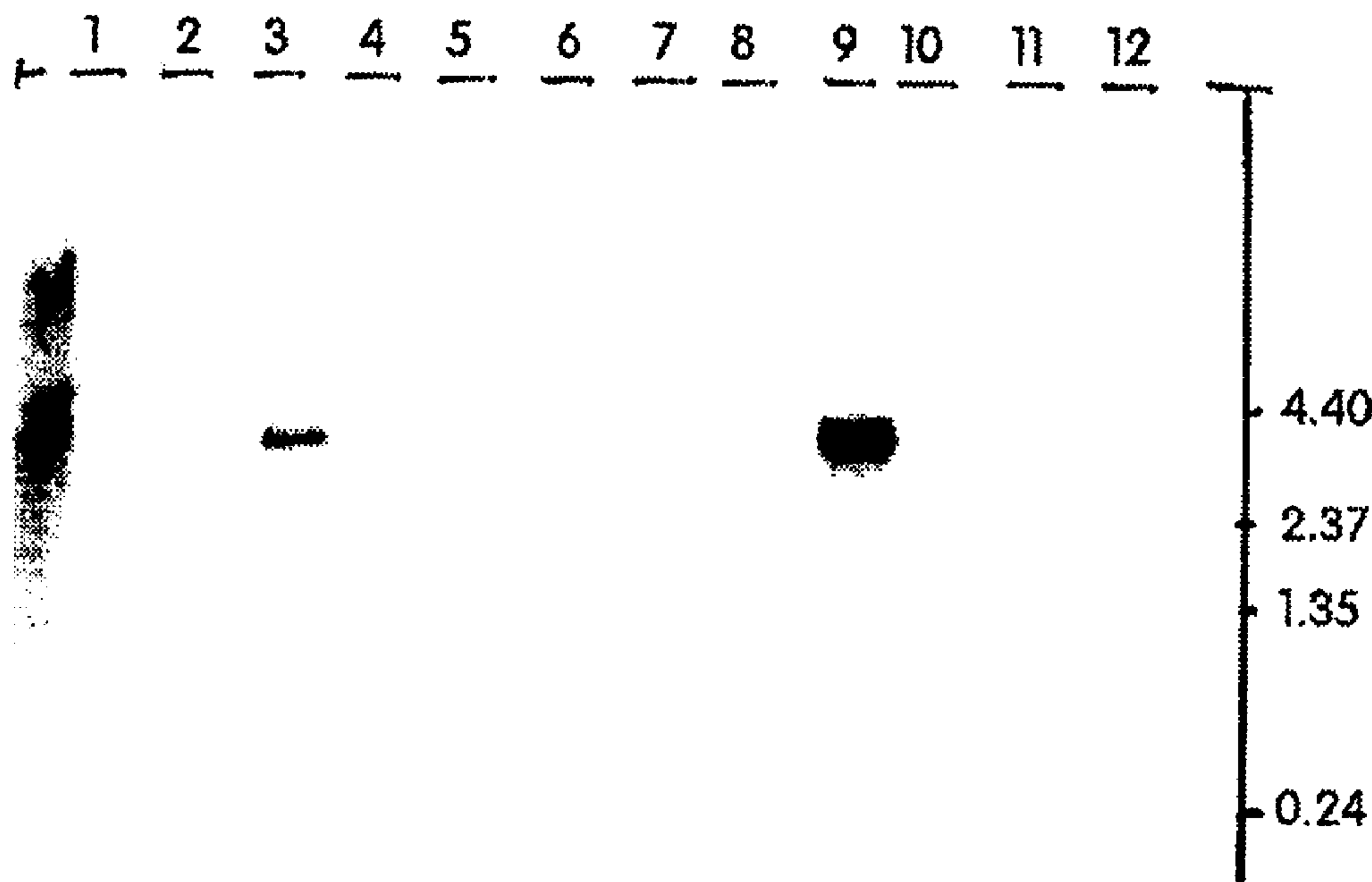




(22) Date de dépôt/Filing Date: 1992/03/11
 (41) Mise à la disp. pub./Open to Public Insp.: 1992/09/17
 (45) Date de délivrance/Issue Date: 2010/05/18
 (62) Demande originale/Original Application: 2 104 678
 (30) Priorités/Priorities: 1991/03/11 (US667,274);
 1991/08/30 (US752,764)

(51) Cl.Int./Int.Cl. *A61K 38/18* (2006.01),
A61K 35/407 (2006.01), *A61K 47/42* (2006.01)
 (72) Inventeurs/Inventors:
 COHEN, CHARLES M., US;
 KUBERASAMPATH, THANGAVEL, US;
 PANG, ROY H. L., US;
 OPPERMAN, HERMANN, US;
 RUEGER, DAVID C., US
 (73) Propriétaire/Owner:
 STRYKER CORPORATION, US
 (74) Agent: RICHES, MCKENZIE & HERBERT LLP

(54) Titre : MORPHOGENESE INDUITE PAR DES PROTEINES
 (54) Title: PROTEIN-INDUCED MORPHOGENESIS



(57) Abrégé/Abstract:

This invention relates to amino acid sequence data, structural features, homologies and various other data characterizing morphogenic proteins. There is also disclosed methods of producing these proteins from natural and recombinant sources and from synthetic constructs. The invention also relates to morphogenic devices comprising these proteins and a suitably modified tissue-specific matrix. Methods of inducing non-chondrogenic tissue growth in mammals are also disclosed. The proteins are useful in inducing the developmental cascade of tissue morphogenesis for a variety of tissues in mammals different from bone or cartilage. The proteins of the morphogenic devices comprise a pair of folded polypeptides, the amino acid sequence of each comprises a sequence sharing at least 70% homology with the C-terminal seven-cysteine domain of human OP-1, mouse OP-1, human OP-2 or mouse OP-2, residues 38-139 of Seq. ID Nos. 5, 6, 7 or 8; or an essentially 102 amino acid sequence having cysteine residues at relative positions 1, 30, 34, 66, 67, 99 and 101 therein, wherein at least 70% of amino acid residues within said sequence are selected independently from residues occupying the corresponding positions within residues 38-139 of Seq. ID Nos., 5, 6, 7 or 8, or conservative substitutions of said corresponding residues.

Abstract of Disclosure

This invention relates to amino acid sequence data, structural features, homologies and various other data characterizing morphogenic proteins. There is also disclosed methods of producing these proteins from natural and recombinant sources and from synthetic constructs. The invention also relates to morphogenic devices comprising these proteins and a suitably modified tissue-specific matrix. Methods of inducing non-chondrogenic tissue growth in mammals are also disclosed. The proteins are useful in inducing the developmental cascade of tissue morphogenesis for a variety of tissues in mammals different from bone or cartilage. The proteins of the morphogenic devices comprise a pair of folded polypeptides, the amino acid sequence of each comprises a sequence sharing at least 70% homology with the C-terminal seven-cysteine domain of human OP-1, mouse OP-1, human OP-2 or mouse OP-2, residues 38-139 of Seq. ID Nos. 5, 6, 7 or 8; or an essentially 102 amino acid sequence having cysteine residues at relative positions 1, 30, 34, 66, 67, 99 and 101 therein, wherein at least 70% of amino acid residues within said sequence are selected independently from residues occupying the corresponding positions within residues 38-139 of Seq. ID Nos., 5, 6, 7 or 8, or conservative substitutions of said corresponding residues.

PROTEIN-INDUCED MORPHOGENESIS

5

This application is a divisional of Canadian Patent Application Serial Number 2,104,678, filed March 11, 1992.

Background of the Invention

10

This invention relates to morphogenic proteins which can induce tissue morphogenesis in mammals; to methods of identifying these proteins and obtaining them from natural sources or producing synthetic forms of these proteins by expressing recombinant DNA encoding the proteins; to the fabrication of tissue-specific acellular matrices; and to methods for promoting tissue stasis, repair and regeneration, and methods for increasing progenitor cell populations using these proteins.

15

Cell differentiation is the central characteristic of morphogenesis which initiates in the embryo, and continues to various degrees throughout the life of an organism in adult tissue repair and regeneration mechanisms. The degree of morphogenesis in adult tissue varies among different tissues and is related, among other things, to the degree of cell turnover in a given tissue. On this basis, tissues can be divided into three broad categories: (1) tissues with static cell populations such as nerve and skeletal muscle where there is no cell division and most of the

20

25

cells formed during early development persist throughout adult life; (2) tissues containing conditionally renewing populations such as liver where there is generally little cell division but, in response to an appropriate stimulus, cells can divide to produce daughters of the same differentially defined type; and (3) tissues with permanently renewing populations including blood, testes and stratified squamous epithelia which are characterized by rapid and continuous cell turnover in the adult. Here, the terminally differentiated cells have a relatively short life span and are replaced through proliferation of a distinct subpopulation of cells, known as stem or progenitor cells.

15

The cellular and molecular events which govern the stimulus for differentiation of these cells is an area of intensive research. In the medical field, it is anticipated that the discovery of factor(s) which control cell differentiation and tissue morphogenesis will significantly advance medicine's ability to repair and regenerate diseased or damaged mammalian tissues and organs. Particularly useful areas include reconstructive surgery and in the treatment of tissue degenerative diseases including arthritis, emphysema, osteoporosis, cardiomyopathy, cirrhosis, and degenerative nerve diseases.

A number of different factors have been isolated in recent years which appear to play a role in cell differentiation. Some of these factors are gene transcription activators such as the NOTCH gene, identified in *Drosophila* and the related XOTCH gene identified in *Xenopus*, as well as a number of transcription activators identified in *Caenorhabditis elegans*.

The hemopoietic system, because of its continually renewing cell population, is an area of concentrated study. Factors identified in this system which may be involved in cell renewal include

5 interleukin 3 (IL-3), erythropoietin, the CSFs (GM-CSF, G-CSF, M-CSF et al.) and various stem cell growth factors.

Other proteins thought to play a role in cell
10 differentiation include proteins that are members of the family of insulin-like growth factors (IGF), members of the family of heparin-binding growth factors, (e.g., FGF - acidic and basic fibroblast growth factors, and ECDGF - embryonal carcinoma-derived
15 growth factor) as well as several transforming oncogenes (hst and int-2, see for example, Heath et al., (1988), J. Cell Sci. Suppl. 10:256-256.) DIF (Differentiation Inducing Factor), identified in Dictyostelium discoideum, is another bioregulatory
20 protein, directing prestock cell differentiation in that organism.

The structurally related proteins of the TGF- β superfamily of proteins also have been identified as
25 involved in a variety of developmental events. For example, TGF- β and the polypeptides of the inhibin/activin group appear to play a role in the regulation of cell growth and differentiation. MIS (Mullerian Inhibiting Substance) causes regression of
30 the Mullerian duct in development of the mammalian male embryo, and DPP, the gene product of the *Drosophila* decapentaplegic complex is required for appropriate dorsal-ventral specification. Similarly, Vg-1 is involved in mesoderm induction in *Xenopus*, and Vgr-1
35 has been identified in a variety of developing murine tissues.

Another source that has revealed a wealth of information is in the area of bone morphogenesis. The development and study of a bone model system has identified the developmental cascade of bone differentiation as consisting of chemotaxis of mesenchymal cells, proliferation of these progenitor cells, differentiation of these cells into chondroblasts, cartilage calcification, vascular invasion, bone formation, remodeling, and finally, marrow differentiation (Reddi (1981) Collagen Rel. Res. 1:209-206). Proteins capable of inducing endochondral bone formation in a mammal when implanted in association with a matrix now have been identified in a number of different mammalian species, as have the genes encoding these proteins, (see, for example, U.S. Patent No. 4,968,590 and U.S. Patent No. 5,011,691, Ozkaynak, et al., (1990) EMBO J 9:2085-2093, and Ozkaynak et al., (1991) Biochem. Biophys. Res. Commun. 179:116-123 and United States patent number 5,266,683 issued on November 30, 1993. These proteins, which share significant amino acid sequence homology with one another as well as structural similarities with various members of the TGF-B super family of proteins, have been shown to induce endochondral bone formation and/or cartilage formation when implanted in a mammal in association with a suitably modified matrix. Proteins capable of inducing a similar developmental cascade of tissue morphogenesis of other tissues have not been identified.

It is an object of this invention to provide morphogenic proteins ("morphogens"), and methods for identifying these proteins, which are capable of inducing the developmental cascade of tissue

morphogenesis for a variety of tissues in mammals different from bone or cartilage. This morphogenic activity includes the ability to induce proliferation and differentiation of progenitor cells, and the

5 ability to support and maintain the differentiated phenotype through the progression of events that results in the formation of adult tissue. Another object is to provide genes encoding these proteins as well as methods for the expression and isolation of

10 these proteins, from either natural sources or biosynthetic sources, using recombinant DNA techniques. Still another object is to provide tissue-specific acellular matrices that may be used in combination with these proteins, and methods for their production.

15 Other objects include providing methods for increasing a progenitor cell population in a mammal, methods for stimulating progenitor cells to differentiate in vivo or in vitro and maintain their differentiated phenotype, methods for inducing tissue-specific growth

20 in vivo and methods for the replacement of diseased or damaged tissue in vivo. These and other objects and features of the invention will be apparent from the description, drawings, and claims which follow.

Summary of the Invention

This invention provides morphogenic proteins ("morphogens") capable of inducing the developmental cascade of tissue morphogenesis in a mammal. In particular, these proteins are capable of inducing the proliferation of uncommitted progenitor cells, and inducing the differentiation of these stimulated progenitor cells in a tissue-specific manner under appropriate environmental conditions. In addition, the morphogens are capable of supporting the growth and maintenance of these differentiated cells. These morphogenic activities allow the proteins of this invention to initiate and maintain the developmental cascade of tissue morphogenesis in an appropriate, morphogenically permissive environment, stimulating stem cells to proliferate and differentiate in a tissue-specific manner, and inducing the progression of events that culminate in new tissue formation. These morphogenic activities also allow the proteins to stimulate the "redifferentiation" of cells previously induced to stray from their differentiation path. Under appropriate environmental conditions it is anticipated that these morphogens also may stimulate the "dedifferentiation" of committed cells (see infra.)

In one aspect of the invention, the proteins and compositions of this invention are useful in the replacement of diseased or damaged tissue in a mammal, particularly when the damaged tissue interferes with normal tissue or organ function. Accordingly, it is anticipated that the proteins of this invention will be useful in the repair of damaged tissue such as, for example, damaged lung tissue resulting from emphysema, cirrhotic kidney or liver tissue, damaged heart or

blood vessel tissue, as may result from cardiomyopathies and/or atherothrombotic or cardioembolic strokes, damaged stomach tissue resulting from ulceric perforations or their repair, damaged
5 neural tissue as may result from physical injury, degenerative diseases such as Alzheimer's disease or multiple sclerosis or strokes, damaged dentin tissue as may result from disease or mechanical injury. When the proteins of this invention are provided to, or their
10 expression stimulated at, a tissue-specific locus, the developmental cascade of tissue morphogenesis is induced (see *infra*). Cells stimulated ex vivo by contact with the proteins or agents capable of stimulating morphogen expression in these cells also
15 may be provided to the tissue locus. In these cases the existing tissue provides the necessary matrix requirements, providing a suitable substratum for the proliferating and differentiating cells in a morphogenically permissive environment, as well as
20 providing the necessary signals for directing the tissue-specificity of the developing tissue. Alternatively, the proteins or stimulated cells may be combined with a formulated matrix and implanted as a device at a locus in vivo. The formulated matrix
25 should be a biocompatible, preferably biodegradable, appropriately modified tissue-specific acellular matrix having the characteristics described below.

In many instances, the loss of tissue function
30 results from scar tissue, formed in response to an initial or repeated injury to the tissue. The degree of scar tissue formation generally depends on the regenerative properties of the injured tissue, and on the degree and type of injury. Thus, in another

aspect, the invention includes morphogens that may be used to prevent or substantially inhibit the formation of scar tissue by providing the morphogens, or morphogen-stimulated cells, to a newly injured tissue
5 loci (see infra).

The morphogens of this invention also may be used to increase or regenerate a progenitor or stem cell population in a mammal. For example, progenitor
10 cells may be isolated from an individual's bone marrow, stimulated ex vivo for a time and at a morphogen concentration sufficient to induce the cells to proliferate, and returned to the bone marrow. Other sources of progenitor cells that may be suitable
15 include biocompatible cells obtained from a cultured cell line, stimulated in culture, and subsequently provided to the body. Alternatively, the morphogen may be provided systemically, or implanted, injected or otherwise provided to a progenitor cell population in
20 an individual to induce its mitogenic activity in vivo. For example, an agent capable of stimulating morphogen expression in the progenitor cell population of interest may be provided to the cells in vivo, for example systemically, to induce mitogenic activity.
25 Similarly, a particular population of hemopoietic stem cells may be increased by the morphogens of this invention, for example by perfusing an individual's blood to extract the cells of interest, stimulating these cells ex vivo, and returning the stimulated cells
30 to the blood. It is anticipated that the ability to augment an individual's progenitor cell population will significantly enhance existing methods for treating disorders resulting from a loss or reduction of a renewable cell population. Two particularly
35 significant applications include the treatment of blood

disorders and impaired or lost immune function. Other cell populations whose proliferation may be exploited include the stem cells of the epidermis, which may be used in skin tissue regeneration, and the stem cells of
5 the gastrointestinal lining, for example, in the healing of ulcers.

In still another aspect of the invention, the morphogens also may be used to support the growth and
10 maintenance of differentiated cells, inducing existing differentiated cells to continue expressing their phenotype. It is anticipated that this activity will be particularly useful in the treatment of tissue disorders where loss of function is caused by cells
15 becoming senescent or quiescent, such as may occur in osteoporosis. Application of the protein directly to the cells to be treated, or providing it by systemic injection, can be used to stimulate these cells to continue expressing their phenotype, thereby
20 significantly reversing the effects of the dysfunction (see *infra*). Alternatively, administration of an agent capable of stimulating morphogen expression in vivo also may be used. In addition, the morphogens of this invention also may be used in gene therapy protocols to
25 stimulate the growth of quiescent cells, thereby potentially enhancing the ability of these cells to incorporate exogenous DNA.

In yet another aspect of the invention, the
30 morphogens of this invention also may be used to induce "redifferentiation" of cells that have strayed from their differentiation pathway, such as can occur during tumorigenesis. It is anticipated that this activity of the proteins will be particularly useful in treatments
35 to reduce or substantially inhibit the growth of

neoplasms. The method also is anticipated to induce the de-and re-differentiation of these cells. As described supra, the proteins may be provided to the cells directly or systemically, or an agent capable of
5 stimulating morphogen expression in vivo may be provided.

Finally, modulations of endogenous morphogen levels may be monitored as part of a method for detecting
10 tissue dysfunction. Specifically, modulations in endogenous morphogen levels are anticipated to reflect changes in tissue or organ stasis. Tissue stasis may be monitored by detecting changes in the levels of the morphogen itself. For example, tissue samples may be
15 obtained at intervals and the concentration of the morphogen present in the tissue detected by standard protein detection means known to those skilled in the art. As an example, a binding protein capable of interacting specifically with the morphogen of
20 interest, such as an anti-morphogen antibody, may be used to detect the morphogen in a standard immunoassay. The morphogen levels detected then may be compared, the changes in the detected levels being indicative of the status of the tissue. Modulations in endogenous
25 morphogen levels also may be monitored by detecting fluctuations in the body's natural antibody titer to morphogens (see infra.)

The morphogenic proteins and compositions of
30 this invention can be isolated from a variety of naturally-occurring sources, or they may be constructed biosynthetically using conventional recombinant DNA technology. Similarly, the matrices may be derived from organ-specific tissue, or they may be formulated
35 synthetically, as described below.

A key to these developments was the discovery and characterization of naturally-occurring osteogenic proteins followed by observation of their remarkable properties. These proteins, originally isolated from bone, are capable of inducing the full developmental cascade of bone formation, including vascularization, mineralization, and bone marrow differentiation, when implanted in a mammalian body in association with a suitably modified matrix. Native proteins capable of inducing this developmental cascade, as well as DNA sequences encoding these proteins now have been isolated and characterized for a number of different species (e.g., human and mouse OP-1, OP-2, and CBMP-2. See, for example, U.S. Patent Nos. 4,968,590 and 5,011,691; U.S. Patent No. 5,266,683 issued on November 30, 1993; Sampath et al. (1990) J. Bio. Chem 265:13198-13205; Ozkaynak, et al. (1990) EMBO J 9:2085-2093 and Ozkaynak, et al. (1991) Biochem. Biophys. Res. Commun. 179:116-123.) The mature forms of these proteins share substantial amino acid sequence homology, especially in the C-terminal regions of the mature proteins. In particular, the proteins share a conserved six or seven cysteine skeleton in this region (e.g., the linear arrangement of these C-terminal cysteine residues is essentially conserved in the different proteins, in addition to other, apparently required amino acids (see Table II, *infra*)).

Polypeptide chains not normally associated with bone or bone formation, but sharing substantial amino acid sequence homology with the C-terminus of the osteogenic proteins, including the conserved six or seven cysteine skeleton, also have been identified as competent for inducing bone in mammals. Among these are amino acid sequences identified in *Drosophila* and

Xenopus, (e.g., DPP and Vgl; see, for example, U.S. Patent No. 5,011,691 and Table II, *infra*). In addition, non-native biosynthetic constructs designed based on extrapolation from these sequence homologies, including the conserved six or seven cysteine skeleton, have been shown to induce endochondral bone formation in mammals when implanted in association with an appropriate matrix (see U.S. Pat. No. 5,011,691 and Table III, *infra*).

