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CA 2345885 A1 2000/04/20

(21) **2 345 885**

(12) **DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION**

(13) **A1**

(86) Date de dépôt PCT/PCT Filing Date: 1999/10/15
(87) Date publication PCT/PCT Publication Date: 2000/04/20
(85) Entrée phase nationale/National Entry: 2001/04/10
(86) N° demande PCT/PCT Application No.: US 99/24263
(87) N° publication PCT/PCT Publication No.: WO 00/21976
(30) Priorité/Priority: 1998/10/15 (60/104,416) US

(51) Cl.Int.⁷/Int.Cl.⁷ C07H 21/04, C12N 15/86, C12N 15/85,
C12N 15/63, C12N 15/00, C07K 16/00, C07K 14/00

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(54) Titre : GENES INDUITS PAR DES PROTEINES MORPHOGENIQUES OSSEUSES ET POLYPEPTIDES
ASSOCIES, ET LEUR UTILISATION DANS DES METHODES DIAGNOSTIQUES ET THERAPEUTIQUES
(54) Title: BONE MORPHOGENIC PROTEIN-INDUCED GENES AND POLYPEPTIDES, AND THEIR USE IN
DIAGNOSTIC AND THERAPEUTIC METHODS

(57) **Abrégé/Abstract:**

The invention provides bone morphogenic protein (BMP)-induced genes, such as CAP6, AAP2, and AAP32, polypeptides encoded by these genes, and diagnostic and therapeutic methods employing these molecules.



PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C07H 21/04, C07K 14/00, 16/00, C12N 15/00, 15/63, 15/85, 15/86	A1	(11) International Publication Number: WO 00/21976 (43) International Publication Date: 20 April 2000 (20.04.00)
(21) International Application Number: PCT/US99/24263 (22) International Filing Date: 15 October 1999 (15.10.99) (30) Priority Data: 60/104,416 15 October 1998 (15.10.98) US (71) Applicant: THE GENERAL HOSPITAL CORPORATION [US/US]; 55 Fruit Street, Boston, MA 02114 (US). (72) Inventors: DEMAY, Marie, B.; The General Hospital Corporation, 55 Fruit Street, Boston, MA 02214 (US). KEARNF, Ann; The General Hospital Corporation, 55 Fruit Street, Boston, MA 02214 (US). (74) Agent: ELBING, Karen; Clark & Elbing, 176 Federal Street, Boston, MA 02110-2214 (US).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: BONE MORPHOGENIC PROTEIN-INDUCED GENES AND POLYPEPTIDES, AND THEIR USE IN DIAGNOSTIC AND THERAPEUTIC METHODS (57) Abstract The invention provides bone morphogenic protein (BMP)-induced genes, such as CAP6, AAP2, and AAP32, polypeptides encoded by these genes, and diagnostic and therapeutic methods employing these molecules.		

5 BONE MORPHOGENIC PROTEIN-INDUCED GENES AND
 POLYPEPTIDES, AND THEIR USE IN DIAGNOSTIC
 AND THERAPEUTIC METHODS

 This invention relates to genes induced by bone morphogenic proteins,
polypeptides encoded by those genes, and diagnostic and therapeutic methods
10 employing these genes and polypeptides.

Background of the Invention

 Bone is a very dense, specialized form of connective tissue, consisting
of tough fibers (type I collagen fibrils), which resist pulling forces, and solid
15 particles (calcium phosphate particles), which resist compression. For all its
rigidity, bone is by no means a permanent and immutable tissue. Throughout
its hard extracellular matrix are channels and cavities occupied by living cells
that are engaged in an unceasing process of remodeling, which involves a
coupled process of bone resorption and bone formation. Osteoclasts degrade
20 bone during the resorption phase by attaching to the mineralized bone matrix
and excavating small pits on the bone surface, thereby releasing bone collagen
and minerals into circulation. In the bone formation phase, osteoblasts replace
the bone collagen removed by osteoclasts by depositing new collagen at the
resorbed areas. In early adulthood, levels of bone resorption and bone
25 formation generally are balanced. Net bone growth occurs when formation
outpaces resorption, for example, in a child during growth or in the healing of a
bone fracture, while net bone loss occurs when resorption outpaces formation,
for example, in osteoporosis or Paget's disease.

 Control of bone growth and development involves a complex interplay

of the activities of growth factors, transcription factors, and other signaling molecules. For example, a number of bone morphogenic proteins (BMPs) have been isolated that induce pluripotent stromal cells to differentiate into osteoblasts. BMPs belong to the TGF- β superfamily, and are highly
5 homologous to proteins involved in pattern formation in lower species. For example, BMP-2 is 74% identical to decapentaplegic, a drosophila protein that plays a critical role in dorso-ventral patterning, and is 58% identical to Vg-1, a protein postulated to determine mesodermal differentiation in *Xenopus* oocytes.

BMPs were first isolated because of their ability to induce new bone
10 formation in ectopic sites in rodents. The development of this new bone is characterized by differentiation of mesenchymal cells into chondrocytes, and subsequent replacement of cartilage by bone. This process is identical to that of endochondral bone formation in the embryo, including the formation of a functional marrow cavity. BMPs, in fact, are normally synthesized at sites of
15 new bone formation early in development, suggesting that they play a role in limb patterning and osteogenesis. In support of their *in vivo* role in skeletal development, mutation of BMP-5 and targeted ablation of BMP-7 both result in a broad range of skeletal defects. These BMPs, therefore, are likely to play critical roles in the commitment of pluripotent cells to form bone.
20 Recombinant BMP-2A (BMP-2) has been shown to induce ectopic bone formation that is histologically indistinguishable from that which is induced by BMP purified from bone extracts.

BMPs have also been shown to potentiate the growth of a large number of tissues, including a variety of connective tissues and organs.

25

Summary of the Invention

The present invention provides substantially pure bone morphogenic

protein (BMP)-induced polypeptides, such as polypeptides selected from the group consisting of CAP6, AAP2, and AAP32. The polypeptides can include amino acid sequences that are substantially identical to the amino acid sequence of SEQ ID NO:2, an amino acid sequence that is substantially
5 identical to that encoded by the nucleic acid sequence of SEQ ID NO:3 or the complement thereof, or an amino acid sequence that is substantially identical to that encoded by the nucleic acid sequence of SEQ ID NO:4 or the complement thereof. The polypeptides can be derived from a mammal such as, for example, a human.

10 The invention also provides substantially pure nucleic acid (*e.g.*, DNA) molecules having sequences encoding BMP-induced polypeptides, for example, CAP6, AAP2, or AAP32. For example, the nucleic acid molecules can encode an amino acid sequence selected from the group consisting of SEQ ID NO:2, an amino acid sequence that is substantially identical to that which is
15 encoded by the nucleic acid sequence of SEQ ID NO:3 or the complement thereof, an amino acid sequence that is substantially identical to that which is encoded by the nucleic acid sequence of SEQ ID NO:4 or the complement thereof, or a fragment thereof. The nucleic acid molecule can have, for example, at least 55% (*e.g.*, 65%, 75%, 85%, 90%, 95%, or 100%) nucleic acid
20 sequence identity to a sequence encoding a BMP-induced polypeptide or a fragment thereof having at least six (*e.g.*, ten, fifteen, twenty, twenty-five, thirty, forty, or fifty) amino acids. In this case, the nucleic acid molecule hybridizes under high stringency conditions to at least a portion of a nucleic acid molecule encoding a bone morphogenic protein-induced polypeptide.

