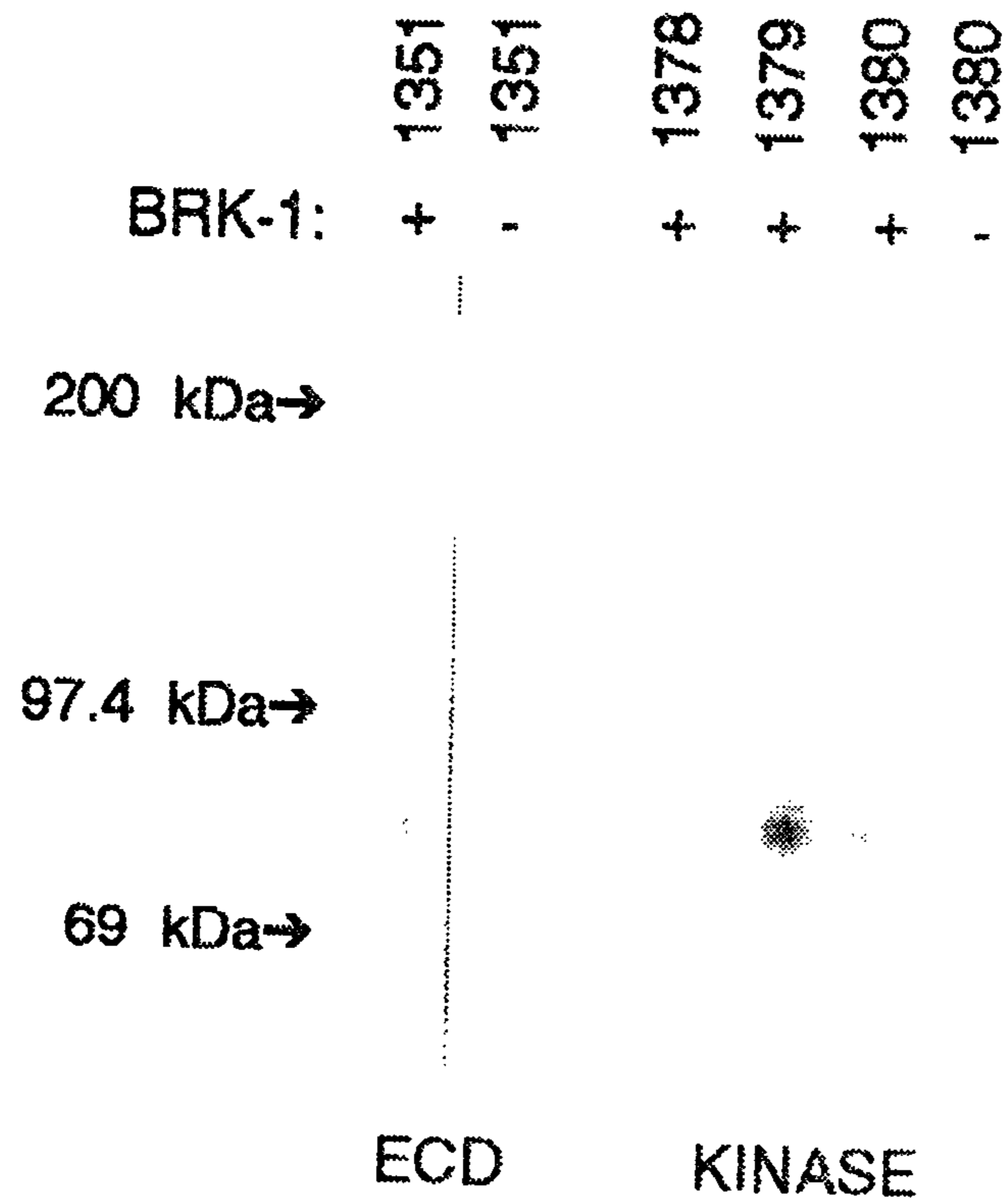


Fig. 7