10

It has now been discovered that this "family" of proteins sharing substantial amino acid sequence homology and the conserved six or seven cysteine skeleton are true morphogens, capable of inducing, in addition to bone and cartilage, tissue-specific morphogenesis for a variety of other organs and tissues. The proteins apparently bind to surface receptors or otherwise contact and interact with progenitor cells, predisposing or stimulating the cells to proliferate and differentiate in a morphogenically permissive environment. The morphogens are capable of inducing the developmental cascade of cellular and molecular events that culminate in the formation of new organ-specific tissue, including any vascularization, connective tissue formation, and nerve enervation as required by the naturally occurring tissue.

It also has been discovered that the way in which the cells differentiate, whether, for example, they differentiate into bone-producing osteoblasts, hemopoietic cells, or liver cells, depends on the nature of their local environment (see *infra*). Thus, in addition to requiring a suitable substratum on which to anchor, the proliferating and differentiating cells also require appropriate signals to direct their

tissue-specificity. These signals may take the form of cell surface markers.

<

5 When the morphogens (or progenitor cells stimulated by these morphogens) are provided at a tissue-specific locus (e.g., by systemic injection or by implantation or injection at a tissue-specific locus, or by administration of an agent capable of
10 stimulating morphogen expression in vivo), the existing tissue at that locus, whether diseased or damaged, has the capacity of acting as a suitable matrix. Alternatively, a formulated matrix may be externally provided together with the stimulated progenitor cells
15 or morphogen, as may be necessary when the extent of injury sustained by the damaged tissue is large. The matrix should be a biocompatible, suitably modified acellular matrix having dimensions such that it allows the influx, differentiation, and proliferation of
20 migratory progenitor cells, and is capable of providing a morphogenically permissive environment (see *infra*). The matrix preferably is tissue-specific, and biodegradable.

25 Formulated matrices may be generated from dehydrated organ-specific tissue, prepared for example, by treating the tissue with solvents to substantially remove the non-structural components from the tissue. Alternatively, the matrix may be formulated
30 synthetically using a biocompatible, preferably in vivo

biodegradable, structural polymer such as collagen in association with suitable tissue-specific cell attachment factors. Currently preferred structural polymers comprise tissue-specific collagens. Currently preferred cell attachment factors include glycosaminoglycans and proteoglycans. The matrix further may be treated with an agent or agents to increase the number of pores and micropits on its surfaces, so as to enhance the influx, proliferation and differentiation of migratory progenitor cells from the body of the mammal.

Among the proteins useful in this invention are proteins originally identified as osteogenic proteins, such as the OP-1, OP-2 and CBMP2 proteins, as well as amino acid sequence-related proteins such as DPP (from *Drosophila*), Vgl (from *Xenopus*), Vgr-1 (from mouse, see Table II and Seq. ID Nos.5-14), and the recently identified GDF-1 protein (Seq. ID No. 14). The members of this family, which include members of the TGF- β super-family of proteins, share substantial amino acid sequence homology in their C-terminal regions. Table I, below, describes the various morphogens identified to date, including their nomenclature as used herein, and Seq. ID references.

TABLE I

30	"OP-1"	Refers generically to the group of morphogenically active proteins expressed from part or all of a DNA sequence encoding OP-1 protein, including allelic and species variants thereof, e.g., human OP-1 ("hOP-1", Seq. ID No. 5, mature
35		

protein amino acid sequence), or mouse
OP-1 ("mOP-1", Seq. ID No. 6, mature
protein amino acid sequence.) The
conserved seven cysteine skeleton is
5 defined by residues 38 to 139 of Seq. ID
Nos. 5 and 6. The cDNA sequences and the
amino acids encoding the full length
proteins are provided in Seq. Id Nos. 16
and 17 (hOP1) and Seq. ID Nos. 18 and 19
10 (mOP1.) The mature proteins are defined
by residues 293-431 (hOP1) and 292-430
(mOP1). The "pro"regions of the proteins,
cleaved to yield the mature,
morphogenically active proteins are
15 defined essentially by residues 30-292
(hOP1) and residues 30-291 (mOP1).

"OP-2"

20 refers generically to the group of active
proteins expressed from part or all of a
DNA sequence encoding OP-2 protein,
including allelic and species variants
thereof, e.g., human OP-2 ("hOP-2", Seq.
ID No. 7, mature protein amino acid
sequence) or mouse OP-2 ("mOP-2", Seq. ID
25 No. 8, mature protein amino acid
sequence). The conserved seven cysteine
skeleton is defined by residues 38 to 139
of Seq. ID Nos. 7 and 8. The cDNA
sequences and the amino acids encoding the
30 full length proteins are provided in Seq.
Id Nos. 20 and 21 (hOP2) and Seq. ID Nos.
22 and 23 (mOP2.) The mature proteins are
defined essentially by residues 264-402
(hOP2) and 261-399 (mOP2). The "pro"
35 regions of the proteins, cleaved to yield

the mature, morphogenically active proteins are defined essentially by residues 18-263 (hOP2) and residues 18-260 (mOP1).

- 5
"CBMP2" refers generically to the morphogenically active proteins expressed from a DNA sequence encoding the CBMP2 proteins, including allelic and species variants thereof, e.g., human CBMP2A ("CBMP2A(fx)", Seq ID No. 9) or human CBMP2B DNA ("CBMP2B(fx)", Seq. ID No. 10).
- 10
- "DPP(fx)" refers to protein sequences encoded by the Drosophila DPP gene and defining the conserved seven cysteine skeleton (seq. ID No. 11).
- 15
- "Vgl(fx)" refers to protein sequences encoded by the Xenopus Vgl gene and defining the conserved seven cysteine skeleton (Seq. ID No. 12).
- 20
- "Vgr-1(fx)" refers to protein sequences encoded by the murine Vgr-1 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 13).
- 25
- "GDF-1(fx)" refers to protein sequences encoded by the human GDF-1 gene and defining the conserved seven cysteine skeleton (seq. ID No. 14).
- 30

The OP-2 proteins have an additional cysteine residue in this region (e.g., see residue 41 of Seq. ID Nos. 7 and 8), in addition to the conserved cysteine skeleton in common with the other proteins in this family. The GDF-1 protein has a four amino acid insert within the conserved skeleton (residues 44-47 of Seq. ID No. 14) but this insert likely does not interfere with the relationship of the cysteines in the folded structure. In addition, the CBMP2 proteins are missing one amino acid residue within the cysteine skeleton.

The morphogens are inactive when reduced, but are active as oxidized homodimers and when oxidized in combination with other morphogens of this invention. Thus, as defined herein, a morphogen of this invention is a dimeric protein comprising a pair of polypeptide chains, wherein each polypeptide chain comprises at least the C-terminal six cysteine skeleton defined by residues 43-139 of Seq. ID No. 5, including functionally equivalent arrangements of these cysteines (e.g., amino acid insertions or deletions which alter the linear arrangement of the cysteines in the sequence but not their relationship in the folded structure), such that, when the polypeptide chains are folded, the dimeric protein species comprising the pair of polypeptide chains has the appropriate three-dimensional structure, including the appropriate intra- or inter-chain disulfide bonds such that the protein is capable of acting as a morphogen as defined herein. Specifically, the protein is capable of any of the following biological functions in a morphogenically permissive environment: stimulating proliferation of progenitor cells; stimulating the differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting the growth and maintenance of differentiated cells, including the

"redifferentiation" of these cells. In addition, it is also anticipated that the morphogens of this invention will be capable of inducing dedifferentiation of committed cells under appropriate environmental
5 conditions.

In one preferred aspect, the morphogens of this invention comprise one of two species of generic amino acid sequences: Generic Sequence 1 (Seq. ID
10 No. 1) or Generic Sequence 2 (Seq. ID No. 2); where each Xaa indicates one of the 20 naturally-occurring L-isomer, α -amino acids or a derivative thereof. Generic Sequence 1 comprises the conserved six cysteine skeleton and Generic Sequence 2 comprises the conserved
15 six cysteine skeleton plus the additional cysteine identified in OP-2 (see residue 36, Seq. ID No. 2). In another preferred aspect, these sequences further comprise the following additional sequence at their N-terminus:

20

Cys Xaa Xaa Xaa Xaa (Seq. ID No. 15)
1 5

Preferred amino acid sequences within the
25 foregoing generic sequences include: Generic Sequence 3 (Seq. ID No. 3) and Generic Sequence 4 (Seq. ID No. 4), listed below, which accommodate the homologies shared among the various preferred members of this morphogen family identified to date (see Table II), as
30 well as the amino acid sequence variation among them. Generic Sequences 3 and 4 are composite amino acid sequences of the proteins presented in Table II and identified in Seq. ID Nos. 5-14. The generic sequences include both the amino acid identity shared by the
35 sequences in Table II, as well as alternative residues

for the variable positions within the sequence. Note that these generic sequences allow for an additional cysteine at position 41 or 46 in Generic Sequences 3 or 4, respectively, providing an appropriate cysteine skeleton where inter- or intramolecular disulfide bonds can form, and contain certain critical amino acids which influence the tertiary structure of the proteins.

Generic Sequence 3

10 Leu Tyr Val Xaa Phe
 1 5
 Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
 10
 Xaa Ala Pro Xaa Gly Xaa Xaa Ala
15 15 20
 Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
 25 30
 Xaa Pro Xaa Xaa Xaa Xaa Xaa
 35
20 Xaa Xaa Xaa Asn His Ala Xaa Xaa
 40 45
 Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa
 50
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
25 55 60
 Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
 65

Xaa Xaa Xaa Leu Xaa Xaa Xaa

70

75

Xaa Xaa Xaa Xaa Val Xaa Leu Xaa

80

5 Xaa Xaa Xaa Xaa Met Xaa Val Xaa

85

90

Xaa Cys Gly Cys Xaa

95

wherein each Xaa is independently selected from a group
 10 of one or more specified amino acids defined as
 follows: "Res." means "residue" and Xaa at res.4 =
 (Ser, Asp or Glu); Xaa at res.6 = (Arg, Gln, Ser or
 Lys); Xaa at res.7 = (Asp or Glu); Xaa at res.8 = (Leu
 or Val); Xaa at res.11 = (Gln, Leu, Asp, His or Asn);
 15 Xaa at res.12 = (Asp, Arg or Asn); Xaa at res.14 = (Ile
 or Val); Xaa at res.15 = (Ile or Val); Xaa at res.18 =
 (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.20 = (Tyr
 or Phe); Xaa at res.21 = (Ala, Ser, Asp, Met, His, Leu
 or Gln); Xaa at res.23 = (Tyr, Asn or Phe); Xaa at
 20 res.26 = (Glu, His, Tyr, Asp or Gln); Xaa at res.28 =
 (Glu, Lys, Asp or Gln); Xaa at res.30 = (Ala, Ser, Pro
 or Gln); Xaa at res.31 = (Phe, Leu or Tyr); Xaa at
 res.33 = (Leu or Val); Xaa at res.34 = (Asn, Asp, Ala
 or Thr); Xaa at res.35 = (Ser, Asp, Glu, Leu or Ala);
 25 Xaa at res.36 = (Tyr, Cys, His, Ser or Ile); Xaa at
 res.37 = (Met, Phe, Gly or Leu); Xaa at res.38 = (Asn
 or Ser); Xaa at res.39 = (Ala, Ser or Gly); Xaa at
 res.40 = (Thr, Leu or Ser); Xaa at res.44 = (Ile or
 Val); Xaa at res.45 = (Val or Leu); Xaa at res.46 =
 30 (Gln or Arg); Xaa at res.47 = (Thr, Ala or Ser); Xaa at
 res.49 = (Val or Met); Xaa at res.50 = (His or Asn);
 Xaa at res.51 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa

at res.52 = (Ile, Met, Asn, Ala or Val); Xaa at res.53
 = (Asn, Lys, Ala or Glu); Xaa at res.54 = (Pro or Ser);
 Xaa at res.55 = (Glu, Asp, Asn, or Gly); Xaa at res.56
 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser or Ala); Xaa at
 5 res.57 = (Val, Ala or Ile); Xaa at res.58 = (Pro or
 Asp); Xaa at res.59 = (Lys or Leu); Xaa at res.60 =
 (Pro or Ala); Xaa at res.63 = (Ala or Val); Xaa at
 res.65 = (Thr or Ala); Xaa at res.66 = (Gln, Lys, Arg
 or Glu); Xaa at res.67 = (Leu, Met or Val); Xaa at
 10 res.68 = (Asn, Ser or Asp); Xaa at res.69 = (Ala, Pro
 or Ser); Xaa at res.70 = (Ile, Thr or Val); Xaa at
 res.71 = (Ser or Ala); Xaa at res.72 = (Val or Met);
 Xaa at res.74 = (Tyr or Phe); Xaa at res.75 = (Phe, Tyr
 or Leu); Xaa at res.76 = (Asp or Asn); Xaa at res.77 =
 15 (Asp, Glu, Asn or Ser); Xaa at res.78 = (Ser, Gln, Asn
 or Tyr); Xaa at res.79 = (Ser, Asn, Asp or Glu); Xaa at
 res.80 = (Asn, Thr or Lys); Xaa at res.82 = (Ile or
 Val); Xaa at res.84 = (Lys or Arg); Xaa at res.85 =
 (Lys, Asn, Gln or His); Xaa at res.86 = (Tyr or His);
 20 Xaa at res.87 = (Arg, Gln or Glu); Xaa at res.88 =
 (Asn, Glu or Asp); Xaa at res.90 = (Val, Thr or Ala);
 Xaa at res.92 = (Arg, Lys, Val, Asp or Glu); Xaa at
 res.93 = (Ala, Gly or Glu); and Xaa at res.97 = (His or
 Arg); and Generic Seq. 4:

25

Generic Sequence 4

	Cys	Xaa	Xaa	Xaa	Xaa	Leu	Tyr	Val	Xaa	Phe
	1				5					10
30	Xaa	Xaa	Xaa	Gly	Trp	Xaa	Xaa	Trp	Xaa	
				15						
	Xaa	Ala	Pro	Xaa	Gly	Xaa	Xaa	Ala		
	20					25				
	Xaa	Tyr	Cys	Xaa	Gly	Xaa	Cys	Xaa		
35			30					35		

Xaa Pro Xaa Xaa Xaa Xaa Xaa
 40
 Xaa Xaa Xaa Asn His Ala Xaa Xaa
 45 50
 5 Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa
 55
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
 60 65
 Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
 10 70
 Xaa Xaa Xaa Leu Xaa Xaa Xaa
 75 80
 Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
 85
 15 Xaa Xaa Xaa Xaa Met Xaa Val Xaa
 90 95
 Xaa Cys Gly Cys Xaa
 100

wherein each Xaa is independently selected from a group
 20 of one or more specified amino acids as defined by the
 following: "Res." means "residue" and Xaa at res.2 =
 (Lys or Arg); Xaa at res.3 = (Lys or Arg); Xaa at res.4
 = (His or Arg); Xaa at res.5 = (Glu, Ser, His, Gly, Arg
 or Pro); Xaa at res.9 = (Ser, Asp or Glu); Xaa at
 25 res.11 = (Arg, Gln, Ser or Lys); Xaa at res.12 = (Asp
 or Glu); Xaa at res.13 = (Leu or Val); Xaa at res.16 =
 (Gln, Leu, Asp, His or Asn); Xaa at res.17 = (Asp, Arg,
 or Asn); Xaa at res.19 = (Ile or Val); Xaa at res.20 =
 (Ile or Val); Xaa at res.23 = (Glu, Gln, Leu, Lys, Pro
 30 or Arg); Xaa at res.25 = (Tyr or Phe); Xaa at res.26 =
 (Ala, Ser, Asp, Met, His, Leu, or Gln); Xaa at res.28 =
 (Tyr, Asn or Phe); Xaa at res.31 = (Glu, His, Tyr, Asp
 or Gln); Xaa at res.33 = (Glu, Lys, Asp or Gln); Xaa at
 res.35 = (Ala, Ser or Pro); Xaa at res.36 = (Phe, Leu
 35 or Tyr); Xaa at res.38 = (Leu or Val); Xaa at res.39 =

(Asn, Asp, Ala or Thr); Xaa at res.40 = (Ser, Asp, Glu, Leu or Ala); Xaa at res.41 = (Tyr, Cys, His, Ser or Ile); Xaa at res.42 = (Met, Phe, Gly or Leu); Xaa at res.44 = (Ala, Ser or Gly); Xaa at res.45 = (Thr, Leu or Ser); Xaa at res.49 = (Ile or Val); Xaa at res.50 = (Val or Leu); Xaa at res.51 = (Gln or Arg); Xaa at res.52 = (Thr, Ala or Ser); Xaa at res.54 = (Val or Met); Xaa at res.55 = (His or Asn); Xaa at res.56 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.57 = (Ile, Met, Asn, Ala or Val); Xaa at res.58 = (Asn, Lys, Ala or Glu); Xaa at res.59 = (Pro or Ser); Xaa at res.60 = (Glu, Asp, or Gly); Xaa at res.61 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser or Ala); Xaa at res.62 = (Val, Ala or Ile); Xaa at res.63 = (Pro or Asp); Xaa at res.64 = (Lys or Leu); Xaa at res.65 = (Pro or Ala); Xaa at res.68 = (Ala or Val); Xaa at res.70 = (Thr or Ala); Xaa at res.71 = (Gln, Lys, Arg or Glu); Xaa at res.72 = (Leu, Met or Val); Xaa at res.73 = (Asn, Ser or Asp); Xaa at res.74 = (Ala, Pro or Ser); Xaa at res.75 = (Ile, Thr or Val); Xaa at res.76 = (Ser or Ala); Xaa at res.77 = (Val or Met); Xaa at res.79 = (Tyr or Phe); Xaa at res.80 = (Phe, Tyr or Leu); Xaa at res.81 = (Asp or Asn); Xaa at res.82 = (Asp, Glu, Asn or Ser); Xaa at res.83 = (Ser, Gln, Asn or Tyr); Xaa at res.84 = (Ser, Asn, Asp or Glu); Xaa at res.85 = (Asn, Thr or Lys); Xaa at res.87 = (Ile or Val); Xaa at res.89 = (Lys or Arg); Xaa at res.90 = (Lys, Asn, Gln or His); Xaa at res.91 = (Tyr or His); Xaa at res.92 = (Arg, Gln or Glu); Xaa at res.93 = (Asn, Glu or Asp); Xaa at res.95 = (Val, Thr or Ala); Xaa at res.97 = (Arg, Lys, Val, Asp or Glu); Xaa at res.98 = (Ala, Gly or Glu); and Xaa at res.102 = (His or Arg).

Particularly useful sequences for use as morphogens in this invention include the C-terminal

domains, e.g., the C-terminal 96-102 amino acid residues of Vgl, Vgr-1, DPP, OP-1, OP-2, CBMP-2A, CBMP-2B and GDF-1 (see Table II, infra, and Seq. ID Nos. 5-14) which include at least the conserved six or
5 seven cysteine skeleton. In addition, biosynthetic constructs designed from the generic sequences, such as COP-1, 3-5, 7, 16 (see Table III, infra) also are useful. Other sequences include the C-terminal CBMP3 and the inhibins/activin proteins (see, for example,
10 U.S. Pat. Nos. 4,968,590 and 5,011,691). Accordingly, other useful sequences are those sharing at least 70% amino acid sequence homology, and preferably 80% homology with any of the sequences above. These are anticipated to include allelic and species variants and
15 mutants, and biosynthetic muteins, as well as novel members of this morphogenic family of proteins. Particularly envisioned in the family of related proteins are those proteins exhibiting morphogenic activity and wherein the amino acid changes from the
20 preferred sequences include conservative changes, e.g., those as defined by Dayoff et al., Atlas of Protein Sequence and Structure; vol. 5, Suppl. 3, pp. 345-362, (M.O. Dayoff, ed., Nat'l BioMed. Research Fdn., Washington, D.C. 1979).

25

The currently most preferred protein sequences useful as morphogens in this invention include those having greater than 60% identity, preferably greater than 65% identity, with the amino acid sequence
30 defining the conserved six cysteine skeleton of hOP1 (e.g., residues 43-139 of Seq. ID No. 5). These most preferred sequences include both allelic and species variants of the OP1 and OP2 proteins.

The invention thus provides proteins comprising any of the polypeptide chains described above, whether isolated from naturally-occurring sources, or produced by recombinant DNA techniques, and
5 includes allelic and species variants of these proteins, naturally-occurring or biosynthetic mutants thereof, as well as various truncated and fusion constructs. Deletion or addition mutants also are envisioned to be active (see infra), including those
10 which may alter the conserved C-terminal cysteine skeleton, provided that the alteration does not functionally disrupt the relationship of these cysteines in the folded structure. Accordingly, such active forms are considered the equivalent of the
15 specifically described constructs disclosed herein. The proteins may include forms having varying glycosylation patterns, varying N-termini, a family of related proteins having regions of amino acid sequence homology, and active truncated or mutated forms of
20 native or biosynthetic proteins, produced by expression of recombinant DNA in host cells.

The morphogenic proteins can be expressed from intact or truncated cDNA or from synthetic DNAs in
25 procaryotic or eucaryotic host cells, and purified, cleaved, refolded, and dimerized to form morphogenically active compositions. Currently preferred host cells include E. coli or mammalian cells, such as CHO, COS or BSC cells.

30 Thus, in view of this disclosure, skilled genetic engineers can isolate genes from cDNA or genomic libraries of various different species which encode appropriate amino acid sequences, or construct
35 DNAs from oligonucleotides, and then can express them

-26-

in various types of host cells, including both prokaryotes and eukaryotes, to produce large quantities of active proteins capable of inducing tissue-specific cell differentiation and tissue morphogenesis in a variety of mammals including humans.