25 The invention also provides vectors including nucleic acid molecules encoding BMP-induced polypeptides, cells containing such vectors, non-human transgenic animals including nucleic acid molecules encoding BMP-induced

polypeptides, and antibodies that specifically bind bone morphogenic protein-induced polypeptides.

Also provided by the invention are detection methods. For example, the invention features a method of detecting a bone morphogenic protein (BMP)-
5 induced polypeptide in a sample. In these methods, a sample is contacted with an antibody that specifically binds to a BMP-induced polypeptide, and binding of the antibody to the polypeptide is detected.

In another example, the invention features a method for identifying a BMP-induced gene, involving culturing cells in the presence of BMP (for
10 example, BMP-2) and identifying genes induced by the BMP.

Also included in the invention are methods for inducing tissue or organ formation (for example, connective tissue, such as bone) formation in a patient, in which an effective amount of a bone morphogenic-induced polypeptide is administered to the patient. These methods can also be used to potentiate
15 growth of other tissues in patients, such as, for example, cartilage, tendon, ligament, nerve, muscle, and epidermal tissues, as well as to potentiate organ (*e.g.*, pancreas, heart, liver, lung, and kidney) development. The invention also includes use of BMP-induced polypeptides, such as those described above, in methods for inducing tissue or organ formation, and in the preparation of
20 medicaments for such uses and others.

By “bone morphogenic protein-induced polypeptide” or “BMP-induced polypeptide” is meant a polypeptide, or fragment thereof, the expression of which is induced in cells, such as stromal cells, in response to bone morphogenic protein (*e.g.*, BMP-2). Preferably, the BMP-induced polypeptide
25 includes a portion having at least 45%, more preferably at least 55%, more preferably at least 70%, and most preferably at least 85% amino acid identity to the amino acid sequence of SEQ ID NO:2 (CAP6), an amino acid sequence that

is encoded by the nucleic acid sequence of SEQ ID NO:3 or the complement thereof, an amino acid sequence that is encoded by the nucleic acid sequence of SEQ ID NO:4 or the complement thereof, or a fragment thereof. It is to be understood that polypeptide products from splice variants of BMP-induced gene sequences are also included in this definition.

A “BMP-induced gene” or a “nucleic acid molecule encoding a BMP-induced polypeptide” is a nucleic acid molecule, such as genomic DNA, cDNA, or mRNA, that encodes a BMP-induced polypeptide (*e.g.*, CAP6, AAP2, or AAP32) or a portion thereof, as defined above.

The term “identity” is used to indicate that a first polypeptide or nucleic acid molecule possesses the same amino acid or nucleotide residue at a given position, compared to a reference polypeptide or nucleic acid molecule to which the sequence of the first molecule is aligned. Sequence identity can be measured using sequence analysis software with the default parameters specified therein, such as the introduction of gaps to achieve an optimal alignment. For example, the Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705, can be used.

The term “substantially identical” is used in reference to a polypeptide or nucleic acid molecule to indicate that the molecule exhibits, over its entire length, at least 50%, preferably at least 60% or 65%, and most preferably 75%, 85%, 90%, or 95% identity to a reference amino acid or nucleic acid sequence. For polypeptides, the length of comparison sequences can be, for example, at least 16 amino acids, preferably at least 20 amino acids, more preferably at least 25 amino acids, and most preferably at least 35 amino acids. For nucleic acid molecules, the length of comparison sequences can be at least 50 nucleotides, preferably at least 60 nucleotides, more preferably at least 75

nucleotides, and most preferably at least 110 nucleotides.

By “probe” or “primer” is meant a single-stranded DNA or RNA molecule of defined sequence that can base pair to a second DNA or RNA molecule that contains a complementary sequence (the “target”). The stability
5 of the resulting hybrid depends upon the extent of the base pairing that occurs, which is affected by parameters such as the degree of complementarity between the probe and the target molecules and the degree of stringency of the hybridization conditions. The degree of hybridization stringency is affected by parameters such as temperature, salt concentration, and the concentration of
10 organic molecules, such as formamide, and appropriate conditions can readily be determined by those skilled in the art. Probes or primers specific for nucleic acid molecules encoding BMP-induced polypeptides can have, preferably, greater than 50% sequence identity, more preferably at least 55-75% sequence identity, still more preferably at least 75-85% sequence identity, yet more
15 preferably at least 85-99% sequence identity, and most preferably 100% sequence identity. Probes can be detectably-labeled, either radioactively, or non-radioactively, by methods well-known to those skilled in the art. Probes are used for methods involving nucleic acid hybridization, such as nucleic acid amplification by polymerase chain reaction (PCR), single stranded
20 conformational polymorphism (SSCP) analysis, restriction fragment polymorphism (RFLP) analysis, Southern hybridization, Northern hybridization, *in situ* hybridization, and electrophoretic mobility shift assay (EMSA).

The term “detectably-labeled” is used to denote any means for marking
25 and identifying the presence of a molecule, *e.g.*, an oligonucleotide probe or primer, a gene or fragment thereof, a cDNA molecule, or an antibody. Methods for detectably-labeling molecule are well known in the art and

include, without limitation, radioactive labeling (*e.g.*, with an isotope such as ^{32}P or ^{35}S) and nonradioactive labeling (*e.g.*, with a fluorescent label, such as fluorescein).

By “substantially pure polypeptide” is meant a polypeptide (or a
5 fragment thereof) that has been separated from the components that accompany it in its natural state. Typically, the polypeptide is substantially pure when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the polypeptide is a BMP-induced polypeptide that is at least 75%, more preferably at least 90%,
10 and most preferably at least 99%, by weight, pure. A substantially pure BMP-induced polypeptide can be obtained, for example, by extraction from a natural source, by expression of a recombinant nucleic acid encoding a BMP-induced polypeptide, or by chemically synthesizing the polypeptide. Purity can be measured by any appropriate method, *e.g.*, by column chromatography,
15 polyacrylamide gel electrophoresis, or HPLC analysis.

A protein is substantially free of naturally associated components when it is separated from those contaminants which accompany it in its natural state. Thus, a protein that is chemically synthesized or produced in a cellular system different from the cell from which it naturally originates can be considered
20 substantially free from its naturally associated components. Accordingly, substantially pure polypeptides not only includes those derived from eukaryotic organisms but also those synthesized in *E. coli* and other prokaryotes.

An antibody is said to “specifically bind” to an antigen, such as a BMP-induced polypeptide, if it recognizes and binds to the BMP-induced
25 polypeptide, but does not substantially recognize and bind other molecules (*e.g.*, other polypeptides) in a sample, *e.g.*, a biological sample, that naturally includes the polypeptide.

By “high stringency conditions” is meant a set of conditions that allow hybridization comparable to hybridization that occurs using a DNA probe of at least 500 nucleotides in length, in a buffer containing 0.5 M NaHPO₄, pH 7.2, 7% SDS, 1 mM EDTA, and 1% BSA, at a temperature of 65°C, or a buffer
5 containing 48% formamide, 4.8 x SSC, 0.2 M Tris-Cl, pH 7.6, 1 x Denhardt’s solution, 10% dextran sulfate, and 0.1% SDS, at a temperature of 42°C (these are typical conditions for high stringency Northern or Southern hybridizations). High stringency hybridization is also relied upon for the success of numerous techniques routinely performed by molecular biologists, such as high
10 stringency PCR, single strand conformational polymorphism analysis, and *in situ* hybridization. In contrast to Northern and Southern hybridizations, these techniques are usually performed with relatively short probes (*e.g.*, usually 16 nucleotides or longer for PCR or sequencing, and 40 nucleotides or longer for *in situ* hybridization). The high stringency conditions used in these techniques
15 are well known to those skilled in the art of molecular biology, and can be found, for example, in Ausubel *et al.*, *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, NY, 1998, which is hereby incorporated by reference.