5 The invention thus further comprises these methods of inducing tissue-specific morphogenesis using the morphogenic proteins of this invention and pharmaceutical and therapeutic agents comprising the morphogens of this invention. The invention further comprises the use of these morphogens in the manufacture of pharmaceuticals for various
10 medical procedures, including procedures for inducing tissue growth, procedures for inducing progenitor cells proliferation, procedures to inhibit neoplasm growth and procedures to promote phenotypic cells expression of differentiated cells.

 In another aspect, the present invention provides a composition
15 composition comprising: (a) a biocompatible acellular matrix that provides an anchoring substratum for mammalian progenitor cells; and (b) a morphogen absorbed on said matrix at a concentration sufficient for inducing a developmental cascade of tissue-specific morphogenesis when said composition is disposed at a non-skeletal tissue locus in
20 vivo, said matrix having components specific for said tissue locus, wherein said morphogen comprises a dimeric protein that induces tissue-specific morphogenesis in said mammal and comprises a pair of folded polypeptides, said morphogen selected from the group consisting of: (i) a morphogen comprising at least 70% homology with
25 the C-terminal seven-cysteine domain of human OP-1, which correspond to residues 38 to 139 of SEQ ID NO: 5, (ii) a morphogen comprising at least 60% identity with the C-terminal seven-cysteine domain of human OP-1, which correspond to residues 38 to 139 of SEQ ID NO: 5; (iii) a morphogen comprising

-26a-

SEQ ID NO: 3 or SEQ ID NO: 4; and (iv) OP-1, OP-2, CBMP2, DPP, Vgl, Vgr-1, and GDF-1.

In another aspect, the present invention provides use of a composition comprising a morphogen selected from the group consisting of: (i) a morphogen comprising at least 70% homology with the C-terminal seven-cysteine domain of human OP-1, which correspond to residues 38 to 139 of SEQ ID NO: 5; (ii) a morphogen comprising at least 60% identity with the C-terminal seven-cysteine domain of human OP-1, which correspond to residues 38 to 139 of SEQ ID NO: 5; (iii) a morphogen comprising SEQ ID NO: 3 or SEQ ID NO: 4; and (iv) OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vgl, Vgr-1, and GDF-1; wherein the morphogen forms a dimeric protein and is capable of inducing ectopic bone formation for preventing or inhibiting the formation of scar tissue.

In another aspect, the present invention provides use of a composition comprising a morphogen selected from the group consisting of: (i) a morphogen comprising at least 70% homology with the C-terminal seven-cysteine domain of human OP-1, which correspond to residues 38 to 139 of SEQ ID NO: 5; (ii) a morphogen comprising at least 60% identity with the C-terminal seven-cysteine domain of human OP-1, which correspond to residues 38 to 139 of SEQ ID NO: 5; (iii) a morphogen comprising SEQ ID NO: 3 or SEQ ID NO: 4; and (iv) OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vgl, Vgr-1, and GDF-1; wherein the morphogen forms a dimeric protein and is capable of inducing ectopic bone formation, for regenerating skin tissue.

In another aspect, the present invention provides use of a composition comprising a morphogen selected from the group consisting of: (i) a morphogen comprising at least 70% homology with the C-terminal seven-cysteine domain of human OP-1, which correspond to residues 38 to 139 of SEQ ID NO: 5; (ii) a morphogen comprising

-26b-

at least 60% identity with the C-terminal seven-cysteine domain of human OP-1, which correspond to residues 38 to 139 of SEQ ID NO: 5; (iii) a morphogen comprising SEQ ID NO: 3 or SEQ ID NO: 4; and (iv) OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vgl, Vgr-1, and GDF-1; wherein the morphogen forms a dimeric protein and is capable of inducing ectopic bone formation for the treatment of noncongenital liver disorders.

In another aspect, the present invention provides use of a composition comprising a morphogen selected from the group consisting of: (i) a morphogen comprising at least 70% homology with the C-terminal seven-cysteine domain of human OP-1, which correspond to residues 38 to 139 of SEQ ID NO: 5; (ii) a morphogen comprising at least 60% identity with the C-terminal seven-cysteine domain of human OP-1, which correspond to residues 38 to 139 of SEQ ID NO: 5; (iii) a morphogen comprising SEQ ID NO: 3 or SEQ ID NO: 4; and (iv) OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vgl, Vgr-1, and GDF-1; wherein the morphogen forms a dimeric protein and is capable of inducing ectopic bone formation, for the treatment of noncongenital blood disorders.

In another aspect, the present invention provides use of a composition comprising a morphogen selected from the group consisting of: (i) a morphogen comprising at least 70% homology with the C-terminal seven-cysteine domain of human OP-1, which correspond to residues 38 to 139 of SEQ ID NO: 5; (ii) a morphogen comprising at least 60% identity with the C-terminal seven-cysteine domain of human OP-1, which correspond to residues 38 to 139 of SEQ ID NO: 5; (iii) a morphogen comprising SEQ ID NO: 3 or SEQ ID NO: 4; and (iv) OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vgl, Vgr-1, and GDF-1; wherein the morphogen forms a dimeric protein and is capable of inducing ectopic bone formation for increasing progenitor cell populations.

In another aspect, the present invention provides use of a composition comprising a morphogen selected from the group consisting of; (i) a morphogen comprising at least 70% homology with

-26c-

the C-terminal seven-cysteine domain of human OP-1, which correspond to residues 38 to 139 of SEQ ID NO: 5; (ii) a morphogen comprising at least 60% identity with the C-terminal seven-cysteine domain of human OP-1, which correspond to residues 38 to 139 of SEQ ID NO: 5; (iii) a morphogen comprising SEQ ID NO: 3 or SEQ ID NO: 4; and (iv) OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vgl, Vgr-1, and GDF-1; wherein the morphogen forms a dimeric protein and is capable of inducing ectopic bone formation, for stimulating the growth and maintaining the phenotype of differentiated cells.

10 In another aspect, the present invention provides use of a composition comprising a morphogen selected from the group consisting of: (i) a morphogen comprising at least 70% homology with the C-terminal seven-cysteine domain of human OP-1, which correspond to residues 38 to 139 of SEQ ID NO: 5; (ii) a morphogen comprising at least 60% identity with the C-terminal seven-cysteine domain of human OP-1, which correspond to residues 38 to 139 of SEQ ID NO: 5; (iii) a morphogen comprising SEQ ID NO: 3 or SEQ ID NO: 4; and (iv) OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vgl, Vgr-1, and GDF-1; wherein the morphogen forms a dimeric protein and is capable of inducing ectopic bone formation for dentin regeneration.

20 In another aspect, the present invention provides use of a composition comprising a morphogen selected from the group consisting of: (i) a morphogen comprising at least 70% homology with the C-terminal seven-cysteine domain of human OP-1, which correspond to residues 38 to 139 of SEQ ID NO: 5; (ii) a morphogen comprising at least 60% identity with the C-terminal seven-cysteine domain of human OP-1, which correspond to residues 38 to 139 of SEQ ID NO: 5; (iii) a morphogen comprising SEQ ID NO: 3 or SEQ ID NO: 4; and (iv) OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vgl, Vgr-1, and GDF-1; wherein the morphogen forms a dimeric protein and is capable of inducing ectopic bone formation for healing of gastrointestinal ulcers.

-26d-

In another aspect, the present invention provides use of a composition comprising a morphogen selected from the group consisting of: (i) a morphogen comprising at least 70% homology with the C-terminal seven-cysteine domain of human OP-1, which correspond
5 to residues 38 to 139 of SEQ ID NO: 5; (ii) a morphogen comprising at least 60% identity with the C-terminal seven-cysteine domain of human OP-1, which correspond to residues 38 to 139 of SEQ ID NO: 5; (iii) a morphogen comprising SEQ ID NO: 3 or SEQ ID NO: 4; (iv) OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vgl, Vgr-1, and GDF-1; wherein the
10 morphogen forms a dimeric protein and is capable of inducing ectopic bone formation for non-skeletal tissue replacement.

In another aspect, the present invention provides use of a composition comprising a morphogen selected from the group consisting of: (i) a morphogen comprising at least 70% homology with
15 the C-terminal seven-cysteine domain of human OP-1, which correspond to residues 38 to 139 of SEQ ID NO: 5; (ii) a morphogen comprising at least 60% identity with the C-terminal seven-cysteine domain of human OP-1, which correspond to residues 38 to 139 of SEQ ID NO: 5; (iii) a morphogen comprising SEQ ID NO: 3 or SEQ ID NO: 4; and (iv)
20 OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vgl, Vgr-1, and GDF-1, wherein the morphogen forms a dimeric protein and is capable of inducing ectopic bone formation for neuroprotection or the treatment of noncongenital degenerative nerve diseases.

In another aspect, the present invention provides use of a
25 composition comprising a morphogen selected from the group consisting of: (i) a morphogen comprising at least 70% homology with the C-terminal seven-cysteine domain of human OP-1, which correspond to residues 38 to 139 of SEQ ID NO: 5; (ii) a morphogen comprising at least 60% identity with the C-terminal seven-cysteine domain of
30 human OP-1, which correspond to residues 38 to 139 of SEQ ID NO: 5; (iii) a morphogen comprising SEQ ID NO: 3 or SEQ ID NO: 4; and (iv) OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vgl, Vgr-1, and GDF-1; wherein the

-26e-

morphogen forms a dimeric protein and is capable of inducing ectopic bone formation for the treatment of lung tissue damaged by emphysema.

5 In another aspect, the present invention provides use of a composition comprising a morphogen selected from the group consisting of: OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vg1, Vgr-1, GDF-1, and a morphogen comprising SEQ ID NO:3 or SEQ ID NO:4; wherein the morphogen forms a dimeric protein and is capable of inducing ectopic bone formation, for regenerating skin tissue.

10 In another aspect, the present invention provides use of a composition comprising a morphogen selected from the group consisting of: OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vg1, Vgr-1, GDF-1, and a morphogen comprising SEQ ID NO:3 or SEQ ID NO:4; wherein the morphogen forms a dimeric protein and is capable of inducing ectopic
15 bone formation, for the treatment of noncongenital liver disorders.

In another aspect, the present invention provides use of a composition comprising a morphogen selected from the group consisting of: OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vg1, Vgr-1, GDF-1, and a
20 morphogen comprising SEQ ID NO:3 or SEQ ID NO:4; wherein the morphogen forms a dimeric protein and is capable of inducing ectopic bone formation, for the treatment of noncongenital blood disorders.

In another aspect, the present invention provides use of a
25 composition comprising a morphogen selected from the group consisting of: OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vg1, Vgr-1, GDF-1, and a morphogen comprising SEQ ID NO:3 or SEQ ID NO:4; wherein the morphogen forms a dimeric protein and is capable of inducing ectopic bone formation, for increasing progenitor cell populations *in vivo*.

30

In another aspect, the present invention provides use of a composition comprising a morphogen selected from the group consisting

-26f-

of; OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vg1, Vgr-1, GDF-1, and a morphogen comprising SEQ ID NO:3 or SEQ ID NO:4; wherein the morphogen forms a dimeric protein and is capable of inducing ectopic bone formation, for stimulating the growth and maintaining the phenotype of differentiated cells.

In another aspect, the present invention provides use of a composition comprising a morphogen selected from the group consisting of: OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vg1, Vgr-1, GDF-1, and a morphogen comprising SEQ ID NO:3 or SEQ ID NO:4; wherein the morphogen forms a dimeric protein and is capable of inducing ectopic bone formation, for the healing of gastrointestinal ulcers.

In another aspect, the present invention provides use of a composition comprising a morphogen selected from the group consisting of: OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vg1, Vgr-1, GDF-1, and a morphogen comprising SEQ ID NO:3 or SEQ ID NO:4; wherein the morphogen forms a dimeric protein and is capable of inducing ectopic bone formation, for neuroprotection or the treatment of noncongenital degenerative nerve diseases.

In another aspect, the present invention provides use of a composition comprising a morphogen selected from the group consisting of: OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vg1, Vgr-1, GDF-1, and a morphogen comprising SEQ ID NO:3 or SEQ ID NO:4; wherein the morphogen forms a dimeric protein and is capable of inducing ectopic bone formation, for the treatment of lung tissue damaged by emphysema.

Brief Description of the Drawings

The foregoing and other objects and features of this invention, as well as the invention itself, may
5 be more fully understood from the following description, when read together with the accompanying drawings, in which:

FIGURE 1 is a photomicrograph of a Northern
10 Blot identifying Vgr-1 specific transcripts in various adult murine tissues;

FIGURE 2 is a photomicrograph of a Northern
Blot identifying mOP-1-specific mRNA expression in
15 various murine tissues prepared from 2 week old mice (panel A) and 5 week old mice (Panel B);

FIGURE 3 is a photomicrograph of Northern
Blots identifying mRNA expression of EF-Tu
20 (A, control), mOP-1 (B, D), and Vgr-1 (C) in (1) 17-day embryos and (2) 3-day post natal mice;

FIGURE 4A and 4B are photomicrographs showing
the presence of OP-1 (by immunofluorescence staining)
25 in the cerebral cortex (A) and spinal cord (B);

FIGURE 5A and 5B are photomicrographs
illustrating the ability of morphogen (OP-1) to induce
undifferentiated NG108 cells (5A) to undergo
30 differentiation of neural morphology (5B).

FIGURE 6A-6D are photomicrographs showing the
effect of morphogen (OP-1) on human embryo carcinoma
cell redifferentiation;
35

FIGURE 7 is a photomicrograph showing the effects of phosphate buffered saline (PBS, animal 1) or morphogen (OP-1, animal 2) on partially hepatectomized rats;

5

FIGURE 8A - 8C are photomicrographs showing the effect of no treatment (8A), carrier matrix treatment (8B) and morphogen treatment (OP-1,8C) on dentin regeneration.

10

Detailed Description

Purification protocols first were developed which enabled isolation of the osteogenic (bone inductive) protein present in crude protein extracts from mammalian bone. (See PCT US 89/01453, and U.S. 4,968,590.) The development of the procedure, coupled with the availability of fresh calf bone, enabled isolation of substantially pure bovine osteogenic protein (BOP). BOP was characterized significantly; its ability to induce cartilage and ultimately endochondral bone growth in cat, rabbit, and rat were demonstrated and studied; it was shown to be able to induce the full developmental cascade of bone formation previously ascribed to unknown protein or proteins in heterogeneous bone extracts. This dose dependent and highly specific activity was present whether or not the protein was glycosylated (see U.S. Patent No. 4,968,958, filed 4/8/88 and Sampath et al., (1990) J. Biol. Chem. 265: pp. 13198-13205). Sequence data obtained from the bovine materials suggested probe designs which were used to isolate genes encoding osteogenic proteins from different species. Human and murine osteogenic protein counterparts have now been identified and characterized (see, for example, U.S. Pat. No. 5,011,691, Ozkaynak, et al., (1990) EMBO J 9:2085-2093, and Ozkaynak et al., (1991) Biochem. Biophys. Res. Commun. 179:116-123, and United States Patent 5,266,683 which issued on November 30, 1993.

Sequence data from the bovine materials also suggested substantial homology with a number of proteins known in the art which were not known to play a role in bone formation. Bone formation assays

performed with these proteins showed that, when these proteins were implanted in a mammal in association with a suitable matrix, cartilage and endochondral bone formation was induced (see, for example, U.S. Patent
5 No. 5,011,691.) One of these proteins is DPP, a *Drosophila* protein known to play a role in dorsal-ventral specification and required for the correct morphogenesis of the imaginal discs. Two other
10 proteins are related sequences identified in *Xenopus* and mouse (Vgl and Vgr-1, respectively), thought to play a role in the control of growth and differentiation during embryogenesis. While DPP and Vgr-1 (or Vgr-1-like) transcripts have been identified in a variety of tissues (embryonic, neonatal and adult,
15 Lyons et al., (1989) PNAS 86:4554-4 558, and see *infra*), Vgl transcripts, which are maternally inherited and spatially restricted to the vegetal endoderm, decline dramatically after gastrulation.

20 From these homologies a generic consensus sequence was derived which encompasses the active sequence required for inducing bone morphogenesis in a mammal when implanted in association with a matrix. The generic sequence has at least a conserved six
25 cysteine skeleton (Generic Sequence 1, Seq. ID No. 1) or, optionally, a 7-cysteine skeleton (Generic Sequence 2, Seq. ID No. 2), which includes the conserved six cysteine skeleton defined by Generic Sequence 1, and an additional cysteine at residue 36,
30 accomodating the additional cysteine residue identified in the OP2 proteins. Each "Xaa" in the generic sequences indicates that any one of the 20 naturally-occurring L-isomer, α -amino acids or a derivative

thereof may be used at that position. Longer generic sequences which also are useful further comprise the following sequence at their N-termini:

5

Cys Xaa Xaa Xaa Xaa (Seq. ID No. 15)
 1 5

Biosynthetic constructs designed from this
 10 generic consensus sequence also have been shown to induce cartilage and/or endochondral bone formation (e.g., COP-1, COP-3, COP-4, COP-5, COP-7 and COP-16, described in U.S. Patent No. 5,011,691 and presented below in Table III.) Table II, set forth below,
 15 compares the amino acid sequences of the active regions of native proteins that have been identified as morphogens, including human OP-1 (hOP-1, Seq. ID Nos. 5 and 16-17), mouse OP-1 (mOP-1, Seq. ID Nos. 6 and 18-19), human and mouse OP-2 (Seq. ID Nos. 7, 8, and 20-22), CBMP2A (Seq. ID No. 9), CBMP2B (Seq. ID No. 10),
 20 DPP (from Drosophila, Seq. ID No. 11), Vgl, (from Xenopus, Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13), and GDF-1 (Seq. ID No. 14.) In the table, three dots indicates that the amino acid in that
 25 position is the same as the amino acid in hOP-1. Three dashes indicates that no amino acid is present in that position, and are included for purposes of illustrating homologies. For example, amino acid residue 60 of CBMP-2A and CBMP-2B is "missing". Of course, both
 30 these amino acid sequences in this region comprise Asn-Ser (residues 58, 59), with CBMP-2A then comprising Lys and Ile, whereas CBMP-2B comprises Ser and Ile.

TABLE II

	hOP-1	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val	
	mOP-1	
5	hOP-2	...	Arg	Arg	
	mOP-2	...	Arg	Arg	
	DPP	...	Arg	Arg	...	Ser	
	Vgl	Lys	Arg	His	
	Vgr-1	Gly	
10	CBMP-2A	Arg	...	Pro	
	CBMP-2B	...	Arg	Arg	...	Ser	
	GDF-1	...	Arg	Ala	Arg	Arg	
		1				5				
15	hOP-1	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp
	mOP-1
	hOP-2	Gln	Leu	...
	mOP-2	Ser	Leu	...
20	DPP	Asp	...	Ser	...	Val	Asp	...
	Vgl	Glu	...	Lys	...	Val	Asn
	Vgr-1	Gln	...	Val
	CBMP-2A	Asp	...	Ser	...	Val	Asn	...
	CBMP-2B	Asp	...	Ser	...	Val	Asn	...
25	GDF-1	Glu	Val	His	Arg
			10					15		
30	hOP-1	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala
	mOP-1
	hOP-2	...	Val	Gln	Ser
	mOP-2	...	Val	Gln	Ser
	DPP	Val	Leu	Asp
	Vgl	...	Val	Gln	Met
	Vgr-1	Lys

	CBMP-2A	Val	Pro	His
	CBMP-2B	Val	Pro	Gln
	GDF-1	...	Val	Arg	...	Phe	Leu
				20					25	
5										
	hOP-1	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala
	mOP-1
	hOP-2	Ser
10	mOP-2
	DPP	His	...	Lys	...	Pro
	Vgl	...	Asn	Tyr	Pro
	Vgr-1	...	Asn	Asp	Ser
	CBMP-2A	...	Phe	His	...	Glu	...	Pro
15	CBMP-2B	...	Phe	His	...	Asp	...	Pro
	GDF-1	...	Asn	Gln	...	Gln
				30					35	
	hOP-1	Phe	Pro	Leu	Asn	Ser	Tyr	Met	Asn	Ala
20	mOP-1
	hOP-2	Asp	...	Cys
	mOP-2	Asp	...	Cys
	DPP	Ala	Asp	His	Phe	...	Ser
	Vgl	Tyr	Thr	Glu	Ile	Leu	...	Gly
25	Vgr-1	Ala	His
	CBMP-2A	Ala	Asp	His	Leu	...	Ser
	CBMP-2B	Ala	Asp	His	Leu	...	Ser
	GDF-1	Leu	...	Val	Ala	Leu	Ser	Gly	Ser**	...
						40				
30										
	hOP-1	Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu
	mOP-1
	hOP-2	Leu	...	Ser	...
	mOP-2	Leu	...	Ser	...
35	DPP	Val

	Vgl	Ser	Leu
	Vgr-1
	CBMP-2A
	CBMP-2B
5	GDF-1	Leu	Val	Leu	Arg	Ala	...
		45					50			
	hOP-1	Val	His	Phe	Ile	Asn	Pro	Glu	Thr	Val
	mOP-1	Asp
10	hOP-2	...	His	Leu	Met	Lys	...	Asn	Ala	...
	mOP-2	...	His	Leu	Met	Lys	...	Asp	Val	...
	DPP	...	Asn	Asn	Asn	Gly	Lys	...
	Vgl	Ser	...	Glu	Asp	Ile
	Vgr-1	Val	Met	Tyr	...
15	CBMP-2A	...	Asn	Ser	Val	...	Ser	---	Lys	Ile
	CBMP-2B	...	Asn	Ser	Val	...	Ser	---	Ser	Ile
	GDF-1	Met	...	Ala	Ala	Ala	...	Gly	Ala	Ala
			55					60		
20	hOP-1	Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln
	mOP-1
	hOP-2	Ala	Lys
	mOP-2	Ala	Lys
25	DPP	Ala	Val
	Vgl	...	Leu	Val	Lys
	Vgr-1	Lys
	CBMP-2A	Ala	Val	Glu
	CBMP-2B	Ala	Val	Glu
30	GDF-1	Asp	Leu	Val	...	Ala	Arg
				65					70	
	hOP-1	Leu	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe
35	mOP-1

	hOP-2	...	Ser	...	Thr	Tyr
	mOP-2	...	Ser	...	Thr	Tyr
	Vgl	Met	Ser	Pro	Met	...	Phe	Tyr
	Vgr-1	Val
5	DPP	...	Asp	Ser	Val	Ala	Met	Leu
	CBMP-2A	...	Ser	Met	Leu
	CBMP-2B	...	Ser	Met	Leu
	GDF-1	...	Ser	Pro	Phe	...
					75					80
10	hOP-1	Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys
	mOP-1
	hOP-2	...	Ser	...	Asn	Arg
	mOP-2	...	Ser	...	Asn	Arg
	DPP	Asn	...	Gln	...	Thr	...	Val
15	Vgl	...	Asn	Asn	Asp	Val	...	Arg
	Vgr-1	Asn
	CBMP-2A	...	Glu	Asn	Glu	Lys	...	Val
	CBMP-2B	...	Glu	Tyr	Asp	Lys	...	Val
	GDF-1	...	Asn	...	Asp	Val	...	Arg
20						85				
	hOP-1	Lys	Tyr	Arg	Asn	Met	Val	Val	Arg	
	mOP-1	
25	hOP-2	...	His	Lys	
	mOP-2	...	His	Lys	
	DPP	Asn	...	Gln	Glu	...	Thr	...	Val	
	Vgl	His	...	Glu	Ala	...	Asp	
	Vgr-1	
30	CBMP-2A	Asn	...	Gln	Asp	Glu	
	CBMP-2B	Asn	...	Gln	Glu	Glu	
	GDF-1	Gln	...	Glu	Asp	Asp	
						90				95
35										

	hOP-1	Ala	Cys	Gly	Cys	His
	mOP-1
	hOP-2
	mOP-2
5	DPP	Gly	Arg
	Vgl	Glu	Arg
	Vgr-1
	CBMP-2A	Gly	Arg
	CBMP-2B	Gly	Arg
10	GDF-1	Glu	Arg

100

**Between residues 43 and 44 of GDF-1 lies the amino acid sequence Gly-Gly-Pro-Pro.

15 Table III, set forth below, compares the amino acid sequence data for six related biosynthetic constructs designated COPs 1, 3, 4, 5, 7, and 16. These sequences also are presented in U.S. Pat. No. 5,011,691. As with Table II, the dots mean that in that
 20 position there is an identical amino acid to that of COP-1, and dashes mean that the COP-1 amino acid is missing at that position.

25

TABLE III

	COP-1	Leu	Tyr	Val	Asp	Phe	Gln	Arg	Asp	Val
	COP-3
	COP-4	Ser	---
30	COP-5	Ser	---
	COP-7	Ser	---
	COP-16	Ser	---

1

5

		COP-1	Gly	Trp	Asp	Asp	Trp	Ile	Ile	Ala
		COP-3	Val	...
		COP-4	Val	...
		COP-5	Val	...
5		COP-7	Asn	Val	...
		COP-16	Asn	Val	...
			10					15		
		COP-1	Pro	Val	Asp	Phe	Asp	Ala	Tyr	Tyr
10		COP-3	...	Pro	Gly	Tyr	Gln	...	Phe	...
		COP-4	...	Pro	Gly	Tyr	Gln	...	Phe	...
		COP-5	...	Pro	Gly	Tyr	Gln	...	Phe	...
		COP-7	...	Pro	Gly	Tyr	His	...	Phe	...
		COP-16	...	Pro	Gly	Tyr	Gln	...	Phe	...
15					20					25
		COP-1	Cys	Ser	Gly	Ala	Cys	Gln	Phe	Pro
		COP-3
20		COP-4
		COP-5	...	His	...	Glu	...	Pro
		COP-7	...	His	...	Glu	...	Pro
		COP-16	...	His	...	Glu	...	Pro
					30					
25										
		COP-1	Ser	Ala	Asp	His	Phe	Asn	Ser	Thr
		COP-3
		COP-4
		COP-5	Leu
30		COP-7	Leu	Leu
		COP-16	Leu
					35					40

		COP-1	Asn	His	Ala	Val	Val	Gln	Thr	Leu	Val
		COP-3
		COP-4
		COP-5
5		COP-7
		COP-16
						45					50
10		COP-1	Asn	Asn	Met	Asn	Pro	Gly	Lys	Val	
		COP-3	
		COP-4	
		COP-5	...	Ser	Val	...	Ser	Lys	Ile	---	
		COP-7	...	Ser	Val	...	Ser	Lys	Ile	---	
15		COP-16	...	Ser	Val	...	Ser	Lys	Ile	---	
						55					
20		COP-1	Pro	Lys	Pro	Cys	Cys	Val	Pro	Thr	
		COP-3	
		COP-4	
		COP-5	Ala	
		COP-7	Ala	
		COP-16	Ala	
25				60					65		
30		COP-1	Glu	Leu	Ser	Ala	Ile	Ser	Met	Leu	
		COP-3	
		COP-4	
		COP-5	
		COP-7	
		COP-16	
35						70					

		COP-1	Tyr	Leu	Asp	Glu	Asn	Ser	Thr	Val
		COP-3	Glu	Lys	...
		COP-4	Glu	Lys	...
5		COP-5	Glu	Lys	...
		COP-7	Glu	Lys	...
		COP-16	Glu	Lys	...
			75					80		
10										
		COP-1	Val	Leu	Lys	Asn	Tyr	Gln	Glu	Met
		COP-3
		COP-4
		COP-5
15		COP-7
		COP-16
					85					90
20										
		COP-1	Thr	Val	Val	Gly	Cys	Gly	Cys	Arg
		COP-3	Val	...	Glu
		COP-4	Val	...	Glu
		COP-5	Val	...	Glu
		COP-7	Val	...	Glu
25		COP-16	Val	...	Glu
								95		

30 As is apparent from the foregoing amino acid
sequence comparisons, significant amino acid changes
can be made within the generic sequences while
retaining the morphogenic activity. For example, while
the GDF-1 protein sequence depicted in Table II shares
35 only about 50% amino acid identity with the hOP1

sequence described therein, the GDF-1 sequence shares greater than 70% amino acid sequence homology with the hOP1 sequence, where homology is defined by allowed conservative amino acid changes within the sequence as
5 defined by Dayoff, et al., Atlas of Protein Sequence and Structure vol.5, supp.3, pp.345-362, (M.O. Dayoff, ed., Nat'l BioMed. Res. Fd'n, Washington D.C. 1979.)

It now has been discovered that the family of
10 proteins described by these sequences also is capable of initiating and maintaining the tissue-specific developmental cascade in tissues other than bone and cartilage. When combined with naive progenitor cells as disclosed herein, these proteins, termed morphogens,
15 are capable of inducing the proliferation and differentiation of the progenitor cells. In the presence of appropriate tissue-specific signals to direct the differentiation of these cells, and a morphogenically permissive environment, these
20 morphogens are capable of reproducing the cascade of cellular and molecular events that occur during embryonic development to yield functional tissue.

A key to these developments was the creation
25 of a mammalian tissue model system, namely a model system for endochondral bone formation, and investigation of the cascade of events important for bone tissue morphogenesis. Work on this system has enabled discovery not only of bone inductive
30 morphogens, but also of tissue inductive morphogens and their activities. The methods used to develop the bone model system, now well known in the art, along with the proteins of this invention, can be used to create model systems for other tissues, such as liver (see infra).

Using the model system for endochondral bone formation, it also has been discovered that the local environment in which the morphogenic material is placed is important for tissue morphogenesis. As used herein, "local environment" is understood to include the tissue structural matrix and the environment surrounding the tissue. For example, in addition to needing an appropriate anchoring substratum for their proliferation, the morphogen-stimulated cells need signals to direct the tissue-specificity of their differentiation. These signals vary for the different tissues and may include cell surface markers. In addition, vascularization of new tissue requires a local environment which supports vascularization. Using the bone model system as an example, it is known that, under standard assay conditions, implanting osteoinductive morphogens into loose mesenchyme in the absence of a tissue-specifying matrix generally does not result in endochondral bone formation unless very high concentrations of the protein are implanted. By contrast, implanting relatively low concentrations of the morphogen in association with a suitably modified bone-derived matrix results in the formation of fully functional endochondral bone (see, for example, Sampath et al. (1981) PNAS 78:7599-7 603 and U.S. Patent No. 4,975,526). In addition, a synthetic matrix comprised of a structural polymer such as tissue-specific collagen and tissue-specific cell attachment factors such as tissue-specific glycosylaminoglycans, will allow endochondral bone formation (see, for example, PCT publication US91/03603, published December 12, 1991 (WO 91/18558). Finally, if the morphogen and a suitable bone or cartilage-specific matrix (e.g., comprising Type I cartilage) are implanted together in loose mesenchyme, cartilage and endochondral bone formation will result, including the formation of bone marrow and

a vascular system. However, if the same composition is provided to a nonvascular environment, such as to cultured cells in vitro or at an cartilage-specific locus, tissue development does not continue beyond
5 cartilage formation (see infra). Similarly, a morphogenic composition containing a cartilage-specific matrix composed of Type 2 collagen is expected to induce formation of non-cartilage tissue in vivo (e.g., hyaline). However, if the composition is provided to a
10 vascular-supporting environment, such as loose mesenchyme, the composition is capable of inducing the differentiation of proliferating progenitor cells into chondrocytes and osteoblasts, resulting in bone formation.

15

It also has been discovered that tissue morphogenesis requires a morphogenically permissive environment. Clearly, in fully-functioning healthy tissue that is not composed of a permanently renewing
20 cell population, there must exist signals to prevent continued tissue growth. Thus, it is postulated that there exists a control mechanism, such as a feedback control mechanism, which regulates the control of cell growth and differentiation. In fact, it is known that
25 both TGF- β , and MIS are capable of inhibiting cell growth when present at appropriate concentrations. In addition, using the bone model system it can be shown that osteogenic devices comprising a bone-derived carrier (matrix) that has been demineralized and
30 guanidine-extracted to substantially remove the noncollagenous proteins does allow endochondral bone formation when implanted in association with an

osteoinductive morphogen. If, however, the bone-derived carrier is not demineralized but rather is washed only in low salt, for example, induction of endochondral bone formation is inhibited, suggesting
5 the presence of one or more inhibiting factors within the carrier.

Another key to these developments was determination of the broad distribution of these
10 morphogens in developing and adult tissue. For example, DPP is expressed in both embryonic and developing *Drosophila* tissue. Vgl has been identified in *Xenopus* embryonic tissue. Vgr-1 transcripts have been identified in a variety of murine tissues,
15 including embryonic and developing brain, lung, liver, kidney and calvaria (dermal bone) tissue. Recently, Vgr-1 transcripts also have been identified in adult murine lung, kidney, heart, and brain tissue, with especially high abundance in the lung (see *infra*).

20
OP-1 and the CBMP2 proteins, both first identified as bone morphogens, have been identified in mouse and human placenta, hippocampus, calvaria and osteosarcoma tissue as determined by identification of
25 OP-1 and CBMP2-specific sequences in cDNA libraries constructed from these tissues (see Ozkaynak, et al., (1990) EMBO J 9:2085-2093, and Ozkaynak et al., (1991) Biochem. Biophys. Res. Commn. 179:116-123). Additionally, the OP-1 protein is present in a variety
30 of embryonic and developing tissues including kidney, liver, heart, adrenal tissue and brain as determined by Western blot analysis and immunolocalization (see *infra*). OP-1-specific transcripts also have been identified in both embryonic and developing tissues,
35 most abundantly in developing kidney, bladder and brain

(see infra). OP-1 also has been identified as a mesoderm inducing factor present during embryogenesis (see infra). Moreover, OP-1 has been shown to be associated with in satellite muscle cells and
5 associated with pluripotential stem cells in bone marrow following damage to adult murine endochondral bone, indicating its morphogenic role in tissue repair and regeneration. In addition, the recently identified protein GDF-1 (see Table II) has been identified in
10 neural tissue (Lee, (1991) PNAS 88 4250-4254).

Exemplification

IDENTIFICATION AND ISOLATION OF MORPHOGENS

15

Among the proteins useful in this invention are proteins originally identified as bone inductive proteins, such as the OP-1, OP-2 and the CBMP proteins, as well as amino acid sequence-related proteins such as
20 DPP (from *Drosophila*), Vgl (from *Xenopus*) and Vgr-1 (from mouse, see Table II and Sequence Listing). The members of this family, which include particular members of the TGF- β super family of structurally related proteins, share substantial amino acid sequence
25 homology in their C-terminal regions. The OP-2 proteins have an extra cysteine residue in this region (position 41 of Seq. ID Nos. 7 and 8), in addition to the conserved cysteine skeleton in common with the other proteins in this family. The proteins are
30 inactive when reduced, but are active as oxidized homodimeric species as well as when oxidized in combination with other morphogens.

Accordingly, the morphogens of this invention
35 can be described by either of the following two species

-45-

of generic amino acid sequences: Generic Sequence 1 or
Generic Sequence 2, (Seq. ID Nos. 1 and 2), where each
Xaa indicates one of the 20 naturally-occurring
L-isomer, α -amino acids or a derivative thereof.
5 Particularly useful sequences that fall within this
family of proteins include the 96-102 C-terminal
residues of Vgl, Vgr-1, DPP, OP-1, OP-2, CBMP-2A,
CBMP-2B, and GDF-1, as well as their intact mature
amino acid sequences. In addition, biosynthetic
10 constructs designed from the generic sequences, such as
COP-1, COP-3-5, COP-7, and COP-16 also are useful (see,
for example, U.S. Pat. No. 5,011,691.)

Generic sequences showing preferred amino
15 acids compiled from sequences identified to date and
useful as morphogens (e.g., Tables II and III) are
described by Generic Sequence 3 (Seq. ID No. 3) and
Generic Sequence 4 (Seq. ID No. 4). Note that these
generic sequences have a 7 or 8-cysteine skeleton where
20 inter- or intramolecular disulfide bonds can form, and
contain certain critical amino acids which influence
the tertiary structure of the proteins. It is also
contemplated that the differing N-termini of the
naturally occurring proteins provide a tissue-specific
25 or other, important modulating activity of these
proteins.

Given the foregoing amino acid and DNA
sequence information, the level of skill in the
30 art, and the disclosures of U.S. Patent Nos.
4,968,590 and 5,011,691, PCT application US 89/01469,
published October 19, 1989 (WO89/09788), and Ozkaynak
et al., (1990) EMBO J 9:2085-2093, and Ozkaynak,
et al., (1991) Biochem. Biophys. Res. Commn. 179:116-
35 123, various DNAs can be constructed which encode

at least the active region of a morphogen of this invention, and various analogs thereof (including allelic variants and those containing genetically engineered mutations), as well as fusion proteins, truncated forms of the mature proteins, deletion and insertion mutants, and similar constructs. Moreover, DNA hybridization probes can be constructed from fragments of the genes encoding any of these proteins, including sequences encoding the active regions or the pro regions of the proteins (see infra), or designed de novo from the generic sequence. These probes then can be used to screen different genomic and cDNA libraries to identify additional morphogenic proteins from different tissues.

15

The DNAs can be produced by those skilled in the art using well known DNA manipulation techniques involving genomic and cDNA isolation, construction of synthetic DNA from synthesized oligonucleotides, and cassette mutagenesis techniques. 15-100mer oligonucleotides may be synthesized on a Biosearch DNA Model 8600 Synthesizer, and purified by polyacrylamide gel electrophoresis (PAGE) in Tris-Borate-EDTA buffer. The DNA then may be electroeluted from the gel. Overlapping oligomers may be phosphorylated by T4 polynucleotide kinase and ligated into larger blocks which also may be purified by PAGE.

The DNA from appropriately identified clones then can be isolated, subcloned (preferably into an expression vector), and sequenced. Plasmids containing sequences of interest then can be transfected into an appropriate host cell for expression of the morphogen and further characterization. The host may be a

procaryotic or eucaryotic cell since the former's inability to glycosylate protein will not destroy the protein's morphogenic activity. Useful host cells include E. coli, Saccharomyces, the insect/baculovirus cell system, myeloma cells, and various other mammalian cells. The vectors additionally may encode various sequences to promote correct expression of the recombinant protein, including transcription promoter and termination sequences, enhancer sequences, preferred ribosome binding site sequences, preferred mRNA leader sequences, preferred signal sequences for protein secretion, and the like.

The DNA sequence encoding the gene of interest also may be manipulated to remove potentially inhibiting sequences or to minimize unwanted secondary and tertiary structure formation. The recombinant morphogen also may be expressed as a fusion protein. After being translated, the protein may be purified from the cells themselves or recovered from the culture medium. All biologically active protein forms comprise dimeric species joined by disulfide bonds or otherwise associated, produced by refolding and oxidizing one or more of the various recombinant polypeptide chains within an appropriate eucaryotic cell or in vitro after expression of individual subunits. A detailed description of morphogens expressed from recombinant DNA in E. coli and in numerous different mammalian cells is disclosed in PCT publication US90/05903, published May 2, 1991 (WO91/05802) and United States Patent 5,266,683 which issued on November 30, 1993.

Alternatively, morphogenic polypeptide chains can be synthesized chemically using conventional peptide synthesis techniques well known to those having

ordinary skill in the art. For example, the proteins may be synthesized intact or in parts on a Biosearch solid phase peptide synthesizer, using standard operating procedures. Completed chains then are
5 deprotected and purified by HPLC (high pressure liquid chromatography). If the protein is synthesized in parts, the parts may be peptide bonded using standard methodologies to form the intact protein. In general, the manner in which the morphogens are made can be
10 conventional and does not form a part of this invention.

MORPHOGEN DISTRIBUTION

15 The generic function of the morphogens of this invention throughout the life of the organism can be evidenced by their expression in a variety of disparate mammalian tissues. Determination of the tissue
20 distribution of morphogens also may be used to identify different morphogens expressed in a given tissue, as well as to identify new, related morphogens. The proteins (or their mRNA transcripts) are readily identified in different tissues using standard
25 methodologies and minor modifications thereof in tissues where expression may be low. For example, protein distribution may be determined using standard Western blot analysis or immunofluorescent techniques, and antibodies specific to the morphogen or morphogens
30 of interest. Similarly, the distribution of morphogen transcripts may be determined using standard Northern hybridization protocols and transcript-specific probes.

Any probe capable of hybridizing specifically to a transcript, and distinguishing the transcript of
35 interest from other, related transcripts may be used.

Because the morphogens of this invention share such high sequence homology in their active, C-terminal domains, the tissue distribution of a specific morphogen transcript may best be determined using a probe specific for the pro region of the immature protein and/or the N-terminal region of the mature protein. Another useful sequence is the 3' non-coding region flanking and immediately following the stop codon. These portions of the sequence vary substantially among the morphogens of this invention, and accordingly, are specific for each protein. For example, a particularly useful Vgr-1-specific probe sequence is the PvuII-SacI fragment, a 265 bp fragment encoding both a portion of the untranslated pro region and the N-terminus of the mature sequence (see Lyons et al. (1989) PNAS 86:4554-4558 for a description of the cDNA sequence). Similarly, particularly useful mOP-1-specific probe sequences are the BstXI-BglI fragment, a 0.68 Kb sequence that covers approximately two-thirds of the mOP-1 pro region; a StuI-StuI fragment, a 0.2 Kb sequence immediately upstream of the 7-cysteine domain; and the Earl-PstI fragment, an 0.3 Kb fragment containing a portion of the 3'untranslated sequence (See Seq. ID No. 18, where the pro region is defined essentially by residues 30-291.) Similar approaches may be used, for example, with hOP1 (Seq. ID No. 16) or human or mouse OP2 (Seq. ID Nos. 20 and 22.)

Using these morphogen-specific probes, which may be synthetically engineered or obtained from cloned sequences, morphogen transcripts can be identified in mammalian tissue, using standard methodologies well known to those having ordinary skill in the art. Briefly, total RNA is prepared from various adult murine tissues (e.g., liver, kidney, testis, heart,

brain, thymus and stomach) by a standard methodology such as by the method of Chomczyaski et al. ((1987) Anal. Biochem 162:156-159) and described below. Poly (A)+ RNA is prepared by using oligo (dT)-cellulose chromatography (e.g., Type 7, from Pharmacia LKB Biotechnology, Inc.). Poly (A)+ RNA (generally 15 μ g) from each tissue is fractionated on a 1% agarose/formaldehyde gel and transferred onto a Nytran membrane (Schleicher & Schuell). Following the transfer, the membrane is baked at 80°C and the RNA is cross-linked under UV light (generally 30 seconds at 1 mW/cm²). Prior to hybridization, the appropriate probe (e.g., the PvuII-SacI Vgr-1 fragment) is denatured by heating. The hybridization is carried out in a lucite cylinder rotating in a roller bottle apparatus at approximately 1 rev/min for approximately 15 hours at 37°C using a hybridization mix of 40% formamide, 5 x Denhardts, 5 x SSPE, and 0.1% SDS. Following hybridization, the non-specific counts are washed off the filters in 0.1 x SSPE, 0.1% SDS at 50°C. Northern blots performed using Vgr-1 probes specific to the variable N terminus of the mature sequence indicate that the Vgr-1 message is approximately 3.5 Kb.