The term “transformation” is used herein to denote any method for
20 introducing a foreign molecule, such as a nucleic acid molecule, into a cell. Lipofection, DEAE-dextran-mediated transfection, microinjection, protoplast fusion, calcium phosphate precipitation, retroviral delivery, electroporation, and biolistic transformation are just a few of the standard methods known to those skilled in the art that can be used. For example, biolistic transformation
25 is a method for introducing foreign molecules into a cell using velocity driven microprojectiles such as tungsten or gold particles. Such velocity-driven methods originate from pressure bursts which include, but are not limited to,

helium-driven, air-driven, and gunpowder-driven techniques. Biolistic transformation can be applied to the transformation or transfection of a wide variety of cell types and intact tissues including, without limitation, intracellular organelles (*e.g.*, mitochondria and chloroplasts), bacteria, yeast, fungi, algae, animal tissue, and cultured cells.

By “transformed cell,” “transfected cell,” or “transduced cell,” is meant a cell (or a descendent of a cell) into which a nucleic acid molecule encoding a polypeptide of the invention has been introduced, by means of recombinant techniques.

By “promoter” is meant a sequence sufficient to direct transcription. If desired, constructs of the invention can include promoter elements that are sufficient to render promoter-dependent gene expression controllable in a cell type-specific, tissue-specific, or temporal-specific manner, or inducible by external signals or agents; such elements can be located in the 5' or 3' or intron sequence regions of the native gene.

The term “operably linked” is used herein to indicate that a gene and one or more regulatory sequences are connected in such a way as to permit gene expression when the appropriate molecules (*e.g.*, transcriptional activator proteins) are bound to the regulatory sequences.

By “sample” is meant a specimen containing a tissue biopsy, cell, blood, serum, urine, stool, or other specimen obtained from a patient or test subject. A sample can be analyzed for the presence of a BMP-induced gene, a BMP-induced polypeptide, or an antibody that binds to a BMP-induced polypeptide to detect, for example, expression levels of a BMP-induced gene or polypeptide using methods that are well known in the art. For example, methods involving nucleic acid hybridization, such as polymerase chain reaction (PCR), reverse transcriptase/polymerase chain reaction (RT/PCR), and Northern hybridization

can be used to detect BMP-induced nucleic acid molecules (*e.g.*, mRNA), and standard immunoassays, such as ELISAs, can be used to measure levels of BMP-induced polypeptides.

Other features and advantages of invention will be apparent from the following detailed description thereof.

Detailed Description of the Invention

The invention provides bone morphogenic protein (BMP)-induced genes, such as CAP6, AAP2, and AAP32, which are expressed in pluripotent stromal cells as they differentiate into osteoblasts in response to bone morphogenic protein-2 (BMP-2), polypeptides encoded by these genes, and diagnostic and therapeutic methods employing these genes and polypeptides.

The BMP-induced genes were isolated by differential display PCR (ddPCR) from a mouse limb bud cell line, MLB13 MYC clone 17, as genes that acquired an osteoblastic phenotype in response to rhBMP-2 treatment. Four true positive ddPCR products were identified, three of which were not represented in currently available sequence databases and which are designated herein as CAP6, AAP2, and AAP32. The fourth gene identified was activin β A. The induction of activin β A by rhBMP-2 in the MLB 13 MYC clone 17 cells peaked at 24 hours and was inhibited by cycloheximide, implicating the need for new protein synthesis. The expression of follistatin, an activin binding protein, is also induced by rhBMP-2, with peak levels being observed at 18 hours post-treatment. Unlike the induction of activin β A, the induction of follistatin did not require new protein synthesis. To examine the temperospatial expression of activin β A during *in vivo* endochondral bone formation, *in situ* hybridization was performed in developing mouse bones. Activin β A message localized to the site of the future joint space in the developing mouse phalanges

at day 14.5.

The full length CAP6 gene was sequenced and its sequence is shown in the sequence listing, where it is labeled SEQ ID NO:1. The amino acid sequence includes a glutamine rich region and does not have a high degree of
5 homology to currently available peptide databases. The protein includes a putative ATP binding loop (consensus: GXGXXG; AGGGLGGG; amino acids 23-30) and catalytic domain (ALK; amino acids 74-76). The mRNA encoding this gene is expressed in several adult mouse tissues, including brain, calvaria, diaphragm, and lung, but not liver. Moreover, sequence analysis reveals that
10 CAP6 is a member of the family of serine-threonine kinases.

The partial sequences of the other two novel nucleic acid molecules encoding AAP2 and AAP32 are shown in the sequence listing as SEQ ID NO:3 and SEQ ID NO:4, respectively. Full length nucleic acid molecules encoding AAP2 and AAP32, as well as nucleic acid molecules encoding allelic variants
15 of CAP6 or a CAP6 polypeptide molecule derived from another source, can be obtained using standard nucleic acid hybridization methods (see, *e.g.*, Ausubel *et al.*, *supra*). For example, a cDNA library can be prepared from a cell line or tissue in which the gene is expressed, and the library can be screened using a probe designed based on the nucleic acid sequences provided herein.
20 Alternatively, the sequences provided herein can be used to design primers for use in the polymerase chain reaction (PCR), which can be carried out to obtain probes suitable for library screening.

BMP-Induced Polypeptides

25 The BMP-induced polypeptides of the invention can be purified from cells in which they are naturally expressed, or produced using recombinant methods, which are described as follows. For example, cell lines can be

produced that over-express BMP-induced polypeptides, allowing their purification for biochemical characterization, large-scale production, antibody production, or patient therapy.

For protein expression, eukaryotic and prokaryotic expression systems
5 can be generated in which nucleic acid molecules containing BMP-induced genes are introduced into a plasmid or other vector, which is then used to transform living cells. Constructs in which BMP-induced cDNAs containing entire open reading frames inserted in the correct orientation into an expression plasmid can be used for protein expression. Alternatively, portions of the
10 BMP-induced gene sequences can be inserted. Prokaryotic and eukaryotic expression systems allow various important functional domains of BMP-induced polypeptides to be recovered, if desired, as fusion proteins, and then used for binding, structural, and functional studies, and also for the generation of appropriate antibodies.

15 Typical expression vectors contain promoters that direct the synthesis of large amounts of mRNA corresponding to the inserted BMP-induced nucleic acid molecule in the plasmid-bearing cells. They can also include eukaryotic or prokaryotic origin of replication sequences allowing for their autonomous replication within the host organism, sequences that encode genetic traits that
20 allow vector-containing cells to be selected for in the presence of otherwise toxic drugs, and sequences that increase the efficiency with which the synthesized mRNA is translated. Stable long-term vectors can be maintained as freely replicating entities by using regulatory elements of, for example, viruses (*e.g.*, the OriP sequences from the Epstein Barr Virus genome). Cell
25 lines can also be produced that have integrated the vector into the genomic DNA, and in this manner the gene product is produced in the cell lines on a continuous basis.