Figure 1 is a photomicrograph representing a Northern blot analysis probing a number of adult murine tissues with the Vgr-1 specific probes: liver, kidney, testis, heart, brain, thymus and stomach, represented in lanes 3-10, respectively. Lanes 1 and 12 are size standards and lanes 2 and 11 are blank. Among the tissues tested, Vgr-1 appears to be expressed most abundantly in adult lung, and to a lesser extent in adult kidney, heart and brain. These results confirm and expand on earlier studies identifying Vgr-1 and Vgr-1-like transcripts in several embryonic and adult

-51-

murine tissue (Lyons et al. (1989) PNAS 86:4554-4558),
as well as studies identifying OP-1 and CBMP2 in
various human cDNA libraries (e.g., placenta,
hippocampus, calvaria, and osteosarcoma, see Ozkaynak
5 et al., (1990) EMBO 9:2085-2093).

Using the same general probing methodology,
mOP-1 transcripts also have been identified in a
variety of murine tissues, including embryo and
10 various developing tissues, as can be seen in Figures
2 and 3. Details of the probing methodology are
disclosed in Ozkaynak, et al., (1991) Biochem.
Biophys. Res. Commn. 179:116-123. The Northern blots
represented in Figure 2 probed RNA prepared from
15 developing brain, spleen, lung, kidney (and adrenal
gland), heart, and liver in 13 day post natal mice
(panel A) or 5 week old mice (panel B). The OP-1
specific probe was a probe containing the 3'
untranslated sequences described supra (0.34 Kb EarI-
20 Pst I fragment). As a control for RNA recovery, EF-
Tu (translational elongation factor) mRNA expression
also was measured (EF-Tu expression is assumed to be
relatively uniform in most tissues).

25 The arrowheads indicate the OP1-specific messages
observed in the various tissues. As can be seen in
Fig. 2, OP-1 expression levels vary significantly in
the spleen, lung, kidney and adrenal tissues, while the
EF-Tu mRNA levels are constant. Uniformly lower levels
30 of EF-Tu mRNA levels were found in the heart, brain and
liver. As can be seen from the photomicrograph, the
highest levels of OP-1 mRNA appear to be in kidney and
adrenal tissue, followed by the brain. By contrast,
heart and liver did not give a detectable signal. Not

shown are additional analyses performed on bladder tissue, which shows significant OP-1 mRNA expression, at levels close to those in kidney/adrenal tissue. The Northern blots also indicate that, like GDF-1, OP-1 mRNA expression may be bicistronic in different tissues. Four transcripts can be seen: 4 Kb, 2.4 Kb, 2.2 Kb, and 1.8 Kb transcripts can be identified in the different tissues, and cross probing with OP-1 specific probes from the proregion and N-terminal sequences of the gene indicate that these transcripts are OP-1 specific.

A side by side comparison of OP-1 and Vgr-1 in Figure 3 shows that the probes distinguish between the morphogens Vgr-1 and OP-1 transcripts in the different tissues, and also highlights the multiple transcription of OP-1 in different tissues. Specifically, Fig. 3 compares the expression of OP-1 (Panels B and D), Vgr-1 (Panel C) and EF-Tu (Panel A) (control) mRNA in 17 day embryos (lane 1) and 3 day post-natal mice (lane 2). The same filter was used for sequential hybridizations with labeled DNA probes specific for OP-1 (Panels B and D), Vgr-1 (Panel C), and EF-Tu (Panel A). Panel A: the EF-Tu specific probe (control) was the 0.4 Kb HindIII-SacI fragment (part of the protein coding region), the SacI site used belonged to the vector; Panel B: the OP-1 specific probe was the 0.68 Kb BstXI-BglI fragment containing pro region sequences; Panel D; the OP-1 specific probe was the 0.34 Kb EarI-PstI fragment containing the 3' untranslated sequence; Panel C: the Vgr-1 specific probe was the 0.26 Kb PvuII-SacI fragment used in the Vgr-1 blots described above.

The 1.8-2.5 Kb OP-1 mRNA appears approximately two times higher in three day post natal mice than in 17 day embryos, perhaps reflecting phases in bone and/or kidney development. In addition, of the four messages
5 found in brain, the 2.2 Kb transcript appears most abundant, whereas in lung and spleen the 1.8 Kb message predominates. Finally, careful separation of the renal and adrenal tissue in five week old mice reveals that the 2.2 Kb transcripts were derived from renal tissue
10 and the 4 Kb mRNA is more prominent in adrenal tissue (see Figure 2).

Similarly, using the same general probing methodology, BMP3 and CBMP2B transcripts recently have
15 been identified in abundance in lung tissue.

Morphogen distribution in embryonic tissue can be determined using five or six-day old mouse embryos and standard immunofluorescence techniques in concert
20 with morphogen-specific antisera. For example, rabbit anti-OP-1 antisera is readily obtained using any of a number of standard antibody protocols well known to those having ordinary skill in the art. The antibodies then are fluorescently labelled using standard
25 procedures. A five or six-day old mouse embryo then is thin-sectioned and the various developing tissues probed with the labelled antibody, again following standard protocols. Using this technique, OP-1 protein has been detected in developing brain and heart.

30

This method also may be used to identify morphogens in adult tissues undergoing repair. For example, a fracture site can be induced in a rat long bone such as the femur. The fracture then is allowed
35 to heal for 2 or 3 days. The animal then is sacrificed

and the fractured site sectioned and probed for the presence of the morphogen e.g., OP-1, with fluorescently labelled rabbit anti-OP-1 antisera using standard immunolocalization methodology. This
5 technique identifies OP-1 in muscle satellite cells, the progenitor cells for the development of muscle, cartilage and endochondral bone. In addition, OP-1 is detected with potential pluripotential stem cells in the bone marrow, indicating its morphogenic role in
10 tissue repair and regeneration.

OP-1 protein also has been identified in rat brain using standard immunofluorescence staining technique. Specifically, adult rat brain (2-3 months old) and
15 spinal cord is frozen and sectioned. Anti-OP-1, raised in rabbits and purified on an OP-1 affinity column prepared using standard methodologies, was added to the sections under standard conditions for specific binding. Goat anti-rabbit IgG, labelled with
20 fluorescence, then was used to visualize OP-1 antibody binding to tissue sections.

As can be seen in FIG 4A and 4B, immunofluorescence staining demonstrates the presence of OP-1 in adult rat
25 central nervous system (CNS.) Similar and extensive staining is seen in both the brain (4A) and spinal cord (4B). OP-1 appears to be predominantly localized to the extracellular matrix of the grey matter, present in all areas except the neuronal cell bodies. In white
30 matter, staining appears to be confined to astrocytes. A similar staining pattern also was seen in newborn rat (10 day old) brain sections.

CELL DIFFERENTIATION

35

The ability of morphogens of this invention to induce cell differentiation can be determined by culturing early mesenchymal cells in the presence of the morphogen and then studying the histology of the cultured cells by staining with toluidine blue. For example, it is known that rat mesenchymal cells destined to become mandibular bone, when separated from the overlying epithelial cells at stage 11 and cultured in vitro under standard tissue culture conditions, will not continue to differentiate. However, if these same cells are left in contact with the overlying endoderm for an additional day, at which time they become stage 12 cells, they will continue to differentiate on their own in vitro to form chondrocytes. Further differentiation into osteoblasts and, ultimately, mandibular bone, requires an appropriate local environment, e.g., a vascularized environment.

It has now been discovered that stage 11 mesenchymal cells, cultured in vitro in the presence of a morphogen, e.g., OP-1, continue to differentiate in vitro to form chondrocytes. These stage 11 cells also continue to differentiate in vitro if they are cultured with the cell products harvested from the overlying endodermal cells. Moreover, OP-1 can be identified in the medium conditioned by endodermal cells either by Western blot or immunofluorescence. This experiment may be performed with other morphogens and with different mesenchymal cells to assess the cell differentiation capability of different morphogens, as well as their distribution in different developing tissues.

As another example of morphogen-induced cell differentiation, the effect of OP-1 on the

differentiation of neuronal cells has been tested in culture. Specifically, the effect of OP-1 on the NG108-15 neuroblastoma x glioma hybrid clonal cell line has been assessed. The cell line shows a fibroblastic-
5 type morphology in culture. The cell line can be induced to differentiate chemically using 0.5 mM butyrate, 1% DMSO or 500 nM Forskolin, inducing the expression of virtually all important neuronal properties of cultured primary neurons. However,
10 chemical induction of these cells also induces cessation of cell division.

In the present experiment NG108-15 cells were subcultured on poly-L-lysine coated 6 well plates.
15 Each well contained 40-50,000 cells in 2.5 ml of chemically defined medium. On the third day 2.5 μ l of OP-1 in 60% ethanol containing 0.025% trifluoroacetic was added to each well. OP-1 concentrations of 0, 1, 10, 40 and 100 ng/ml were tested. The media was
20 changed daily with new aliquots of OP-1. After four days with 40 and 100 ng OP-1/ml concentrations, OP-1 induced differentiation of the NG108-15 cells. Figure 5 shows the morphological changes that occur. The OP-1 induces clumping and rounding of the cells and
25 the production of neurite outgrowths (processes). Compare FIG 5A (naive NG108-15 cells) with FIG 5B, showing the effects of OPI-treated cells. Thus the OP-1 can induce the cells to differentiate into a neuronal cell morphology. Some of the outgrowths
30 appear to join in a synaptic-type junction. This effect was not seen in cells incubated with TGF-B1 at concentrations of 1 to 100 ng/ml.

The neuroprotective effects of OP-1 were
35 demonstrated by comparison with chemical

differentiation agents on the NG108-15 cells. 50,000 cells were plated on 6 well plates and treated with butyrate, DMSO, Forskolin or OP-1 for four days. Cell counts demonstrated that in the cultures containing the
5 chemical agents the differentiation was accompanied by a cessation of cell division. In contrast, the cells induced to differentiate by OP-1 continued to divide, as determined by H³-thymidine uptake. The data suggest that OP-1 is capable of maintaining the stability of
10 the cells in culture after differentiation.

As yet another, related example, the ability of the morphogens of this invention to induce the "redifferentiation" of transformed cells also has been
15 assessed. Specifically, the effect of OP-1 on human EC cells (embryo carcinoma cells, NTERA-Z CL.D1) is disclosed herein. In the absence of an external stimulant these cells can be maintained as undifferentiated stem cells, and can be induced to grow
20 in serum free media (SFM). In the absence of morphogen treatment the cells proliferate rampantly and are anchorage-independent. The effect of morphogen treatment is seen in Figs. 6A-D. Figs 6A and 6B show 4 days of growth in SFM in the presence of OP-1
25 (25ng/ml, 6A) or the absence of morphogen (6B). Figs. 6C and 6D are 5 days growth in the presence of 10ng/ml OP-1 (6C) or no morphogen (6D). Figs. 6C and 6D are at 10x and 20x magnification compared to FIGs 6A and 5B. As can readily be seen, in the presence of
30 OP-1, EC cells grow as flattened cells, becoming anchorage dependent. In addition, growth rate is reduced approximately 10 fold. Finally, the cells are induced to differentiate.

35

MAINTENANCE OF PHENOTYPE

The morphogens of this invention also may be used to maintain a cell's differentiated phenotype. This morphogenic capability is particularly useful for
5 inducing the continued expression of phenotype in senescent or quiescent cells.

The phenotypic maintenance capability of morphogens is readily assessed. A number of
10 differentiated cells become senescent or quiescent after multiple passages under standard tissue culture conditions in vitro. However, if these cells are cultivated in vitro in association with a morphogen of this invention, the cells are induced to maintain
15 expression of their phenotype through multiple passages. For example, the alkaline phosphatase activity of cultured osteoblasts, like cultured osteosarcoma cells and calvaria cells, is significantly reduced after multiple passages in vitro.
20 However, if the cells are cultivated in the presence of a morphogen (e.g., OP-1), alkaline phosphatase activity is maintained over extended periods of time. Similarly, phenotypic expression of myocytes also is maintained in the presence of the morphogen. This
25 experiment may be performed with other morphogens and different cells to assess the phenotypic maintenance capability of different morphogens on cells of differing origins.

30 Phenotypic maintenance capability also may be assessed in vivo, using a rat model for osteoporosis, disclosed in co-pending Canadian patent application serial number 2,116,559. As disclosed therein, Long Evans rats are ovariectomized to produce
35 an osteoporotic condition resulting from decreased

estrogen production. Eight days after ovariectomy, rats are systemically provided with phosphate buffered saline (PBS) or OP-1 (21 μ g or 20 μ g) for 22 days. The rats then are sacrificed and serum alkaline phosphatase levels, serum calcium levels, and serum osteocalcin levels are determined, using standard methodologies. Three-fold higher levels of osteocalcin levels are found in rats provided with 1 or 20 μ g of OP-1. Increased alkaline phosphatase levels also were seen. Histomorphometric analysis on the tibial diaphysical bone shows OP-1 can reduce bone mass lost due to the drop in estrogen levels.

CELL STIMULATION

The ability of the morphogens of this invention to stimulate the proliferation of progenitor cells also can be assayed readily in vitro. Useful naive stem cells include pluripotential stem cells, which may be isolated from bone marrow or umbilical cord blood using conventional methodologies, (see, for example, Faradji et al., (1988) Vox Sang. 55 (3):133-138 or Broxmeyer et al., (1989) PNAS 86 (10):3828-3832), as well as naive stem cells obtained from blood. Alternatively, embryonic cells (e.g., from a cultured mesodermal cell line) may be useful.

Another method for obtaining progenitor cells and for determining the ability of morphogens to stimulate cell proliferation is to capture progenitor cells from an in vivo source. For example, a biocompatible matrix material able to allow the influx of migratory progenitor cells may be implanted at an in vivo site long enough to allow the influx of migratory progenitor cells. For example, a bone-derived,

guanidine-extracted matrix, formulated as disclosed for example in Sampath et al. ((1983) PNAS 80:6591-6595), or U.S. Patent No. 4,975,526, may be implanted into a rat at a subcutaneous site, essentially following the method of Sampath et al. (ibid). After three days the implant is removed, and the progenitor cells associated with the matrix dispersed and cultured.

Progenitor cells, however obtained, then are incubated in vitro with a suspected morphogen under standard cell culture conditions well known to those having ordinary skill in the art. In the absence of external stimuli, the progenitor cells do not, or minimally proliferate on their own in culture. However, if the cells are cultured in the presence of a morphogen, such as OP-1, they are stimulated to proliferate. Cell growth can be determined visually or spectrophotometrically using standard methods well known in the art.

20

PROLIFERATION OF PROGENITOR CELL POPULATIONS

Progenitor cells may be stimulated to proliferate in vivo or ex vivo. The cells may be stimulated in vivo by injecting or otherwise providing a sterile preparation containing the morphogen into the individual. For example, the hemopoietic pluripotential stem cell population of an individual may be stimulated to proliferate by injecting or otherwise providing an appropriate concentration of the morphogen to the individual's bone marrow.

Progenitor cells may be stimulated ex vivo by contacting progenitor cells of the population to be enhanced with a morphogen under sterile conditions at a

concentration and for a time sufficient to stimulate proliferation of the cells. In general, a period of from about 10 minutes to about 24 hours should be sufficient. The stimulated cells then are provided to
5 the individual as, for example, by injecting the cells to an appropriate in vivo locus. Suitable biocompatible progenitor cells may be obtained by any of the methods known in the art or described herein.

10 REGENERATION OF DAMAGED OR DISEASED TISSUE

The morphogens of this invention may be used to repair diseased or damaged mammalian tissue. The tissue to be repaired is preferably assessed, and
15 excess necrotic or interfering scar tissue removed as needed, by surgical, chemical, ablating or other methods known in the medical arts.

The morphogen then may be provided directly to
20 the tissue locus as part of a sterile, biocompatible composition, either by surgical implantation or injection. Alternatively, a sterile, biocompatible composition containing morphogen-stimulated progenitor cells may be provided to the tissue locus. The
25 existing tissue at the locus, whether diseased or damaged, provides the appropriate matrix to allow the proliferation and tissue-specific differentiation of progenitor cells. In addition, a damaged or diseased tissue locus, particularly one that has been further
30 assaulted by surgical means, provides a morphogenically permissive environment. For some tissues, it is envisioned that systemic provision of the morphogen will be sufficient.

In some circumstances, particularly where tissue damage is extensive, the tissue may not be capable of providing a sufficient matrix for cell influx and proliferation. In these instances, it may
5 be necessary to provide the morphogen or morphogen-stimulated progenitor cells to the tissue locus in association with a suitable, biocompatible formulated matrix, prepared by any of the means described below. The matrix preferably is tissue-specific, in vivo
10 biodegradable, and comprises particles having dimensions within the range of 70-850 μ m, most preferably 150-420 μ m.

The morphogens of this invention also may be used
15 to prevent or substantially inhibit scar tissue formation following an injury. If a morphogen is provided to a newly injured tissue locus, it can induce tissue morphogenesis at the locus, preventing the aggregation of migrating fibroblasts into non-
20 differentiated connective tissue. The morphogen preferably is provided as a sterile pharmaceutical preparation injected into the tissue locus within five hours of the injury. Several non-limiting examples follow, illustrating the morphogens regenerate
25 capabilities in different tissues. The proteins of this invention previously have been shown to be capable of inducing cartilage and endochondral bone formation (See, for example U.S. Patent No. 5,011,691).

30 As an example, protein-induced morphogenesis of substantially injured liver tissue following a partial hepatectomy is disclosed. Variations on this general protocol may be used to test morphogen activity in other different tissues. The general method involves
35 excising an essentially nonregenerating portion of a

-63-

tissue and providing the morphogen, preferably as a soluble pharmaceutical preparation to the excised tissue locus, closing the wound and examining the site at a future date. Like bone, liver has a potential to regenerate upon injury during post-fetal life.

Morphogen, (e.g., purified recombinant human OP-1, mature form), was solubilized (1 mg/ml) in 50% ethanol (or compatible solvent) containing 0.1% trifluoroacetic acid (or compatible acid). The injectable OP-1 solution was prepared by diluting one volume of OP-1/solvent-acid stock solution with 9 volumes of 0.2% rat serum albumin in sterile PBS (phosphate-buffered saline).

Growing rats or aged rats were anesthetized by using ketamine. Two of the liver lobes (left and right) were cut out (approximately 1/3 of the lobe) and the OP-1 was injected locally at multiple sites along the cut ends. The amount of OP-1 injected was 100 µg in 100 of PBS/RSA (phosphate buffered saline/rat serum albumin) injection buffer. Placebo samples are injection buffer without OP-1. Five rats in each group were used. The wound was closed and the rats were allowed to eat normal food and drink tap water.

After 12 days, the rats were sacrificed and liver regeneration was observed visually. The photomicrograph in Fig. 7 illustrates dramatically the regenerative effects of OP-1 on liver regeneration. The OP-1-injected group showed complete liver tissue regeneration and no sign remained of any cut in the liver (animal 2 on the right hand side). The arrow in Figure 7 denotes liver tissue that has been regenerated. By contrast, in the control group into which only P BS was injected only minimal regeneration was evidenced (animal 1 on the left hand side). In addition, the incision remains in this sample.

As another example, the ability of the morphogens of this invention to induce dentinogenesis also was assessed. To date, the unpredictable response of dental pulp tissue to injury is a basic clinical
5 problem in dentistry. Cynomolgus monkeys were chosen as primate models as monkeys are presumed to be more indicative of human dental biology than models based on lower non-primate mammals.

10

Using standard dental surgical procedures, small areas (e.g., 2mm) of dental pulps were surgically exposed by removing the enamel and dentin immediately above the pulp (by drilling) of sample teeth,
15 performing a partial amputation of the coronal pulp tissue, inducing hemostasis, application of the pulp treatment, and sealing and filling the cavity by standard procedures.

20 Pulp treatments used were: OP-1 dispersed in a carrier matrix; carrier matrix alone and no treatment. Twelve teeth per animal (four for each treatment) were prepared, and two animals were used. At four weeks, teeth were extracted and processed histologically for
25 analysis of dentin formation, and/or ground to analyze dentin mineralization. FIG.8 illustrates dramatically the effect of morphogen on osteodentin reparation. FIG. 8A is a photomicrograph of the control treatment (PBS) and shows little or no reparation. FIG. 8B is a
30 photomicrograph of treatment with carrier alone, showing minimal reparation. By contrast, treatment with morphogen (FIG. 8C) shows significant reparation. The results of FIG. 8 indicate that OP-1-CM (OP-1 plus

carrier matrix) reliably induced formation of reparative or osteodentin bridges on surgically exposed healthy dental pulps. By contrast, pulps treated with carrier matrix alone, or not treated failed to form
5 reparative dentin.

As another example, the morphogen-induced regenerative effects on central nervous system (CNS) repair may be assessed using a rat brain stab model.
10 Briefly, male Long Evans rats are anesthetized and the head area prepared for surgery. The calvariae is exposed using standard surgical procedures and a hole drilled toward the center of each lobe using a 0.035K wire, just piercing the calvariae. 25 μ l solutions
15 containing either morphogen (OP-1, 25 μ g) or PBS then is provided to each of the holes by Hamilton syringe. Solutions are delivered to a depth approximately 3 mm below the surface, into the underlying cortex, corpus callosum and hippocampus. The skin then is sutured and
20 the animal allowed to recover.

Three days post surgery, rats are sacrificed by decapitation and their brains processed for sectioning. Scar tissue formation is evaluated by immunofluorescence
25 staining for glial fibrillary acidic protein, a marker protein for glial scarring, to qualitatively determine the degree of scar formation. Sections also are probed with anti-OP-1 antibodies to determine the presence of OP-1. Reduced levels of glial fibrillary acidic
30 protein are anticipated in the tissue sections of animals treated with morphogen, evidencing the ability of morphogen to inhibit glial scar formation, thereby stimulating nerve regeneration.