Expression of foreign sequences in bacteria, such as *Escherichia coli*, can be accomplished by the insertion of a BMP-induced nucleic acid molecule into a bacterial expression vector. Such plasmid vectors contain several elements required for the propagation of the plasmid in bacteria, and for
5 expression of the DNA inserted into the plasmid. Propagation of only plasmid-bearing bacteria can be achieved by introducing into the plasmid selectable marker-encoding sequences that allow plasmid-bearing bacteria to grow in the presence of otherwise toxic drugs. The plasmid also contains a transcriptional promoter capable of producing large amounts of mRNA from the cloned gene.
10 Such promoters can be, but are not necessarily, inducible promoters that initiate transcription upon induction with a particular compound. The plasmid also preferably contains a polylinker to simplify insertion of the gene in the correct orientation within the vector.

Once the appropriate expression vectors containing a BMP-induced
15 gene, fragment, fusion, or mutant thereof are constructed, they are introduced into appropriate host cells by a transformation technique, such as, for example, calcium phosphate transfection, DEAE-dextran transfection, electroporation, microinjection, protoplast fusion, or liposome-mediated transfection. The host cells that are transfected with the vectors of the invention can include, but are
20 not limited to, *E. coli* or other bacteria, yeast, fungi, insect cells (using, for example, baculoviral vectors for expression), or cells derived from mice, humans, or other animals. Mammalian cells can also be used to express the BMP-induced protein using a vaccinia virus expression system described, for example, by Ausubel *et al.*, *supra*.

25 *In vitro* expression of BMP-induced polypeptides, fusions, or fragments encoded by cloned DNA is also possible using the T7 late promoter expression system. This system depends on the regulated expression of T7 RNA

polymerase, an enzyme encoded in the DNA of bacteriophage T7. The T7 RNA polymerase initiates transcription at a specific 23 basepair promoter sequence called the T7 late promoter. Copies of the T7 late promoter are located at several sites on the T7 genome, but none is present in *E. coli* chromosomal DNA. As a result, in T7-infected cells, T7 RNA polymerase catalyzes transcription of viral genes, but not of *E. coli* genes. In this expression system, recombinant *E. coli* cells are first engineered to carry the gene encoding T7 RNA polymerase next to the *lac* promoter. In the presence of IPTG, these cells transcribe the T7 polymerase gene at a high rate and synthesize abundant amounts of T7 RNA polymerase. These cells are then transformed with plasmid vectors that carry a copy of the T7 late promoter protein. When IPTG is added to the culture medium containing these transformed *E. coli* cells, large amounts of T7 RNA polymerase are produced. The polymerase then binds to the T7 late promoter on the plasmid expression vectors, catalyzing transcription of the inserted cDNA at a high rate. Since each *E. coli* cell contains many copies of the expression vector, large amounts of mRNA corresponding to the cloned cDNA can be produced in this system and the resulting protein can be radioactively labeled. Plasmid vectors containing late promoters and the corresponding RNA polymerases from related bacteriophages such as T3, T5, and SP6 can also be used for *in vitro* production of proteins from cloned DNA. *E. coli* can also be used for expression using an M13 phage, such as mGPI-2. Furthermore, vectors that contain phage lambda regulatory sequences, or vectors that direct the expression of fusion proteins, for example, a maltose-binding protein fusion protein or a glutathione-S-transferase fusion protein, also can be used for expression in *E. coli*.

Eukaryotic expression systems are also useful for expressing BMP-

induced polypeptides, particularly for obtaining appropriate post-translational modification of expressed proteins. Transient transfection of a eukaryotic expression plasmid allows the transient production of BMP-induced polypeptides by a transfected host cell. BMP-induced polypeptides can also be produced by a stably-transfected mammalian cell line. A number of vectors suitable for stable transfection of mammalian cells are available to the public (see, e.g., Pouwels *et al.*, *Cloning Vectors: A Laboratory Manual*, 1985, Supp. 1987), as are methods for constructing such cell lines (see, e.g., Ausubel *et al.*, *supra*). In one example, cDNA encoding a BMP-induced polypeptide, fusion, or fragment is cloned into an expression vector that includes the dihydrofolate reductase (DHFR) gene. Integration of the plasmid and, therefore, integration of the BMP-induced polypeptide-encoding gene into the host cell chromosome, is selected for by inclusion of 0.01-300 μ M methotrexate in the cell culture medium (as is described, for example, by Ausubel *et al.*, *supra*). This dominant selection can be accomplished in most cell types. Recombinant protein expression can be increased by DHFR-mediated amplification of the transfected gene. Methods for selecting cell lines bearing gene amplifications are described by Ausubel *et al.*, *supra*. These methods generally involve extended culture in medium containing gradually increasing levels of methotrexate. The most commonly used DHFR-containing expression vectors are pCVSEII-DHFR and pAdD26SV(A) (described, for example, in Ausubel *et al.*, *supra*). The host cells described above or, preferably, a DHFR-deficient CHO cell line (e.g., CHO DHFR⁻ cells, ATCC Accession No. CRL 9096) are among those most preferred for DHFR selection of a stably-transfected cell line or DHFR-mediated gene amplification.

Eukaryotic cell expression of BMP-induced polypeptides facilitates studies of BMP-induced genes and gene products, including determination of

proper expression and post-translational modifications for biological activity, identifying regulatory elements located in the 5', 3', and intron regions of BMP-induced genes, and determining their roles in tissue regulation of BMP-induced polypeptide expression. It also permits the production of large amounts of

5 these polypeptides for isolation and purification, and the use of cells expressing BMP-induced polypeptides as a functional assay system for antibodies generated against the proteins. Eukaryotic cells expressing BMP-induced polypeptides can be used to test the effectiveness of pharmacological agents on BMP-induced polypeptide associated diseases or as means by which to study

10 BMP-induced polypeptides as components of a transcriptional activation system. Expression of BMP-induced polypeptides, fusions, and polypeptide fragments in eukaryotic cells also enables the study of the function of the normal complete protein, specific portions of the protein, or of naturally occurring polymorphisms and artificially-produced mutated proteins. The

15 BMP-induced DNA sequences can be altered using procedures known in the art, such as restriction endonuclease digestion, DNA polymerase fill-in, exonuclease deletion, terminal deoxynucleotide transferase extension, ligation of synthetic or cloned DNA sequences, and site-directed sequence alteration using specific oligonucleotides, together with PCR.

20 Another preferred eukaryotic expression system is the baculovirus system using, for example, the vector pBacPAK9, which is available from Clontech (Palo Alto, CA). If desired, this system can be used in conjunction with other protein expression techniques, for example, the myc tag approach described by Evan *et al.* (*Mol. Cell Biol.* 5:3610-3616, 1985).

25 Once the recombinant protein is expressed, it can be isolated from the expressing cells by cell lysis followed by protein purification techniques, such as affinity chromatography. In this example, an anti-BMP-induced polypeptide

antibody, which can be produced by the methods described herein, can be attached to a column and used to isolate the recombinant BMP-induced polypeptides. Lysis and fractionation of BMP-induced polypeptide-harboring cells prior to affinity chromatography can be performed by standard methods (see, *e.g.*, Ausubel *et al.*, *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, NY, 1998). Once isolated, the recombinant protein can, if desired, be purified further by, *e.g.*, high performance liquid chromatography (HPLC; *e.g.*, see Fisher, *Laboratory Techniques In Biochemistry And Molecular Biology*, Work and Burdon, Eds., Elsevier, 1980).

Polypeptides of the invention, particularly short BMP-induced polypeptide fragments and longer fragments of the N-terminus and C-terminus of the BMP-induced polypeptide, can also be produced by chemical synthesis (*e.g.*, by the methods described in *Solid Phase Peptide Synthesis*, 2nd ed., 1984, The Pierce Chemical Co., Rockford, IL). These general techniques of polypeptide expression and purification can also be used to produce and isolate useful BMP-induced polypeptide fragments or analogs, as described herein.

Those skilled in the art of molecular biology will understand that a wide variety of expression systems can be used to produce the recombinant BMP-induced polypeptides. The precise host cell used is not critical to the invention. The BMP-induced polypeptides can be produced in a prokaryotic host (*e.g.*, *E. coli*) or in a eukaryotic host (*e.g.*, *S. cerevisiae*, insect cells, such as Sf9 cells, or mammalian cells, such as COS-1, NIH 3T3, or HeLa cells). These cells are commercially available from, for example, the American Type Culture Collection, Rockville, Maryland (see also Ausubel *et al.*, *supra*). The method of transformation and the choice of expression vehicle (*e.g.*, expression vector) will depend on the host system selected. Transformation and transfection methods are described, *e.g.*, in Ausubel *et al.*, *supra*, and expression vehicles

can be chosen from those provided, *e.g.*, in Pouwels *et al.*, *Cloning Vectors: A Laboratory Manual*, 1985, Supp. 1987. Polypeptides of the invention can be tested for bone inducing activity using any of the assays described, for example, in U.S. Patent No. 5,728,679.

5 Polypeptide fragments that incorporate various portions of BMP-induced polypeptides are useful in identifying the domains important for the biological activities of BMP-induced polypeptides. Methods for generating such fragments are well known in the art (see, for example, Ausubel *et al.*, *supra*) using the nucleotide sequences provided herein. For example, a BMP-
10 induced polypeptide fragment can be generated by PCR amplifying the desired fragment using oligonucleotide primers designed based upon the BMP-induced nucleic acid sequences provided herein. Preferably the oligonucleotide primers include unique restriction enzyme sites that facilitate insertion of the fragment into the cloning site of a mammalian expression vector. This vector can then
15 be introduced into a mammalian cell by artifice by various techniques known in the art, for example, those described herein, resulting in the production of a BMP-induced gene fragment.

BMP-induced polypeptide fragments can be used in evaluating portions of the protein involved in important biological activities, such as protein-
20 protein interactions. These fragments can be used alone or as chimeric fusion proteins. BMP-induced polypeptide fragments can also be used to raise antibodies specific for various regions of BMP-induced polypeptides.

BMP-Induced Polypeptide Antibodies

25 To prepare polyclonal antibodies, BMP-induced polypeptides, fragments of BMP-induced polypeptides, or fusion proteins containing defined portions of BMP-induced polypeptide can be synthesized in bacteria by expression of

corresponding DNA sequences in a suitable cloning vehicle. Fusion proteins are commonly used as a source of antigen for producing antibodies. Two widely used expression systems for *E. coli* are *lacZ* fusions using the pUR series of vectors and *trpE* fusions using the pATH vectors. The proteins can be
5 purified, coupled to a carrier protein and mixed with Freund's adjuvant, to enhance stimulation of the antigenic response in an inoculated animal, and injected into rabbits or other laboratory animals. Alternatively, protein can be isolated from BMP-induced polypeptide-expressing cultured cells. Following booster injections at bi-weekly intervals, the rabbits or other laboratory animals
10 are bled and sera isolated. The sera can be used directly or can be purified prior to use by various methods, including affinity chromatography employing reagents such as Protein A-Sepharose, antigen-Sepharose, or anti-mouse-Ig-Sepharose. The sera can then be used to probe protein extracts from BMP-induced polypeptide-expressing tissue electrophoretically fractionated on a
15 polyacrylamide gel to identify BMP-induced polypeptides. Alternatively, synthetic peptides can be made that correspond to the antigenic portions of the protein and used to inoculate the animals.

To generate peptide or full-length proteins for use in making, for example, BMP-induced polypeptide-specific antibodies, a BMP-induced
20 polypeptide coding sequence can be expressed as a C-terminal fusion with glutathione S-transferase (GST; Smith *et al.*, *Gene* 67:31-40, 1988). The fusion protein can be purified on glutathione-Sepharose beads, eluted with glutathione, cleaved with thrombin (at the engineered cleavage site), and purified to the degree required to successfully immunize rabbits. Primary
25 immunizations can be carried out with Freund's complete adjuvant and subsequent immunizations performed with Freund's incomplete adjuvant. Antibody titers are monitored by Western blot and immunoprecipitation

analyses using the thrombin-cleaved BMP-induced polypeptide fragment of the GST-BMP-induced polypeptide fusion protein. Immune sera are affinity purified using CNBr-Sepharose-coupled BMP-induced polypeptide. Antiserum specificity can be determined using a panel of unrelated GST fusion proteins.

5 Alternatively, monoclonal BMP-induced polypeptide antibodies can be produced by using, as an antigen, a BMP-induced polypeptide isolated from BMP-induced polypeptide-expressing cultured cells or BMP-induced polypeptide isolated from tissues. The cell extracts, or recombinant protein extracts containing BMP-induced polypeptide, can, for example, be injected
10 with Freund's adjuvant into mice. Several days after being injected, the mouse spleens are removed, the tissues are disaggregated, and the spleen cells are suspended in phosphate-buffered saline (PBS). The spleen cells serve as a source of lymphocytes, some of which are producing antibody of the appropriate specificity. These are then fused with permanently growing
15 myeloma partner cells, and the products of the fusion are plated into a number of tissue culture wells in the presence of a selective agent such as hypoxanthine, aminopterin, and thymidine (HAT). The wells are then screened by ELISA to identify those containing cells making antibody capable of binding a BMP-induced polypeptide or polypeptide fragment or mutant
20 thereof. These are then replated and after a period of growth, these wells are again screened to identify antibody-producing cells. Several cloning procedures are carried out until over 90% of the wells contain single clones that are positive for antibody production. From this procedure a stable line of clones that produce the antibody is established. The monoclonal antibody can
25 then be purified by affinity chromatography using Protein A Sepharose, ion-exchange chromatography, as well as variations and combinations of these techniques. Truncated versions of monoclonal antibodies can also be produced

by recombinant methods in which plasmids are generated that express the desired monoclonal antibody fragment(s) in a suitable host.

As an alternate or adjunct immunogen to GST fusion proteins, peptides corresponding to relatively unique hydrophilic regions of BMP-induced polypeptide can be generated and coupled to keyhole limpet hemocyanin (KLH) through an introduced C-terminal lysine. Antiserum to each of these peptides is similarly affinity-purified on peptides conjugated to BSA, and specificity is tested by ELISA and Western blotting using peptide conjugates, and by Western blotting and immunoprecipitation using a BMP-induced polypeptide, for example, expressed as a GST fusion protein.