35

MORPHOGEN ACTIVITY MODULATION

Antibodies to morphogens of this invention have been identified in healthy human sera. In addition, implanting devices comprising morphogens (e.g., OP-1) have been discovered to induce an increase in anti-morphogen antibodies (e.g., anti-OP-1 antibodies). It is anticipated that these antibodies comprise part of the body's regulation of morphogen activity in vivo. The presence of the antibodies, and fluctuations in their levels, which are readily monitored, can provide a useful method for monitoring tissue stasis and tissue viability (e.g., identification of a pathological state). For example, standard radioimmunoassays or ELISA may be used to detect and quantify endogenous anti-morphogen antibodies in sera. Antibodies or other binding proteins capable of detecting anti-morphogen antibodies may be obtained using standard methodologies.

20

MATRIX PREPARATION

The morphogens of this invention may be implanted surgically, dispersed in a biocompatible, preferably in vivo biodegradable matrix appropriately modified to provide a structure in which the morphogen may be dispersed and which allows the influx, differentiation and proliferation of migrating progenitor cells. The matrix also should provide signals capable of directing the tissue specificity of the differentiating cells, as well as a morphogenically permissive environment, being essentially free of growth inhibiting signals.

In the absence of these features the matrix does not appear to be suitable as part of a morphogenic composition. Recent studies on osteogenic devices

(morphogens dispersed within a formulated matrix) using matrices formulated from polylactic acid and/or polyglycolic acid biopolymers, ceramics (α-tri-calcium-phosphate), or hydroxyapatite show that these materials, by themselves, are unable to provide the appropriate environment for inducing de novo endochondral bone formation in rats by themselves. In addition, matrices formulated from commercially available highly purified, reconstituted collagens or naturally-derived non-bone, species-specific collagen (e.g., from rat tail tendon) also are unsuccessful in inducing bone when implanted in association with an osteogenic protein. These matrices apparently lack specific structurally-related features which aid in directing the tissue specificity of the morphogen-stimulated, differentiating progenitor cells.

The formulated matrix may be shaped as desired in anticipation of surgery or may be shaped by the physician or technician during surgery. Thus, the material may be used in topical, subcutaneous, intraperitoneal, or intramuscular implants to repair tissue or to induce its growth de novo. The matrix preferably is biodegradable in vivo, being slowly absorbed by the body and replaced by new tissue growth, in the shape or very nearly in the shape of the implant.

Details of how to make and how to use the matrices useful in this invention are disclosed below.

TISSUE-DERIVED MATRICES

Suitable biocompatible, in vivo biodegradable acellular matrices may be prepared from naturally-

occurring tissue. The tissue is treated with suitable agents to substantially extract the cellular, nonstructural components of the tissue. The agents also should be capable of extracting any growth
5 inhibiting components associated with the tissue. The resulting material is a porous, acellular matrix, substantially depleted in nonstructurally-associated components.

10 The matrix also may be further treated with agents that modify the matrix, increasing the number of pores and micropits on its surfaces. Those skilled in the art will know how to determine which agents are best suited to the extraction of nonstructural
15 components for different tissues. For example, soft tissues such as liver and lung may be thin-sectioned and exposed to a nonpolar solvent such as, for example, 100% ethanol, to destroy the cellular structure of the tissue and extract nonstructural components. The
20 material then is dried and pulverized to yield nonadherent porous particles. Structural tissues such as cartilage and dentin where collagen is the primary component may be demineralized and extracted with guanidine, essentially following the method of Sampath
25 et al. (1983) PNAS 80:6591-6595. For example, pulverized and demineralized dentin is extracted with five volumes of 4M guanidine-HCl, 50mM Tris-HCl, pH 7.0 for 16 hours at 4°C. The suspension then is filtered. The insoluble material that remains is collected and
30 used to fabricate the matrix. The material is mostly collagenous in manner. It is devoid of morphogenic activity. The matrix particles may further be treated with a collagen fibril-modifying agent that extracts potentially unwanted components from the matrix, and
35 alters the surface structure of the matrix material.

Useful agents include acids, organic solvents or heated aqueous media. A detailed description of these matrix treatments are disclosed in U.S. Patent No. 4,975,526 and PCT publication US90/00912, published September 7, 5 1990 (WO90/10018).

The currently most preferred agent is a heated aqueous fibril-modifying medium such as water, to increase the matrix particle surface area and porosity. 10 The currently most preferred aqueous medium is an acidic aqueous medium having a pH of less than about 4.5, e.g., within the range of about pH 2 - pH 4 which may help to "swell" the collagen before heating. 0.1% acetic acid, which has a pH of about 3, currently is 15 most preferred. 0.1 M acetic acid also may be used.

Various amounts of delipidated, demineralized guanidine-extracted bone collagen are heated in the aqueous medium (1g matrix/30ml aqueous medium) under 20 constant stirring in a water jacketed glass flask, and maintained at a given temperature for a predetermined period of time. Preferred treatment times are about one hour, although exposure times of between about 0.5 to two hours appear acceptable. The temperature 25 employed is held constant at a temperature within the range of about 37°C to 65°C. The currently preferred heat treatment temperature is within the range of about 45°C to 60°C.

30 After the heat treatment, the matrix is filtered, washed, lyophilized and used for implant. Where an acidic aqueous medium is used, the matrix also is preferably neutralized prior to washing and lyophilization. A currently preferred neutralization 35 buffer is a 200mM sodium phosphate buffer, pH 7.0. To

neutralize the matrix, the matrix preferably first is allowed to cool following thermal treatment, the acidic aqueous medium (e.g., 0.1% acetic acid) then is removed and replaced with the neutralization buffer and the matrix agitated for about 30 minutes. The neutralization buffer then may be removed and the matrix washed and lyophilized.

Other useful fibril-modifying treatments include acid treatments (e.g., trifluoroacetic acid and hydrogen fluoride) and solvent treatments such as dichloromethane, acetonitrile, isopropanol and chloroform, as well as particular acid/solvent combinations.

15

After contact with the fibril-modifying agent, the treated matrix may be washed to remove any extracted components, following a form of the procedure set forth below:

20

1. Suspend matrix preparation in TBS (Tris-buffered saline) 1g/200 ml and stir at 4°C for 2 hrs; or in 6 M urea, 50 mM Tris-HCl, 500 mM NaCl, pH 7.0 (UTBS) or water and stir at room temperature (RT) for 30 minutes (sufficient time to neutralize the pH);

2. Centrifuge and repeat wash step; and

3. Centrifuge; discard supernatant; water wash residue; and then lyophilize.

30

SYNTHETIC TISSUE-SPECIFIC MATRICES

35

In addition to the naturally-derived tissue-

specific matrices described above, useful tissue-specific matrices may be formulated synthetically if appropriately modified. These porous biocompatible, in vivo biodegradable synthetic matrices are disclosed in
5 PCT publication US91/03603, published December 12, 1991 (WO91/18558). Briefly, the matrix comprises a porous crosslinked structural polymer of biocompatible, biodegradable collagen and appropriate, tissue-specific glycosaminoglycans as tissue-specific
10 cell attachment factors. Collagen derived from a number of sources may be suitable for use in these synthetic matrices, including insoluble collagen, acid-soluble collagen, collagen soluble in neutral or basic aqueous solutions, as well as those
15 collagens which are commercially available.

Glycosaminoglycans (GAGs) or mucopolysaccharides are hexosamine-containing
20 polysaccharides of animal origin that have a tissue specific distribution, and therefore may be used to help determine the tissue specificity of the morphogen-stimulated differentiating cells. Reaction with the GAGs also provides collagen with another valuable
25 property, i.e., inability to provoke an immune reaction (foreign body reaction) from an animal host.

Chemically, GAGs are made up of residues of hexoseamines glycosidically bound and alternating in a
30 more-or-less regular manner with either hexouronic acid or hexose moieties (see, e.g., Dodgson et al. in Carbohydrate Metabolism and its Disorders (Dickens et al., eds.) Vol. 1, Academic Press (1968)). Useful GAGs include hyaluronic acid, heparin, heparin sulfate,
35 chondroitin 6-sulfate, chondroitin 4-sulfate, dermatan

-72-

sulfate, and keratin sulfate. Other GAGs are suitable for forming the matrix described herein, and those skilled in the art will either know or be able to ascertain other suitable GAGs using no more than routine experimentation. For a more detailed description of mucopolysaccharides, see Aspinall, Polysaccharides, Pergamon Press, Oxford (1970). For example, as disclosed in U.S. Patent No. 5,645,591, issued July 8, 1997, chondroitin-6-sulfate can be used where endochondral bone formation is desired. Heparin sulfate, on the other hand, may be used to formulate synthetic matrices for use in lung tissue repair.

Collagen can be reacted with a GAG in aqueous acidic solutions, preferably in diluted acetic acid solutions. By adding the GAG dropwise into the aqueous collagen dispersion, coprecipitates of tangled collagen fibrils coated with GAG results. This tangled mass of fibers then can be homogenized to form a homogeneous dispersion of fine fibers and then filtered and dried.

Insolubility of the collagen-GAG products can be raised to the desired degree by covalently cross-linking these materials, which also serves to raise the resistance to resorption of these materials. In general, any covalent cross-linking method suitable for cross-linking collagen also is suitable for cross-linking these composite materials, although crosslinking by a dehydrothermal process is preferred.

When dry, the crosslinked particles are essentially spherical, with diameters of about 500 μm . Scanning electron microscopy shows pores of about 20 μm on the surface and 40 μm on the interior. The interior is made up of both fibrous and sheet-like

structures, providing surfaces for cell attachment. The voids interconnect, providing access to the cells throughout the interior of the particle. The material appears to be roughly 99.5% void volume, making the material very efficient in terms of the potential cell mass that can be grown per gram of microcarrier.

The morphogens described herein can be combined and dispersed in an appropriately modified tissue-specific matrix using any of the methods described below:

1. Ethanol Precipitation

Matrix is added to the morphogen dissolved in guanidine-HCl. Samples are vortexed and incubated at a low temperature. Samples are then further vortexed. Cold absolute ethanol is added to the mixture which is then stirred and incubated. After centrifugation (microfuge, high speed) the supernatant is discarded. The matrix is washed with cold concentrated ethanol in water and then lyophilized.

2. Acetonitrile Trifluoroacetic Acid Lyophilization

In this procedure, morphogen in an acetonitrile trifluoroacetic acid (ACN/TFA) solution is added to the carrier material. Samples are vigorously vortexed many times and then lyophilized.

3. Buffered Saline Lyophilization

Morphogen preparations in physiological saline may also be vortexed with the matrix and lyophilized to

produce morphogenically active material.

BIOASSAY

5

The following sets forth various procedures for evaluating the in vivo morphogenic utility of the morphogens and morphogenic compositions of this invention. The proteins and compositions may be
10 injected or surgically implanted in a mammal, following any of a number of procedures well known in the art. For example, surgical implant bioassays may be performed essentially following the procedure of Sampath et al. (1983) PNAS 80:6591-6595.

15

Histological Evaluation

Histological sectioning and staining is preferred to determine the extent of morphogenesis in
20 vivo, particularly in tissue repair procedures. Excised implants are fixed in Bouins Solution, embedded in paraffin, and cut into 6-8 μ m sections. Staining with toluidine blue or hemotoxylin/eosin demonstrates clearly the ultimate development of the new tissue.
25 Twelve day implants are usually sufficient to determine whether the implants contain newly induced tissue.

Successful implants exhibit a controlled progression through the stages of induced tissue
30 development allowing one to identify and follow the tissue-specific events that occur. For example, in endochondral bone formation the stages include:
(1) leukocytes on day one; (2) mesenchymal cell migration and proliferation on days two and three;
35 (3) chondrocyte appearance on days five and six;

- (4) cartilage matrix formation on day seven;
(5) cartilage calcification on day eight; (6) vascular invasion, appearance of osteoblasts, and formation of new bone on days nine and ten; (7) appearance of
5 osteoblastic and bone remodeling and dissolution of the implanted matrix on days twelve to eighteen; and
(8) hematopoietic bone marrow differentiation in the ossicle on day twenty-one.

10 Biological Markers

In addition to histological evaluation, biological markers may be used as a marker for tissue morphogenesis. Useful markers include tissue-specific
15 enzymes whose activities may be assayed (e.g., spectrophotometrically) after homogenization of the implant. These assays may be useful for quantitation and for obtaining an estimate of tissue formation quickly after the implants are removed from the animal.
20 For example, alkaline phosphatase activity may be used as a marker for osteogenesis.

Incorporation of systemically provided morphogens may be followed using tagged morphogens
25 (e.g., radioactively labelled) and determining their localization in new tissue, and/or by monitoring their disappearance from the circulatory system using a standard pulse-chase labeling protocol. The morphogen also may be provided with a tissue-specific molecular
30 tag, whose uptake may be monitored and correlated with the concentration of morphogen provided. As an example, ovary removal in female rats results in reduced bone alkaline phosphatase activity, rendering the rats predisposed to osteoporosis. If the female
35 rats now are provided with a morphogen, e.g., OP-1, a

reduction in the systemic concentration of calcium (CA^{2+}) is seen, which correlates with the presence of the provided morphogen and can be shown to correspond to increased alkaline phosphatase activity.

5

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The present embodiments are therefore to be considered in all
10 respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be
15 embraced therein.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: COHEN, CHARLES M.
KUBERASAMPATH, THANGAVEL
PANG, ROY H.L.
OPPERMANN, HERMANN
RUEGER, DAVID C.
- (ii) TITLE OF INVENTION: PROTEIN-INDUCED MORPHOGENESIS
- (iii) NUMBER OF SEQUENCES: 23
- (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: TESTA, HURWITZ & THIBEAULT
(B) STREET: 53 STATE STREET
(C) CITY: BOSTON
(D) STATE: MASSACHUSETTS
(E) COUNTRY: U.S.A.
(F) ZIP: 02109
- (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
- (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 667,274
(B) FILING DATE: 11-MAR-1991
- (viii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 752,764
(B) FILING DATE: 30-AUG-1991

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 amino acids
(B) TYPE: amino acids
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: Generic Sequence 1

(D) OTHER INFORMATION: Each Xaa indicates one of the 20 naturally-occurring L-isomer, α -amino acids or a derivative thereof.

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

```

Xaa Xaa Xaa Xaa Xaa Xaa
  1                               5
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 10                               15
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
 20                               25
Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 30                               35
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 40                               45                               50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
 55                               60
Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 65                               70
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 75                               80
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
 85                               90
Xaa Cys Xaa
 95

```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 97 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein
- (ix) FEATURE:
 - (A) NAME: Generic Sequence 2
 - (D) OTHER INFORMATION: Each Xaa indicates one of the 20 naturally-occurring L-isomer, α -amino acids or a derivative thereof.
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

                Xaa Xaa Xaa Xaa Xaa Xaa
                  1             5
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
              10             15
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
              20             25
Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
              30             35
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
              40             45             50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
              55             60
Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
              65             70
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
              75             80
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
              85             90
Xaa Cys Xaa
              95

```

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 97 amino acids
 - (B) TYPE: amino acids
 - (C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: Generic Sequence 3

(D) OTHER INFORMATION: wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification.

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

```

Leu Tyr Val Xaa Phe
  1                               5
Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
                               10
Xaa Ala Pro Gly Xaa Xaa Xaa Ala
 15                               20
Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
                               25                               30
Xaa Pro Xaa Xaa Xaa Xaa Xaa
                               35
Xaa Xaa Xaa Asn His Ala Xaa Xaa
                               40                               45
Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa
                               50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
                               55                               60
Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
                               65
Xaa Xaa Xaa Leu Xaa Xaa Xaa
 70                               75
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
                               80
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
 85                               90
Xaa Cys Gly Cys Xaa
                               95

```


(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: Generic Sequence 4

(D) OTHER INFORMATION: wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification.

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

Cys Xaa Xaa Xaa Xaa Leu Tyr Val Xaa Phe
 1 5 10
 Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
 15
 Xaa Ala Pro Xaa Gly Xaa Xaa Ala
 20 25
 Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
 30 35
 Xaa Pro Xaa Xaa Xaa Xaa Xaa
 40
 Asn Xaa Xaa Asn His Ala Xaa Xaa
 45 50
 Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa
 55
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
 60 65
 Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
 70

Xaa Xaa Xaa Leu Xaa Xaa Xaa
 75 80
 Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
 85
 Xaa Xaa Xaa Xaa Met Xaa Val Xaa
 90 95
 Xaa Cys Gly Cys Xaa
 100

(2)

INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 139 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: hOP-1 (mature form)

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

Ser	Thr	Gly	Ser	Lys	Gln	Arg	Ser	Gln
1				5				
Asn	Arg	Ser	Lys	Thr	Pro	Lys	Asn	Gln
10					15			
Glu	Ala	Leu	Arg	Met	Ala	Asn	Val	Ala
	20					25		
Glu	Asn	Ser	Ser	Ser	Asp	Gln	Arg	Gln
		30					35	
Ala	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val
			40					45
Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp
				50				
Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala
55					60			
Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala
	65					70		

Phe	Pro	Leu	Asn	Ser	Tyr	Met	Asn	Ala
		75					80	
Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu
			85					90
Val	His	Phe	Ile	Asn	Pro	Glu	Thr	Val
				95				
Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln
100					105			
Leu	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe
	110					115		
Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys
		120					125	
Lys	Tyr	Arg	Asn	Met	Val	Val	Arg	Ala
			130					135
Cys	Gly	Cys	His					

- (2) INFORMATION FOR SEQ ID NO:6:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 139 amino acids
- (B) TYPE: amino acids
- (C) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (ix) FEATURE:
- (A) NAME: mOP-1 (mature form)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ser	Thr	Gly	Gly	Lys	Gln	Arg	Ser	Gln
1				5				
Asn	Arg	Ser	Lys	Thr	Pro	Lys	Asn	Gln
10					15			
Glu	Ala	Leu	Arg	Met	Ala	Ser	Val	Ala
	20					25		

Glu	Asn	Ser	Ser	Ser	Asp	Gln	Arg	Gln
		30					35	
Ala	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val
			40					45
Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp
				50				
Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala
55					60			
Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala
	65					70		
Phe	Pro	Leu	Asn	Ser	Tyr	Met	Asn	Ala
		75					80	
Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu
			85					90
Val	His	Phe	Ile	Asn	Pro	Asp	Thr	Val
				95				
Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln
100					105			
Leu	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe
	110					115		
Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys
		120					125	
Lys	Tyr	Arg	Asn	Met	Val	Val	Arg	Ala
			130					135
Cys	Gly	Cys	His					

- (2) INFORMATION FOR SEQ ID NO:7:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 139 amino acids
- (B) TYPE: amino acids
- (C) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (ix) FEATURE:
- (A) NAME: hOP-2 (mature form)

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

Ala	Val	Arg	Pro	Leu	Arg	Arg	Arg	Gln
1				5				
Pro	Lys	Lys	Ser	Asn	Glu	Leu	Pro	Gln
10					15			
Ala	Asn	Arg	Leu	Pro	Gly	Ile	Phe	Asp
	20					25		
Asp	Val	His	Gly	Ser	His	Gly	Arg	Gln
		30					35	
Val	Cys	Arg	Arg	His	Glu	Leu	Tyr	Val
			40					45
Ser	Phe	Gln	Asp	Leu	Gly	Trp	Leu	Asp
				50				
Trp	Val	Ile	Ala	Pro	Gln	Gly	Tyr	Ser
55					60			
Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ser
	65					70		
Phe	Pro	Leu	Asp	Ser	Cys	Met	Asn	Ala
		75					80	
Thr	Asn	His	Ala	Ile	Leu	Gln	Ser	Leu
			85					90
Val	His	Leu	Met	Lys	Pro	Asn	Ala	Val
				95				
Pro	Lys	Ala	Cys	Cys	Ala	Pro	Thr	Lys
100					105			
Leu	Ser	Ala	Thr	Ser	Val	Leu	Tyr	Tyr
	110					115		
Asp	Ser	Ser	Asn	Asn	Val	Ile	Leu	Arg
		120					125	
Lys	His	Arg	Asn	Met	Val	Val	Lys	Ala
			130					135
Cys	Gly	Cys	His					

- (2) INFORMATION FOR SEQ ID NO:8:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 139 amino acids
 (B) TYPE: amino acids
 (C) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (ix) FEATURE:
 (A) NAME: mOP-2 (mature form)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ala	Ala	Arg	Pro	Leu	Lys	Arg	Arg	Gln
1				5				
Pro	Lys	Lys	Thr	Asn	Glu	Leu	Pro	His
10					15			
Pro	Asn	Lys	Leu	Pro	Gly	Ile	Phe	Asp
	20					25		
Asp	Gly	His	Gly	Ser	Arg	Gly	Arg	Glu
		30					35	
Val	Cys	Arg	Arg	His	Glu	Leu	Tyr	Val
			40					45
Arg	Phe	Arg	Asp	Leu	Gly	Trp	Leu	Asp
				50				
Trp	Val	Ile	Ala	Pro	Gln	Gly	Tyr	Ser
55					60			
Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala
	65					70		
Phe	Pro	Leu	Asp	Ser	Cys	Met	Asn	Ala
		75					80	
Thr	Asn	His	Ala	Ile	Leu	Gln	Ser	Leu
			85					90
Val	His	Leu	Met	Lys	Pro	Asp	Val	Val
				95				
Pro	Lys	Ala	Cys	Cys	Ala	Pro	Thr	Lys
100					105			

Leu	Ser	Ala	Thr	Ser	Val	Leu	Tyr	Tyr
	110					115		
Asp	Ser	Ser	Asn	Asn	Val	Ile	Leu	Arg
		120					125	
Lys	His	Arg	Asn	Met	Val	Val	Lys	Ala
			130					135
Cys	Gly	Cys	His					

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 96 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: CBMP-2A(fx)

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

Cys	Lys	Arg	His	Pro	Leu	Tyr	Val	Asp	Phe	Ser
1				5					10	
Asp	Val	Gly	Trp	Asn	Asp	Trp	Ile	Val	Ala	Pro
		15						20		
Pro	Gly	Tyr	His	Ala	Phe	Tyr	Cys	His	Gly	Glu
	25					30				
Cys	Pro	Phe	Pro	Leu	Ala	Asp	His	Leu	Asn	Ser
	35					40				
Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu	Val	Asn
45				50						55
Ser	Val	Asn	Ser	Lys	Ile	Pro	Lys	Ala	Cys	Cys
			60						65	
Val	Pro	Thr	Glu	Leu	Ser	Ala	Ile	Ser	Met	Leu
		70						75		
Tyr	Leu	Asp	Glu	Asn	Glu	Lys	Val	Val	Leu	Lys
		80						85		

Asn Tyr Gln Asp Met Val Val Glu Gly Cys Gly
 90 95
 Cys Arg
 100

- (2) INFORMATION FOR SEQ ID NO:10:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 101 amino acids
 (B) TYPE: amino acids
 (C) TOPOLOGY: linear
 (ii) MOLECULE TYPE: protein
 (ix) FEATURE:
 (A) NAME: CBMP-2B(fx)
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Cys Arg Arg His Ser
 1 5
 Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn
 10 15
 Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala
 20 25
 Phe Tyr Cys His Gly Asp Cys Pro Phe Pro Leu
 30 35
 Ala Asp His Leu Asn Ser Thr Asn His Ala Ile
 40 45
 Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser
 50 55 60
 Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu
 65 70
 Ser Ala Ile Ser Met Leu Tyr Leu Asp Glu Tyr
 75 80
 Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met
 85 90

Val Val Glu Gly Cys Gly Cys Arg
 95 100

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: DPP(fx)

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

Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser
 1 5 10
 Asp Val Gly Trp Asp Asp Trp Ile Val Ala Pro
 15 20
 Leu Gly Tyr Asp Ala Tyr Tyr Cys His Gly Lys
 25 30
 Cys Pro Phe Pro Leu Ala Asp His Phe Asn Ser
 35 40
 Thr Asn His Ala Val Val Gln Thr Leu Val Asn
 45 50 55
 Asn Asn Asn Pro Gly Lys Val Pro Lys Ala Cys
 60 65
 Cys Val Pro Thr Gln Leu Asp Ser Val Ala Met
 70 75
 Leu Tyr Leu Asn Asp Gln Ser Thr Val Val Leu
 80 85
 Lys Asn Tyr Gln Glu Met Thr Val Val Gly Cys
 90 95
 Gly Cys Arg
 100

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: Vgl(fx)

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

Cys Lys Lys Arg His Leu Tyr Val Glu Phe Lys
 1 5 10
 Asp Val Gly Trp Gln Asn Trp Val Ile Ala Pro
 15 20
 Gln Gly Tyr Met Ala Asn Tyr Cys Tyr Gly Glu
 25 30
 Cys Pro Tyr Pro Leu Thr Glu Ile Leu Asn Gly
 35 40
 Ser Asn His Ala Ile Leu Gln Thr Leu Val His
 45 50 55
 Ser Ile Glu Pro Glu Asp Ile Pro Leu Pro Cys
 60 65
 Cys Val Pro Thr Lys Met Ser Pro Ile Ser Met
 70 75
 Leu Phe Tyr Asp Asn Asn Asp Asn Val Val Leu
 80 85
 Arg His Tyr Glu Asn Met Ala Val Asp Glu Cys
 90 95
 Gly Cys Arg
 100

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: Vgr-1(fx)

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

Cys Lys Lys His Glu Leu Tyr Val Ser Phe Gln
 1 5 10
 Asp Val Gly Trp Gln Asp Trp Ile Ile Ala Pro
 15 20
 Xaa Gly Tyr Ala Ala Asn Tyr Cys Asp Gly Glu
 25 30
 Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala
 35 40
 Thr Asn His Ala Ile Val Gln Thr Leu Val His
 45 50 55
 Val Met Asn Pro Glu Tyr Val Pro Lys Pro Cys
 60 65
 Cys Ala Pro Thr Lys Val Asn Ala Ile Ser Val
 70 75
 Leu Tyr Phe Asp Asp Asn Ser Asn Val Ile Leu
 80 85
 Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys
 90 95
 Gly Cys His
 100

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 106 amino acids

(B) TYPE: protein

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: human
 (F) TISSUE TYPE: BRAIN

(ix) FEATURE:
 (D) OTHER INFORMATION:
 /product= "GDF-1 (fx)"

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

Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly
 1 5 10
 Trp His Arg Trp Val Ile Ala Pro Arg Gly Phe Leu Ala Asn Tyr
 15 20 25
 Cys Gln Gly Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly
 30 35 40
 Gly Pro Pro Ala Leu Asn His Ala Val Leu Arg Ala Leu Met His
 45 50 55
 Ala Ala Ala Pro Gly Ala Ala Asp Leu Pro Cys Cys Val Pro Ala
 60 65 70
 Arg Leu Ser Pro Ile Ser Val Leu Phe Phe Asp Asn Ser Asp Asn
 75 80 85
 Val Val Leu Arg Gln Tyr Glu Asp Met Val Val Asp Glu Cys Gly
 90 95 100
 Cys Arg
 105

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 5 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

Cys Xaa Xaa Xaa Xaa
 1 5

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1822 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: HOMO SAPIENS
- (F) TISSUE TYPE: HIPPOCAMPUS

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 49..1341
- (D) OTHER INFORMATION: /standard_name= "hOP1"

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