Alternatively, monoclonal antibodies can be prepared using the BMP-induced polypeptides described above and standard hybridoma technology (see, e.g., Kohler *et al.*, *Nature* 256:495, 1975; Kohler *et al.*, *Eur. J. Immunol.* 6:511, 1976; Kohler *et al.*, *Eur. J. Immunol.* 6:292, 1976; Hammerling *et al.*, In *Monoclonal Antibodies and T Cell Hybridomas*, Elsevier, New York, NY, 1981; Ausubel *et al.*, *supra*). Once produced, monoclonal antibodies are also tested for specific BMP-induced polypeptide recognition by Western blot or immunoprecipitation analysis (for example, by the methods described in Ausubel *et al.*, *supra*). Monoclonal and polyclonal antibodies that specifically recognize a BMP-induced polypeptide (or a fragment thereof) are considered useful in the invention.

Antibodies of the invention can be produced using BMP-induced polypeptide amino acid sequences that do not reside within highly conserved regions, and that appear likely to be antigenic, as analyzed by criteria such as those provided by the Peptide Structure Program (Genetics Computer Group Sequence Analysis Package, Program Manual for the GCG Package, Version 7, 1991) using the algorithm of Jameson and Wolf (*CABIOS* 4:181, 1988). These

fragments can be generated by standard techniques, *e.g.*, by PCR, and cloned into the pGEX expression vector (Ausubel *et al.*, *supra*). GST fusion proteins are expressed in *E. coli* and purified using a glutathione-agarose affinity matrix as described in Ausubel *et al.*, *supra*. To generate rabbit polyclonal antibodies, and to minimize the potential for obtaining antisera that are non-specific, or exhibit low-affinity binding to a BMP-induced polypeptide, two or three fusions are generated for each protein, and each fusion is injected into at least two rabbits. Antisera are raised by injections in series, preferably including at least three booster injections.

10 In addition to intact monoclonal and polyclonal anti-BMP-induced polypeptide antibodies, the invention features various genetically engineered antibodies, humanized antibodies, and antibody fragments, including F(ab')₂, Fab', Fab, Fv, and sFv fragments. Antibodies can be humanized by methods known in the art, *e.g.*, monoclonal antibodies with a desired binding specificity can be commercially humanized (Scotgene, Scotland; Oxford Molecular, Palo Alto, CA). Fully human antibodies, such as those expressed in transgenic animals, are also features of the invention (Green *et al.*, *Nature Genetics* 7:13-21, 1994).

Ladner (U.S. Patent Nos. 4,946,778 and 4,704,692) describes methods for preparing single polypeptide chain antibodies. Ward *et al.* (*Nature* 341:544-546, 1989) describe the preparation of heavy chain variable domains, which they term "single domain antibodies," and which have high antigen-binding affinities. McCafferty *et al.* (*Nature* 348:552-554, 1990) show that complete antibody V domains can be displayed on the surface of fd bacteriophage, that the phage bind specifically to antigen, and that rare phage (one in a million) can be isolated after affinity chromatography. Boss *et al.* (U.S. Patent No. 4,816,397) describe various methods for producing

immunoglobulins, and immunologically functional fragments thereof, which include at least the variable domains of the heavy and light chain in a single host cell. Cabilly *et al.* (U.S. Patent No. 4,816,567) describe methods for preparing chimeric antibodies.

5 Antibodies to BMP-induced polypeptide can be used, as noted above, to detect BMP-induced polypeptides or to inhibit the biological activities of BMP-induced polypeptides. For example, a nucleic acid molecule encoding an antibody or a portion of an antibody can be expressed within a cell to inhibit BMP-induced polypeptide function. In addition, the antibodies can be coupled
10 to compounds for diagnostic and/or therapeutic uses, such as radionuclides and liposomes carrying therapeutic compounds.

Use of BMP-Induced Genes and Polypeptides

Consistent with the role of BMPs in the potentiation of a number of
15 different tissues, BMP-induced genes and polypeptides also find use in therapeutic and diagnostic applications involving growth potentiation in diverse tissues, including, for example, cartilage, tendon, ligament, nerve, muscle, and epidermal tissues, as well as organs such as pancreas, heart, liver, lung, and kidney.

20 For example, therapies can be designed to circumvent or overcome inadequate or excessive expression of BMP-induced genes. Inadequate expression of such genes may be a characteristic of conditions associated with bone loss, such as osteoporosis, Paget's disease, and osteogenesis imperfecta, which can thereby be treated by administration of BMP-induced polypeptides
25 or genes that encode them.

As is noted above, CAP6 has been identified as a member of the family of serine-threonine kinases. Misexpression of these kinases has been

associated with malignancy. Thus, compounds that inhibit the activity of CAP6, which can be identified according to the invention, can be used in methods to prevent or to treat malignancies, such as cancer. Further, CAP6 includes a long glutamine repeat region, and expansion of such triplet repeats
5 has been associated with heritable diseases (*e.g.*, Huntington's disease). Thus, compounds that inhibit this expansion, which can be identified according to the invention, can be used to prevent or to treat such diseases. CAP6, as well as corresponding peptide fragments and nucleic acid molecules can also be used to diagnose such conditions.

10 In considering various therapies, it is to be understood that such therapies are preferably targeted to the effected or potentially effected organs. Reagents that modulate BMP-induced polypeptide biological activity include, without limitation, full length BMP-induced polypeptides, or fragments thereof, BMP-induced polypeptide mRNA or antisense RNA, or any compound that
15 modulates BMP-induced polypeptide biological activity, expression, or stability. Multiple active components can be administered together, for example, molecules of the invention can be administered with each other or with, *e.g.*, BMPs or related molecules.

Treatment or prevention of diseases that would benefit from BMP-
20 induced polypeptide expression can be accomplished by replacing a mutant BMP-induced polypeptide gene with a normal BMP-induced polypeptide gene, by modulating the function a mutant protein, by delivering normal BMP-induced polypeptide to the appropriate cells, or by altering the levels of normal or mutant protein. It is also possible to modify the pathophysiologic pathway
25 (*e.g.*, a signal transduction pathway) in which the protein participates to correct the physiological defect.

To replace a mutant protein with normal protein, or to add protein to

cells that no longer express sufficient BMP-induced polypeptides, it may be necessary to obtain large amounts of pure BMP-induced polypeptide from cultured cell systems that can express the protein. Delivery of the protein to the effected tissue can then be accomplished using appropriate packaging or administration systems. Alternatively, small molecule analogs that act as BMP-induced polypeptide agonists or antagonists can be administered to produce a desired physiological effect.

Gene therapy is another therapeutic approach for preventing or ameliorating diseases related to BMP-induced polypeptide expression. Nucleic acid molecules encoding BMP-induced polypeptides can be delivered to cells, where it must be in a form in which it can be taken up and direct expression of sufficient protein to provide effective function. Transducing retroviral, adenoviral, and human immunodeficiency viral (HIV) vectors can be used for somatic cell gene therapy especially because of their high efficiency of infection and stable integration and expression; see, *e.g.*, Cayouette *et al.*, *Hum. Gene Therapy*, 8:423-430, 1997; Kido *et al.*, *Curr. Eye Res.*, 15:833-844, 1996; Bloomer *et al.*, *J. Virol.*, 71:6641-6649, 1997; Naldini *et al.*, *Science* 272:263-267, 1996; and Miyoshi *et al.*, *Proc. Nat. Acad. Sci., U.S.A.*, 94:10319-1032, 1997. For example, a full length BMP-induced polypeptide gene, or a portion thereof, can be cloned into a retroviral vector and driven from its endogenous promoter or from the retroviral long terminal repeat or from a promoter specific for the target cell type of interest (such as neurons). Other viral vectors which can be used include adenovirus, adeno-associated virus, vaccinia virus, bovine papilloma virus, or a herpes virus such as Epstein-Barr virus.

Gene transfer can also be achieved using non-viral means requiring infection *in vitro*. This would include calcium phosphate, DEAE dextran, electroporation, and protoplast fusion. Liposomes can also be beneficial for

delivery of DNA into a cell.

Transplantation of normal genes into the affected tissues of a patient can also be accomplished by transferring a normal BMP-induced polypeptide gene into a cultivatable cell type *ex vivo*, after which the cells are injected into the
5 targeted tissue(s).

Retroviral vectors, adenoviral vectors, adenovirus-associated viral vectors, or other viral vectors with the appropriate tropism for cells likely to be involved in BMP-induced polypeptide-related diseases can be used as gene transfer delivery systems for therapeutic BMP-induced polypeptide gene
10 constructs. Numerous vectors useful for this purpose are generally known (see, for example, Miller, *Human Gene Therapy* 15-14, 1990; Friedman, *Science* 244:1275-1281, 1989; Eglitis and Anderson, *BioTechniques* 6:608-614, 1988; Tolstoshev and Anderson, *Curr. Opin. Biotech.* 1:55-61, 1990; Sharp, *The Lancet* 337:1277-1278, 1991; Cornetta *et al.*, *Nucl. Acid Res. and Mol. Biol.*
15 36:311-322, 1987; Anderson, *Science* 226:401-409, 1984; Moen, *Blood Cells* 17:407-416, 1991; Miller *et al.*, *Biotech.* 7:980-990, 1989; Le Gal La Salle *et al.*, *Science* 259:988-990, 1993; and Johnson, *Chest* 107:77S-83S, 1995). Retroviral vectors are particularly well developed and have been used in clinical settings (Rosenberg *et al.*, *N. Engl. J. Med* 323:370, 1990; Anderson *et al.*, U.S. Patent No. 5,399,346).
20

Non-viral approaches can also be employed for the introduction of therapeutic DNA into cells predicted to be subject to diseases involving BMP-induced polypeptides. For example, a BMP-induced polypeptide nucleic acid molecule or antisense nucleic acid molecule can be introduced into a cell by
25 lipofection (Felgner *et al.*, *Proc. Natl. Acad. Sci. USA* 84:7413, 1987; Ono *et al.*, *Neurosci. Lett.* 117:259, 1990; Brigham *et al.*, *Am. J. Med. Sci.* 298:278, 1989; Staubinger *et al.*, *Meth. Enz.* 101:512, 1983), asialoorosomucoid-

polylysine conjugation (Wu *et al.*, *J. Biol. Chem.* 263:14621, 1988; Wu *et al.*, *J. Biol. Chem.* 264:16985, 1989), or, less preferably, micro-injection under surgical conditions (Wolff *et al.*, *Science* 247:1465, 1990).

In these constructs, BMP-induced polypeptide cDNA expression can be
5 directed from any suitable promoter (*e.g.*, the human cytomegalovirus (CMV),
simian virus 40 ((SV40), or metallothionein promoters), and regulated by any
appropriate mammalian regulatory element. For example, if desired, enhancers
known to preferentially direct gene expression in specific cell types can be used
to direct BMP-induced polypeptide expression. The enhancers used include,
10 without limitation, those that are characterized as tissue- or cell-specific
enhancers. Alternatively, if a BMP-induced polypeptide genomic clone is used
as a therapeutic construct (such clones can be identified by hybridization with
the BMP-induced polypeptide cDNA described above), regulation can be
mediated by the cognate regulatory sequences or, if desired, by regulatory
15 sequences derived from a heterologous source, including any of the promoters
or regulatory elements described above.

Antisense-based strategies can be employed to explore BMP-induced
polypeptide gene function and as a basis for therapeutic drug design. The
principle is based on the hypothesis that sequence-specific suppression of gene
20 expression (via transcription or translation) can be achieved by intracellular
hybridization between genomic DNA or mRNA and a complementary
antisense species. The formation of a hybrid RNA duplex interferes with
transcription of the target BMP-induced polypeptide-encoding genomic DNA,
or processing, transport, translation, and/or stability of the target BMP-induced
25 polypeptide mRNA.

Antisense molecules can be delivered by a variety of approaches. For
example, antisense oligonucleotides or antisense RNA can be directly

administered (*e.g.*, by intravenous injection) to a subject in a form that allows uptake into cells. Alternatively, viral or plasmid vectors that encode antisense RNA (or RNA fragments) can be introduced into a cell *in vivo* or *ex vivo*.

Antisense effects can be induced by control (sense) sequences; however, the
5 extent of phenotypic changes are highly variable. Phenotypic effects induced by antisense effects are based on changes in criteria such as protein levels, protein activity measurement, and target mRNA levels.

For example, BMP-induced polypeptide gene therapy can also be accomplished by direct administration of antisense BMP-induced polypeptide
10 mRNA to a cell that is expected to be adversely affected by the expression of wild-type or mutant BMP-induced polypeptide. The antisense BMP-induced polypeptide mRNA can be produced and isolated by any standard technique, but is most readily produced by *in vitro* transcription using an antisense BMP-induced polypeptide cDNA under the control of a high efficiency promoter
15 (*e.g.*, the T7 promoter). Administration of antisense BMP-induced polypeptide mRNA to cells can be carried out by any of the methods for direct nucleic acid administration described above.

An alternative strategy for inhibiting BMP-induced polypeptide function using gene therapy involves intracellular expression of an anti-BMP-induced
20 polypeptide antibody or a portion of an anti-BMP-induced polypeptide antibody. For example, the gene (or gene fragment) encoding a monoclonal antibody that specifically binds to BMP-induced polypeptide and inhibits its biological activity can be placed under the transcriptional control of a tissue-specific gene regulatory sequence.

25 Another therapeutic approach within the invention involves administration of recombinant BMP-induced polypeptide, either directly to the site of a potential or actual disease-affected tissue (for example, by injection) or

systemically (for example, by any conventional recombinant protein administration technique). The dosage of BMP-induced polypeptide depends on a number of factors, including the size and health of the individual patient, but, generally, between 0.1 mg and 100 mg inclusive are administered per day to an adult in any pharmaceutically acceptable formulation.

The methods of the instant invention can be used to diagnose or treat the disorders described herein in any mammal, for example, humans, domestic pets, or livestock. Where a non-human mammal is treated or diagnosed, the BMP-induced polypeptide, nucleic acid, or antibody employed is preferably specific for that species.

A BMP-induced polypeptide, gene, or modulator of a BMP-induced polypeptide can be administered within a pharmaceutically-acceptable diluent, carrier, or excipient, in unit dosage form. Conventional pharmaceutical practice can be employed to provide suitable formulations or compositions to administer BMP-induced polypeptides, neutralizing BMP-induced polypeptide antibodies, or BMP-induced polypeptide-inhibiting compounds (*e.g.*, antisense molecules) to patients suffering from a BMP-induced polypeptide-related disease, such as disease characterized by bone resorption, such as osteoporosis. Administration can begin before the patient is symptomatic. Any appropriate route of administration can be employed, for example, administration can be parenteral, intravenous, intra-arterial, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, aerosol, by suppositories, or oral. Therapeutic formulations can be in the form of liquid solutions or suspensions; for oral administration, formulations can be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols.