```

GGTGCGGGCC CGGAGCCCGG AGCCCGGGTA GCGCGTAGAG CCGGCGCG ATG CAC GTG      57
                                     Met His Val
                                     1

CGC TCA CTG CGA GCT GCG GCG CCG CAC AGC TTC GTG GCG CTC TGG GCA      105
Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala
      5                               10                               15

CCC CTG TTC CTG CTG CGC TCC GCC CTG GCC GAC TTC AGC CTG GAC AAC      153
Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn
      20                               25                               30                               35

GAG GTG CAC TCG AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG      201
Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg
                               40                               45                               50

CGG GAG ATG CAG CGC GAG ATC CTC TCC ATT TTG GGC TTG CCC CAC CGC      249
Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg
                               55                               60                               65

CCG CGC CCG CAC CTC CAG GGC AAG CAC AAC TCG GCA CCC ATG TTC ATG      297
Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met
                               70                               75                               80

CTG GAC CTG TAC AAC GCC ATG GCG GTG GAG GAG GGC GGC GGG CCC GGC      345
Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly Gly Pro Gly
      85                               90                               95

GGC CAG GGC TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC      393
Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly
      100                               105                               110                               115
    
```

CCC CCT CTG GCC AGC CTG CAA GAT AGC CAT TTC CTC ACC GAC GCC GAC Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp 120 125 130	441
ATG GTC ATG AGC TTC GTC AAC CTC GTG GAA CAT GAC AAG GAA TTC TTC Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe 135 140 145	489
CAC CCA CGC TAC CAC CAT CGA GAG TTC CGG TTT GAT CTT TCC AAG ATC His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile 150 155 160	537
CCA GAA GGG GAA GCT GTC ACG GCA GCC GAA TTC CGG ATC TAC AAG GAC Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp 165 170 175	585
TAC ATC CGG GAA CGC TTC GAC AAT GAG ACG TTC CGG ATC AGC GTT TAT Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile Ser Val Tyr 180 185 190 195	633
CAG GTG CTC CAG GAG CAC TTG GGC AGG GAA TCG GAT CTC TTC CTG CTC Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu Phe Leu Leu 200 205 210	681
GAC AGC CGT ACC CTC TGG GCC TCG GAG GAG GGC TGG CTG GTG TTT GAC Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp 215 220 225	729
ATC ACA GCC ACC AGC AAC CAC TGG GTG GTC AAT CCG CGG CAC AAC CTG Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu 230 235 240	777
GGC CTG CAG CTC TCG GTG GAG ACG CTG GAT GGG CAG AGC ATC AAC CCC Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro 245 250 255	825
AAG TTG GCG GGC CTG ATT GGG CGG CAC GGG CCC CAG AAC AAG CAG CCC Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro 260 265 270 275	873
TTC ATG GTG GCT TTC TTC AAG GCC ACG GAG GTC CAC TTC CGC AGC ATC Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe Arg Ser Ile 280 285 290	921
CGG TCC ACG GGG AGC AAA CAG CGC AGC CAG AAC CGC TCC AAG ACG CCC Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro 295 300 305	969
AAG AAC CAG GAA GCC CTG CGG ATG GCC AAC GTG GCA GAG AAC AGC AGC Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser 310 315 320	1017

AGC GAC CAG AGG CAG GCC TGT AAG AAG CAC GAG CTG TAT GTC AGC TTC	1065
Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe	
325 330 335	
CGA GAC CTG GGC TGG CAG GAC TGG ATC ATC GCG CCT GAA GGC TAC GCC	1113
Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala	
340 345 350 355	
GCC TAC TAC TGT GAG GGG GAG TGT GCC TTC CCT CTG AAC TCC TAC ATG	1161
Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met	
360 365 370	
AAC GCC ACC AAC CAC GCC ATC GTG CAG ACG CTG GTC CAC TTC ATC AAC	1209
Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn	
375 380 385	
CCG GAA ACG GTG CCC AAG CCC TGC TGT GCG CCC ACG CAG CTC AAT GCC	1257
Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala	
390 395 400	
ATC TCC GTC CTC TAC TTC GAT GAC AGC TCC AAC GTC ATC CTG AAG AAA	1305
Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys	
405 410 415	
TAC AGA AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCCTCC	1351
Tyr Arg Asn Met Val Arg Ala Cys Gly Cys His	
420 425 430	
GAGAATTCAG ACCCTTTGGG GCCAAGTTTT TCTGGATCCT CCATTGCTCG CCTTGGCCAG	1411
GAACCAGCAG ACCAACTGCC TTTTGTGAGA CCTTCCCCTC CCTATCCCA ACTTTAAAGG	1471
TGTGAGAGTA TTAGGAAACA TGAGCAGCAT ATGGCTTTTG ATCAGTTTTT CAGTGGCAGC	1531
ATCCAATGAA CAAGATCCTA CAAGCTGTGC AGGCAAAACC TAGCAGGAAA AAAAAACAAC	1591
GCATAAAGAA AAATGGCCGG GCCAGGTCAT TGGCTGGGAA GTCTCAGCCA TGCACGGACT	1651
CGTTTCCAGA GGTAATTATG AGCGCCTACC AGCCAGGCCA CCCAGCCGTG GGAGGAAGGG	1711
GCGTGGCAA GGGGTGGCA CATTGGTGTG TGTGCGAAAG GAAAATTGAC CCGGAAGTTC	1771
CTGTAATAAA TGTCACAATA AAACGAATGA ATGAAAAAAAA AAAAAAAAAA A	1822

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 431 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

(ix) FEATURE:

(D) OTHER INFORMATION: /Product="OP1-PP"

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

```

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

```


Ile Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn
 260 265 270
 Lys Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe
 275 280 285
 Arg Ser Ile Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser
 290 295 300
 Lys Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu
 305 310 315 320
 Asn Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr
 325 330 335
 Val Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu
 340 345 350
 Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn
 355 360 365
 Ser Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His
 370 375 380
 Phe Ile Asn Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln
 385 390 395 400
 Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile
 405 410 415
 Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
 420 425 430

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1873 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: MURIDAE
- (F) TISSUE TYPE: EMBRYO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 104..1393
- (D) OTHER INFORMATION: /note= "MOP1 (CDNA)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTGCAGCAAG TGACCTCGGG TCGTGGACCG CTGCCCTGCC CCCTCCGCTG CCACCTGGGG	60
CGGCGCGGGC CCGGTGCCCC GGATCGCGCG TAGAGCCGGC GCG ATG CAC GTG CGC	115
	Met His Val Arg
	1
TCG CTG CGC GCT GCG GCG CCA CAC AGC TTC GTG GCG CTC TGG GCG CCT	163
Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala Pro	
5 10 15 20	
CTG TTC TTG CTG CGC TCC GCC CTG GCC GAT TTC AGC CTG GAC AAC GAG	211
Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn Glu	
	25 30 35
GTG CAC TCC AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG CGG	259
Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg Arg	
	40 45 50
GAG ATG CAG CGG GAG ATC CTG TCC ATC TTA GGG TTG CCC CAT CGC CCG	307
Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg Pro	
	55 60 65
CGC CCG CAC CTC CAG GGA AAG CAT AAT TCG GCG CCC ATG TTC ATG TTG	355
Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met Leu	
	70 75 80
GAC CTG TAC AAC GCC ATG GCG GTG GAG GAG AGC GGG CCG GAC GGA CAG	403
Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly Pro Asp Gly Gln	
	85 90 95 100
GGC TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC CCC CCT	451
Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly Pro Pro	
	105 110 115
TTA GCC AGC CTG CAG GAC AGC CAT TTC CTC ACT GAC GCC GAC ATG GTC	499
Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp Met Val	
	120 125 130
ATG AGC TTC GTC AAC CTA GTG GAA CAT GAC AAA GAA TTC TTC CAC CCT	547
Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe His Pro	
	135 140 145
CGA TAC CAC CAT CGG GAG TTC CGG TTT GAT CTT TCC AAG ATC CCC GAG	595
Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile Pro Glu	
	150 155 160
GGC GAA GCG GTG ACC GCA GCC GAA TTC AGG ATC TAT AAG GAC TAC ATC	643
Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp Tyr Ile	
	165 170 175 180

CGG GAG CGA TTT GAC AAC GAG ACC TTC CAG ATC ACA GTC TAT CAG TGG Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr Val Tyr Gln Trp 185 190 195	691
CTC CAG GAG CAC TCA GGC AGG GAG TCG GAC CTC TTC TTG CTG GAC AGC Leu Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe Leu Leu Asp Ser 200 205 210	739
CGC ACC ATC TGG GCT TCT GAG GAG GGC TGG TTG GTG TTT GAT ATC ACA Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp Ile Thr 215 220 225	787
GCC ACC AGC AAC CAC TGG GTG GTC AAC CCT CGG CAC AAC CTG GGC TTA Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu Gly Leu 230 235 240	835
CAG CTC TCT GTG GAG ACC CTG GAT GGG CAG AGC ATC AAC CCC AAG TTG Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro Lys Leu 245 250 255 260	883
GCA GGC CTG ATT GGA CGG CAT GGA CCC CAG AAC AAG CAA CCC TTC ATG Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro Phe Met 265 270 275	931
GTG GCC TTC TTC AAG GCC ACG GAA GTC CAT CTC CGT AGT ATC CGG TCC Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg Ser Ile Arg Ser 280 285 290	979
ACG GGG GGC AAG CAG CGC AGC CAG AAT CGC TCC AAG ACG CCA AAG AAC Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys Asn 295 300 305	1027
CAA GAG GCC CTG AGG ATG GCC AGT GTG GCA GAA AAC AGC AGC AGT GAC Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn Ser Ser Ser Asp 310 315 320	1075
CAG AGG CAG GCC TGC AAG AAA CAT GAG CTG TAC GTC AGC TTC CGA GAC Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp 325 330 335 340	1123
CTT GGC TGG CAG GAC TGG ATC ATT GCA CCT GAA GGC TAT GCT GCC TAC Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr 345 350 355	1171
TAC TGT GAG GGA GAG TGC GCC TTC CCT CTG AAC TCC TAC ATG AAC GCC Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn Ala 360 365 370	1219
ACC AAC CAC GCC ATC GTC CAG ACA CTG GTT CAC TTC ATC AAC CCA GAC Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Asp 375 380 385	1267

ACA GTA CCC AAG CCC TGC TGT GCG CCC ACC CAG CTC AAC GCC ATC TCT	1315
Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser	
390 395 400	
GTC CTC TAC TTC GAC GAC AGC TCT AAT GTC ATC CTG AAG AAG TAC AGA	1363
Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg	
405 410 415 420	
AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCTTCC TGAGACCCTG	1413
Asn Met Val Val Arg Ala Cys Gly Cys His	
425 430	
ACCTTTGCGG GGCCACACCT TTCCAAATCT TCGATGTCTC ACCATCTAAG TCTCTCACTG	1473
CCCACCTTGG CGAGGAGAAC AGACCAACCT CTCCTGAGCC TTCCCTCACC TCCCAACCGG	1533
AAGCATGTAA GGGTTCCAGA AACCTGAGCG TGCAGCAGCT GATGAGCGCC CTTTCCTTCT	1593
GGCACGTGAC GGACAAGATC CTACCAGCTA CCACAGCAA CGCCTAAGAG CAGGAAAAAT	1653
GTCTGCCAGG AAAGTGTCCA GTGTCCACAT GGCCCTGGC GCTCTGAGTC TTTGAGGAGT	1713
AATCGCAAGC CTCGTTGAGC TGCAGCAGAA GGAAGGGCTT AGCCAGGGTG GCGGCTGGCG	1773
TCTGTGTTGA AGGGAAACCA AGCAGAAGCC ACTGTAATGA TATGTCACAA TAAAACCCAT	1833
GAATGAAAAA AAAAAAAAAA AAAAAAAAAA AAAAGAATTC	1873

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 430 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (ix) FEATURE:
 - (D) OTHER INFORMATION: /product= "mOP1-PP"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala	
1 5 10 15	
Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser	
20 25 30	
Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser	
35 40 45	

Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
 50 55 60
 Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
 65 70 75 80
 Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly
 85 90 95
 Pro Asp Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr
 100 105 110
 Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp
 115 120 125
 Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu
 130 135 140
 Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser
 145 150 155 160
 Lys Ile Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr
 165 170 175
 Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr
 180 185 190
 Val Tyr Gln Trp Leu Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe
 195 200 205
 Leu Leu Asp Ser Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val
 210 215 220
 Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His
 225 230 235 240
 Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile
 245 250 255
 Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys
 260 265 270
 Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg
 275 280 285
 Ser Ile Arg Ser Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys
 290 295 300
 Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn
 305 310 315 320
 Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val
 325 330 335

Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly
 340 345 350
 Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser
 355 360 365
 Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe
 370 375 380
 Ile Asn Pro Asp Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu
 385 390 395 400
 Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu
 405 410 415
 Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
 420 425 430

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1723 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens
- (F) TISSUE TYPE: HIPPOCAMPUS

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 490..1696
- (D) OTHER INFORMATION: /note= "hOP2 (cDNA)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GGCGCCGGCA GAGCAGGAGT GGCTGGAGGA GCTGTGGTTG GAGCAGGAGG TGGCACGGCA 60
 GGGCTGGAGG GCTCCCTATG AGTGGCGGAG ACGGCCCAGG AGGCGCTGGA GCAACAGCTC 120
 CCACACCGCA CCAAGCGGTG GCTGCAGGAG CTCGCCCATC GCCCCTGCGC TGCTCGGACC 180
 GCGGCCACAG CCGGACTGGC GGGTACGGCG GCGACAGAGG CATTGGCCGA GAGTCCCAGT 240
 CCGCAGAGTA GCCCCGGCCT CGAGGCGGTG GCGTCCCGGT CCTCTCCGTC CAGGAGCCAG 300
 GACAGGTGTC GCGCGGCGGG GCTCCAGGGA CCGCGCCTGA GGCCGGCTGC CCGCCCGTCC 360
 CGCCCCGCCC CGCCGCCC GC CCGCCGCGA GCCCAGCCTC CTTGCCGTCG GGGCGTCCCC 420

AGGCCCTGGG TCGGCCGCGG AGCCGATGCG CGCCCGCTGA GCGCCCCAGC TGAGCGCCCC 480

CGGCCTGCC ATG ACC GCG CTC CCC GGC CCG CTC TGG CTC CTG GGC CTG 528
 Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu
 1 5 10

GCG CTA TGC GCG CTG GGC GGG GGC GGC CCC GGC CTG CGA CCC CCG CCC 576
 Ala Leu Cys Ala Leu Gly Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro
 15 20 25

GGC TGT CCC CAG CGA CGT CTG GGC GCG CGC GAG CGC CGG GAC GTG CAG 624
 Gly Cys Pro Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln
 30 35 40 45

CGC GAG ATC CTG GCG GTG CTC GGG CTG CCT GGG CGG CCC CGG CCC CGC 672
 Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg
 50 55 60

GCG CCA CCC GCC GCC TCC CGG CTG CCC GCG TCC GCG CCG CTC TTC ATG 720
 Ala Pro Pro Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met
 65 70 75

CTG GAC CTG TAC CAC GCC ATG GCC GGC GAC GAC GAC GAG GAC GGC GCG 768
 Leu Asp Leu Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala
 80 85 90

CCC GCG GAG CGG CGC CTG GGC CGC GCC GAC CTG GTC ATG AGC TTC GTT 816
 Pro Ala Glu Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val
 95 100 105

AAC ATG GTG GAG CGA GAC CGT GCC CTG GGC CAC CAG GAG CCC CAT TGG 864
 Asn Met Val Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp
 110 115 120 125

AAG GAG TTC CGC TTT GAC CTG ACC CAG ATC CCG GCT GGG GAG GCG GTC 912
 Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val
 130 135 140

ACA GCT GCG GAG TTC CGG ATT TAC AAG GTG CCC AGC ATC CAC CTG CTC 960
 Thr Ala Ala Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu
 145 150 155

AAC AGG ACC CTC CAC GTC AGC ATG TTC CAG GTG GTC CAG GAG CAG TCC 1008
 Asn Arg Thr Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser
 160 165 170

AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT CTT CAG ACG CTC CGA GCT 1056
 Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala
 175 180 185

GGA GAC GAG GGC TGG CTG GTG CTG GAT GTC ACA GCA GCC AGT GAC TGC 1104
 Gly Asp Glu Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys
 190 195 200 205

TGG TTG CTG AAG CGT CAC AAG GAC CTG GGA CTC CGC CTC TAT GTG GAG Trp Leu Leu Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu 210 215 220	1152
ACT GAG GAC GGG CAC AGC GTG GAT CCT GGC CTG GCC GGC CTG CTG GGT Thr Glu Asp Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly 225 230 235	1200
CAA CGG GCC CCA CGC TCC CAA CAG CCT TTC GTG GTC ACT TTC TTC AGG Gln Arg Ala Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg 240 245 250	1248
GCC AGT CCG AGT CCC ATC CGC ACC CCT CGG GCA GTG AGG CCA CTG AGG Ala Ser Pro Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg 255 260 265	1296
AGG AGG CAG CCG AAG AAA AGC AAC GAG CTG CCG CAG GCC AAC CGA CTC Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu 270 275 280 285	1344
CCA GGG ATC TTT GAT GAC GTC CAC GGC TCC CAC GGC CGG CAG GTC TGC Pro Gly Ile Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys 290 295 300	1392
CGT CGG CAC GAG CTC TAC GTC AGC TTC CAG GAC CTC GGC TGG CTG GAC Arg Arg His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp 305 310 315	1440
TGG GTC ATC GCT CCC CAA GGC TAC TCG GCC TAT TAC TGT GAG GGG GAG Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu 320 325 330	1488
TGC TCC TTC CCA CTG GAC TCC TGC ATG AAT GCC ACC AAC CAC GCC ATC Cys Ser Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile 335 340 345	1536
CTG CAG TCC CTG GTG CAC CTG ATG AAG CCA AAC GCA GTC CCC AAG GCG Leu Gln Ser Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala 350 355 360 365	1584
TGC TGT GCA CCC ACC AAG CTG AGC GCC ACC TCT GTG CTC TAC TAT GAC Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp 370 375 380	1632
AGC AGC AAC AAC GTC ATC CTG CGC AAA CAC CGC AAC ATG GTG GTC AAG Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn Met Val Val Lys 385 390 395	1680
GCC TGC GGC TGC CAC T GAGTCAGCCC GCCCAGCCCT ACTGCAG Ala Cys Gly Cys His 400	1723

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 402 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) OTHER INFORMATION: /product= "hOP2-PP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```

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