Methods well known in the art for making formulations are found, for example, in *Remington's Pharmaceutical Sciences*, (18th edition), ed. A. Gennaro, 1990, Mack Publishing Company, Easton, PA. Formulations for parenteral administration can, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated naphthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers can be used to control the release of the compounds. Other potentially useful parenteral delivery systems for BMP-induced polypeptide modulatory compounds include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation can contain excipients, for example, lactose, or can be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or can be oily solutions for administration in the form of nasal drops, or as a gel.

In addition, BMP-induced genes and polypeptides have a number of diagnostic uses. For example, as discussed above, maintenance of bone structure requires a balance between the bone resorbing activity of osteoclasts and the bone formation activity of osteoblasts. Throughout life, a dynamic process of bone remodeling takes place through the opposing activities of these cells, with the net effect, under normal circumstances, of maintaining bone structure. Disruption of this balance is a hallmark of bone injury, as well as several diseases of bones, such as osteoporosis, Paget's disease, and osteogenesis imperfecta.

As noted above, the genes of the invention are induced in the differentiation of the bone formation cells, osteoblasts. Thus, the genes of the invention and, in particular, the gene products they encode, can be used as

markers of bone formation and resorption, facilitating diagnosis of, for example, pathological alterations in bone turnover and to measure responses to therapy. This is of significant medical relevance, as numerous and prevalent debilitating diseases are associated with loss of the balance between bone loss and formation. In particular, osteoporosis and Paget's disease are associated with net bone loss.

Standard diagnostic methods, such as immunoassays, including enzyme-linked immunosorbent assays (ELISAs), quantitative PCR, and reverse transcriptase/polymerase chain reaction (RT/PCR)-based assays can be readily adapted for use in the diagnostic methods of the invention (see, *e.g.*, Ausubel *et al.*, *supra*; Ehrlich (Ed.) *PCR Technology: Principles and Applications for DNA Amplification*, Stockton Press, NY; Yap *et al.*, *Nucl. Acids. Res.* 19:4294, 1991). Materials from which samples may be obtained for use in diagnosis include, for example, urine, blood samples (*e.g.*, serum) and bone marrow, which can be obtained using standard methods. Of course, use of samples obtained by minimally invasive techniques are preferred, provided sufficient levels of marker are present in the sample for detection.

Standard immunoassays can be used to detect or to monitor BMP-induced polypeptide expression in a biological sample. BMP-induced polypeptide-specific polyclonal or monoclonal antibodies (produced as described above) can be used in any standard immunoassay format (*e.g.*, ELISA, Western blot, or RIA) to measure BMP-induced polypeptide levels. These levels can be compared to wild-type BMP-induced polypeptide levels in control samples. For example, a decrease in BMP-induced polypeptide production can indicate a condition or a predisposition to a condition involving insufficient BMP-induced polypeptide biological activity. Examples of immunoassays are described, *e.g.*, in Ausubel *et al.*, *supra*.

Immunohistochemical techniques can also be utilized for BMP-induced polypeptide detection. For example, a tissue sample can be obtained from a patient, sectioned, and stained for the presence of BMP-induced polypeptides using an anti-BMP-induced polypeptide antibody and any standard detection
5 system (*e.g.*, one which includes a secondary antibody conjugated to horseradish peroxidase). General guidance regarding such techniques can be found in, *e.g.*, Bancroft and Stevens (*Theory and Practice of Histological Techniques*, Churchill Livingstone, 1982) and Ausubel *et al.*, *supra*.

Alternatively, diagnosis of BMP-related disorders may be evaluated by
10 an examination of BMP-induced genes and a determination of whether such genes include mutations characteristic of disease. Moreover, as discussed above, such BMP-induced genes and polypeptides may be used for diagnostic purposes for detecting alterations in any of a variety of issues including, for example, cartilage, tendon, ligament, nerve, muscle, and epidermal tissues, as
15 well as organs such as pancreas, heart, liver, lung, and kidney.

Additional uses and applications for the molecules of the invention are described, for example, in U.S. Patent Nos. 5,661,007, 5,728,679, 5,703,043, 5,700,911, 5,688,678, 5,658,882, 5,639,638, 5,637,480, 5,635,372, 5,459,047, and 5,543,394, which, as all other references cited herein, are hereby
20 incorporated by reference.

What is claimed is:

WO 00/21976

PCT/US99/24263

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SEQUENCE LISTING

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1. A substantially pure bone morphogenic protein-induced polypeptide.
2. The polypeptide of claim 1, wherein said polypeptide is selected from the group consisting of CAP6, AAP2, and AAP32.
- 5 3. The polypeptide of claim 2, wherein said polypeptide comprises (i) an amino acid sequence that is substantially identical to the amino acid sequence of SEQ ID NO:2, or (ii) an amino acid sequence that is encoded by the nucleic acid sequence of SEQ ID NO:3 or SEQ ID NO:4.
- 10 4. The polypeptide of claim 1, wherein said polypeptide is derived from a mammal.
5. The polypeptide of claim 4, wherein said mammal is a human.
- 15 6. A substantially pure nucleic acid molecule comprising a sequence encoding a bone morphogenic protein-induced polypeptide.
7. The nucleic acid molecule of claim 6, wherein said polypeptide is
20 selected from the group consisting of CAP6, AAP2, and AAP32.
8. The nucleic acid molecule of claim 7, wherein said nucleic acid molecule encodes an amino acid sequence selected from the group consisting of SEQ ID NO:2, an amino acid sequence that is encoded by the nucleic acid
25 sequence of SEQ ID NO:3, an amino acid sequence that is encoded by the nucleic acid sequence of SEQ ID NO:4, or a fragment thereof.

9. The nucleic acid molecule of claim 8, wherein said nucleic acid molecule is DNA.

10. A nucleic acid molecule having at least 55% nucleic acid sequence
5 identity to a sequence encoding a bone morphogenic protein-induced polypeptide or a fragment thereof comprising at least six amino acids, wherein said nucleic acid molecule hybridizes under high stringency conditions to at least a portion of a nucleic acid molecule encoding a bone morphogenic protein-induced polypeptide.

10

11. A vector comprising the nucleic acid molecule of claim 6 or claim
10.

12. A cell comprising the vector of claim 11.

15

13. A non-human transgenic animal comprising the nucleic acid molecule of claim 6 or claim 10.

14. An antibody that specifically binds to a bone morphogenic protein-
20 induced polypeptide.

15. The antibody of claim 14, wherein said bone morphogenic protein-induced polypeptide is induced by BMP-2, and said bone morphogenic protein-induced polypeptide is CAP6, AAP2, or AAP32.

25

16. A method of detecting a bone morphogenic protein-induced polypeptide in a sample, said method comprising contacting said sample with

an antibody that specifically binds said bone morphogenic protein-induced polypeptide and assaying for binding of said antibody to said polypeptide.

17. A method for identifying a bone morphogenic protein-induced gene,
5 said method comprising culturing cells in the presence of bone morphogenic protein and identifying genes induced by said bone morphogenic protein.

18. The method of claim 17, wherein said bone morphogenic protein-induced polypeptide is induced by BMP-2.

10

19. Use of a bone morphogenic-induced polypeptide for inducing tissue or organ formation in a patient, said use comprising administering to said patient an effective amount of a bone morphogenic protein-induced polypeptide.

15

20. The use of claim 19, wherein said tissue is connective tissue or bone.

21. The use of claim 19, wherein said bone morphogenic protein-induced polypeptide is induced by BMP-2.

20

22. The use of claim 19, wherein said bone morphogenic protein-induced polypeptide is CAP6, AAP2, or AAP32.