```

Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Glu Asp
 210 215 220
 Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly Gln Arg Ala
 225 230 235 240
 Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg Ala Ser Pro
 245 250 255
 Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg Arg Arg Gln
 260 265 270
 Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu Pro Gly Ile
 275 280 285
 Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys Arg Arg His
 290 295 300
 Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp Trp Val Ile
 305 310 315 320
 Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ser Phe
 325 330 335
 Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser
 340 345 350
 Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala Cys Cys Ala
 355 360 365
 Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn
 370 375 380
 Asn Val Ile Leu Arg Lys His Arg Asn Met Val Val Lys Ala Cys Gly
 385 390 395 400
 Cys His

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1926 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: MURIDAE
 - (F) TISSUE TYPE: EMBRYO
- (ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 93..1289
 (D) OTHER INFORMATION: /note= "mOP2 cDNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GCCAGGCACA GGTGCGCCGT CTGGTCCTCC CCGTCTGGCG TCAGCCGAGC	50
CCGACCAGCT ACCAGTGGAT GCGCGCCGGC TGAAAGTCCG AG ATG GCT ATG CGT Met Ala Met Arg 1	104
CCC GGG CCA CTC TGG CTA TTG GGC CTT GCT CTG TGC GCG CTG GGA GGC Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys Ala Leu Gly Gly 5 10 15 20	152
GGC CAC GGT CCG CGT CCC CCG CAC ACC TGT CCC CAG CGT CGC CTG GGA Gly His Gly Pro Arg Pro Pro His Thr Cys Pro Gln Arg Arg Leu Gly 25 30 35	200
GCG CGC GAG CGC CGC GAC ATG CAG CGT GAA ATC CTG GCG GTG CTC GGG Ala Arg Glu Arg Arg Asp Met Gln Arg Glu Ile Leu Pro Val Leu Gly 40 45 50	248
CTA CCG GGA CGG CCC CGA CCC CGT GCA CAA CCC GCG GCT GCC CGG CAG Leu Pro Gly Arg Pro Arg Pro Arg Ala Gln Pro Ala Ala Ala Arg Gln 55 60 65	296
CCA GCG TCC GCG CCC CTC TTC ATG TTG GAC CTA TAC CAC GCC ATG ACC Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala Met Thr 70 75 80	344
GAT GAC GAC GAC GGC GGG CCA CCA CAG GCT CAC TTA GGC CGT GCC GAC Asp Asp Asp Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg Ala Asp 85 90 95 100	392
CTG GTC ATG AGC TTC GTC AAC ATG GTG GAA CGC GAC CGT ACC CTG GGC Leu Val Met Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr Leu Gly 105 110 115	440
TAC CAG GAG CCA CAC TGG AAG GAA TTC CAC TTT GAC CTA ACC CAG ATC Tyr Gln Glu Pro His Trp Lys Glu Phe His Phe Asp Leu Thr Gln Ile 120 125 130	488
CCT GCT GGG GAG GCT GTC ACA GCT GCT GAG TTC CGG ATC TAC AAA GAA Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Glu 135 140 145	536
CCC AGC ACC CAC CCG CTC AAC ACA ACC CTC CAC ATC AGC ATG TTC GAA Pro Ser Thr His Pro Leu Asn Thr Thr Leu His Ile Ser Met Phe Glu 150 155 160	584

GTG	GTC	CAA	GAG	CAC	TCC	AAC	AGG	GAG	TCT	GAC	TTG	TTC	TTT	TTG	GAT	632
Val	Val	Gln	Glu	His	Ser	Asn	Arg	Glu	Ser	Asp	Leu	Phe	Phe	Leu	Asp	
165					170					175					180	
CTT	CAG	ACG	CTC	CGA	TCT	GGG	GAC	GAG	GGC	TGG	CTG	GTG	CTG	GAC	ATC	680
Leu	Gln	Thr	Leu	Arg	Ser	Gly	Asp	Glu	Gly	Trp	Leu	Val	Leu	Asp	Ile	
			185						190					195		
ACA	GCA	GCC	AGT	GAC	CGA	TGG	CTG	CTG	AAC	CAT	CAC	AAG	GAC	CTG	GGA	728
Thr	Ala	Ala	Ser	Asp	Arg	Trp	Leu	Leu	Asn	His	His	Lys	Asp	Leu	Gly	
			200					205					210			
CTC	CGC	CTC	TAT	GTG	GAA	ACC	GCG	GAT	GGG	CAC	AGC	ATG	GAT	CCT	GGC	776
Leu	Arg	Leu	Tyr	Val	Glu	Thr	Ala	Asp	Gly	His	Ser	Met	Asp	Pro	Gly	
		215					220					225				
CTG	GCT	GGT	CTG	CTT	GGA	CGA	CAA	GCA	CCA	CGC	TCC	AGA	CAG	CCT	TTC	824
Leu	Ala	Gly	Leu	Leu	Gly	Arg	Gln	Ala	Pro	Arg	Ser	Arg	Gln	Pro	Phe	
	230					235					240					
ATG	GTA	ACC	TTC	TTC	AGG	GCC	AGC	CAG	AGT	CCT	GTG	CGG	GCC	CCT	CGG	872
Met	Val	Thr	Phe	Phe	Arg	Ala	Ser	Gln	Ser	Pro	Val	Arg	Ala	Pro	Arg	
245					250					255					260	
GCA	GCG	AGA	CCA	CTG	AAG	AGG	AGG	CAG	CCA	AAG	AAA	ACG	AAC	GAG	CTT	920
Ala	Ala	Arg	Pro	Leu	Lys	Arg	Arg	Gln	Pro	Lys	Lys	Thr	Asn	Glu	Leu	
				265					270					275		
CCG	CAC	CCC	AAC	AAA	CTC	CCA	GGG	ATC	TTT	GAT	GAT	GGC	CAC	GGT	TCC	968
Pro	His	Pro	Asn	Lys	Leu	Pro	Gly	Ile	Phe	Asp	Asp	Gly	His	Gly	Ser	
			280					285						290		
CGC	GGC	AGA	GAG	GTT	TGC	CGC	AGG	CAT	GAG	CTC	TAC	GTC	AGC	TTC	CGT	1016
Arg	Gly	Arg	Glu	Val	Cys	Arg	Arg	His	Glu	Leu	Tyr	Val	Ser	Phe	Arg	
		295					300					305				
GAC	CTT	GGC	TGG	CTG	GAC	TGG	GTC	ATC	GCC	CCC	CAG	GGC	TAC	TCT	GCC	1064
Asp	Leu	Gly	Trp	Leu	Asp	Trp	Val	Ile	Ala	Pro	Gln	Gly	Tyr	Ser	Ala	
	310					315					320					
TAT	TAC	TGT	GAG	GGG	GAG	TGT	GCT	TTC	CCA	CTG	GAC	TCC	TGT	ATG	AAC	1112
Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala	Phe	Pro	Leu	Asp	Ser	Cys	Met	Asn	
325					330					335					340	
GCC	ACC	AAC	CAT	GCC	ATC	TTG	CAG	TCT	CTG	GTG	CAC	CTG	ATG	AAG	CCA	1160
Ala	Thr	Asn	His	Ala	Ile	Leu	Gln	Ser	Leu	Val	His	Leu	Met	Lys	Pro	
				345					350					355		
GAT	GTT	GTC	CCC	AAG	GCA	TGC	TGT	GCA	CCC	ACC	AAA	CTG	AGT	GCC	ACC	1208
Asp	Val	Val	Pro	Lys	Ala	Cys	Cys	Ala	Pro	Thr	Lys	Leu	Ser	Ala	Thr	
			360					365					370			

Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Gln Pro Ala Ala
 50 55 60 65
 Ala Arg Gln Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala
 70 75 80
 Met Thr Asp Asp Asp Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg
 85 90 95
 Ala Asp Leu Val Met Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr
 100 105 110
 Leu Gly Tyr Gln Glu Pro His Trp Lys Glu Phe His Phe Asp Leu Thr
 115 120 125 130
 Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr
 135 140 145
 Lys Glu Pro Ser Thr His Pro Leu Asn Thr Thr Leu His Ile Ser Met
 150 155 160
 Phe Glu Val Val Gln Glu His Ser Asn Arg Glu Ser Asp Leu Phe Phe
 165 170 175
 Leu Asp Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu Val Leu
 180 185 190
 Asp Ile Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn His His Lys Asp
 195 200 205 210
 Leu Gly Leu Arg Leu Tyr Val Glu Thr Ala Asp Gly His Ser Met Asp
 215 220 225
 Pro Gly Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser Arg Gln
 230 235 240
 Pro Phe Met Val Thr Phe Phe Arg Ala Ser Gln Ser Pro Val Arg Ala
 245 250 255
 Pro Arg Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn
 260 265 270
 Glu Leu Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His
 275 280 285 290
 Gly Ser Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser
 295 300 305
 Phe Arg Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr
 310 315 320

Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys
325 330 335

Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met
340 345 350

Lys Pro Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser
355 360 365 370

Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg
375 380 385

Lys His Arg Asn Met Val Val Lys Ala Cys Gly Cys His
390 395

CLAIMS:

1. Use of a composition comprising a morphogen selected from the group consisting of: OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vg1, Vgr-1, GDF-1, and a morphogen comprising SEQ ID NO:3 or SEQ ID NO:4; wherein the morphogen forms a dimeric protein and is capable of inducing ectopic bone formation, for regenerating skin tissue.
2. Use of a composition comprising a morphogen selected from the group consisting of: OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vg1, Vgr-1, GDF-1, and a morphogen comprising SEQ ID NO:3 or SEQ ID NO:4; wherein the morphogen forms a dimeric protein and is capable of inducing ectopic bone formation, for the treatment of noncongenital liver disorders.
3. Use of a composition comprising a morphogen selected from the group consisting of: OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vg1, Vgr-1, GDF-1, and a morphogen comprising SEQ ID NO:3 or SEQ ID NO:4; wherein the morphogen forms a dimeric protein and is capable of inducing ectopic bone formation, for the treatment of noncongenital blood disorders.
4. Use of a composition comprising a morphogen selected from the group consisting of: OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vg1, Vgr-1, GDF-1, and a morphogen comprising SEQ ID NO:3 or SEQ ID NO:4; wherein the morphogen forms a dimeric protein and is capable of inducing ectopic bone formation, for increasing progenitor cell populations *in vivo*.
5. Use of a composition comprising a morphogen selected from the group consisting of; OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vg1, Vgr-1, GDF-1, and a morphogen comprising SEQ ID NO:3 or SEQ ID NO:4; wherein the morphogen forms a dimeric protein and is capable of

inducing ectopic bone formation, for stimulating the growth and maintaining the phenotype of differentiated cells.

6. Use of a composition comprising a morphogen selected from the group consisting of: OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vg1, Vgr-1, GDF-1, and a morphogen comprising SEQ ID NO:3 or SEQ ID NO:4; wherein the morphogen forms a dimeric protein and is capable of inducing ectopic bone formation, for the healing of gastrointestinal ulcers.

7. Use of a composition comprising a morphogen selected from the group consisting of: OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vg1, Vgr-1, GDF-1, and a morphogen comprising SEQ ID NO:3 or SEQ ID NO:4; wherein the morphogen forms a dimeric protein and is capable of inducing ectopic bone formation, for neuroprotection or the treatment of noncongenital degenerative nerve diseases.

8. Use of a composition comprising a morphogen selected from the group consisting of: OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vg1, Vgr-1, GDF-1, and a morphogen comprising SEQ ID NO:3 or SEQ ID NO:4; wherein the morphogen forms a dimeric protein and is capable of inducing ectopic bone formation, for the treatment of lung tissue damaged by emphysema.

9. The use of any one of claims 1 to 8, wherein the morphogen is selected from the group consisting of OP-1, OP-2, CBMP2A, CBMP2B, DPP, Vg1, Vgr-1, and GDF-1

10. The use of any one of claims 1 to 8, wherein said morphogen is OP-1.

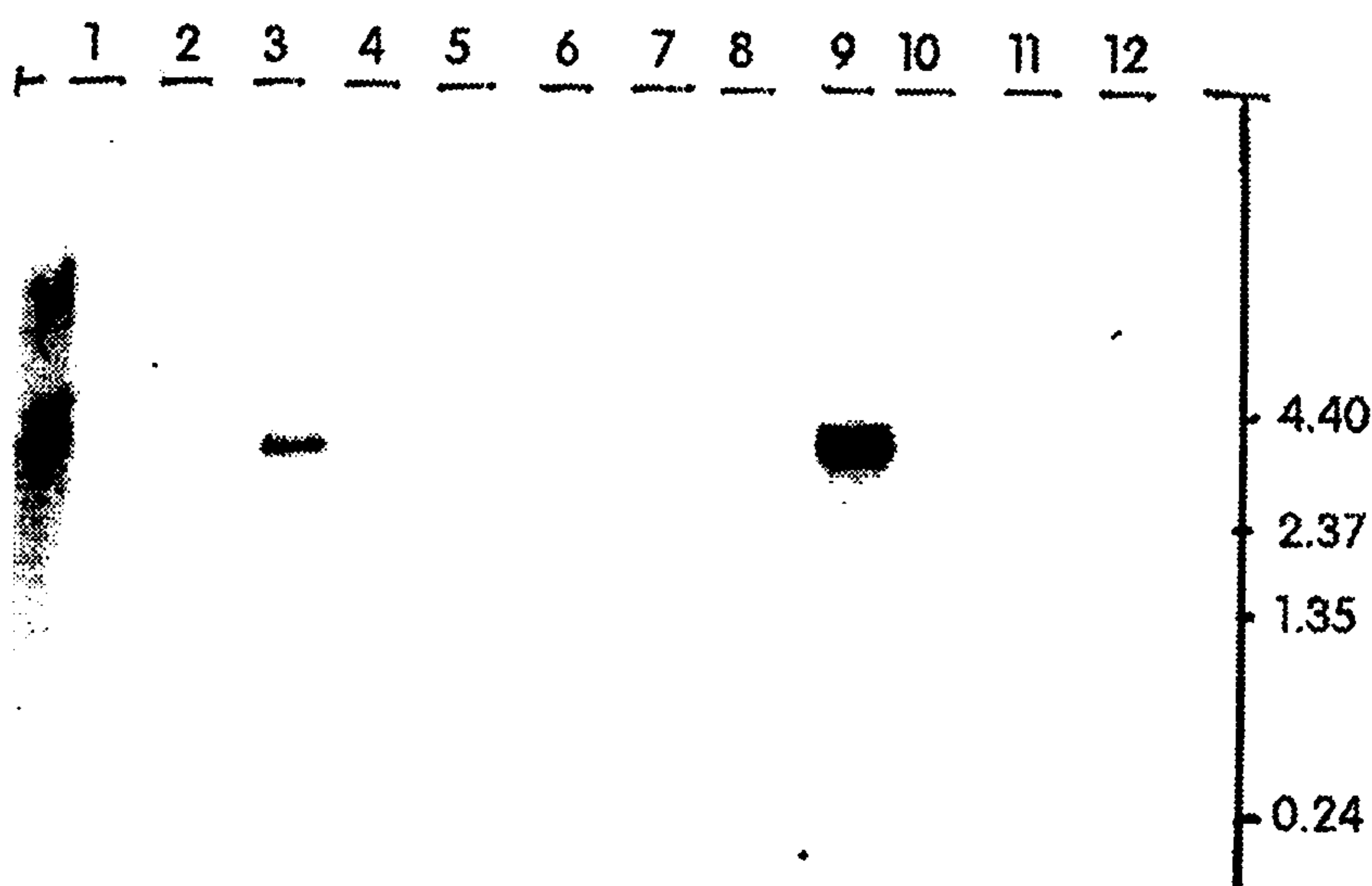


Fig. 1

SUBSTITUTE SHEET

2/11

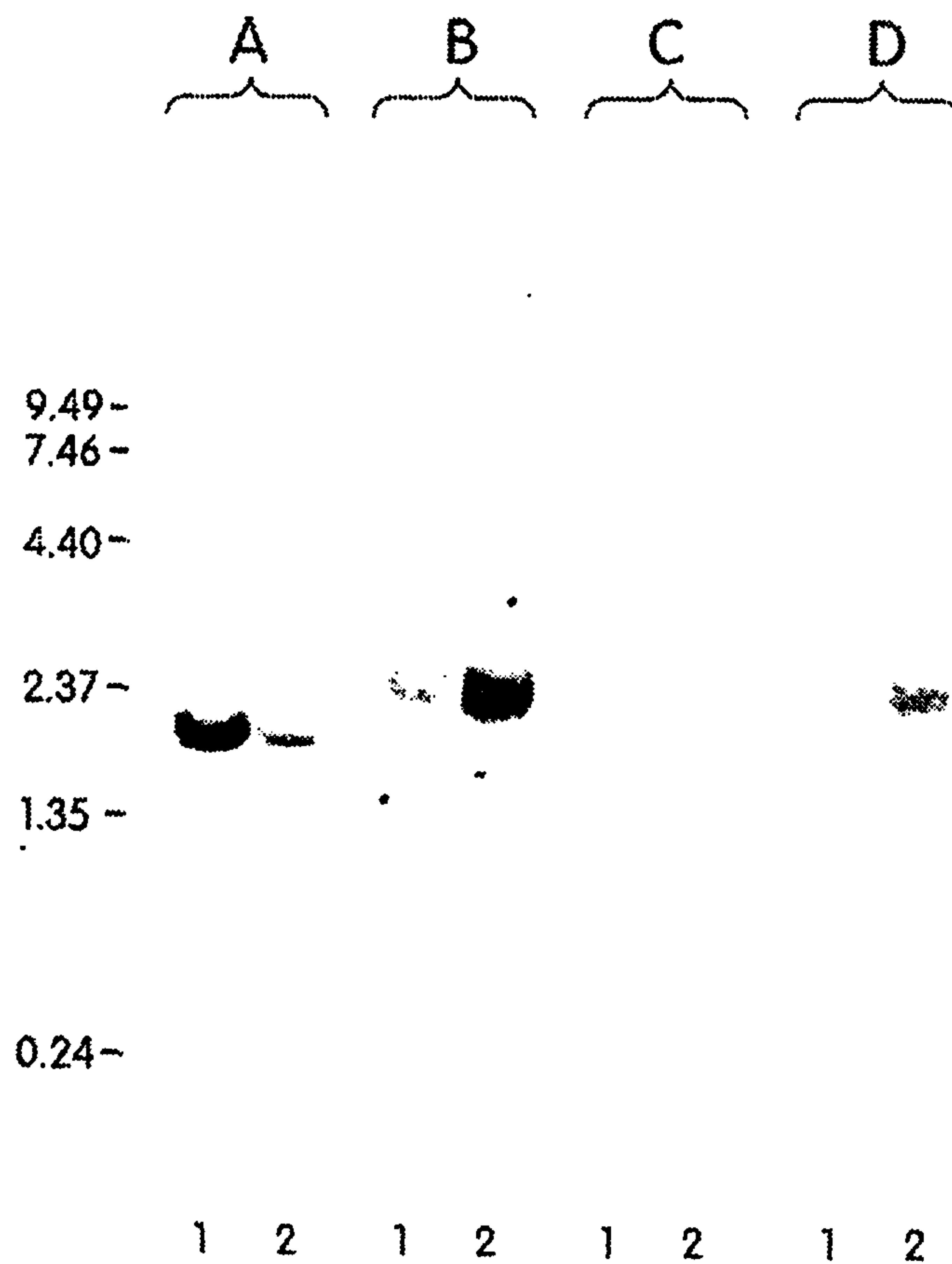


Fig. 2

SUBSTITUTE SHEET

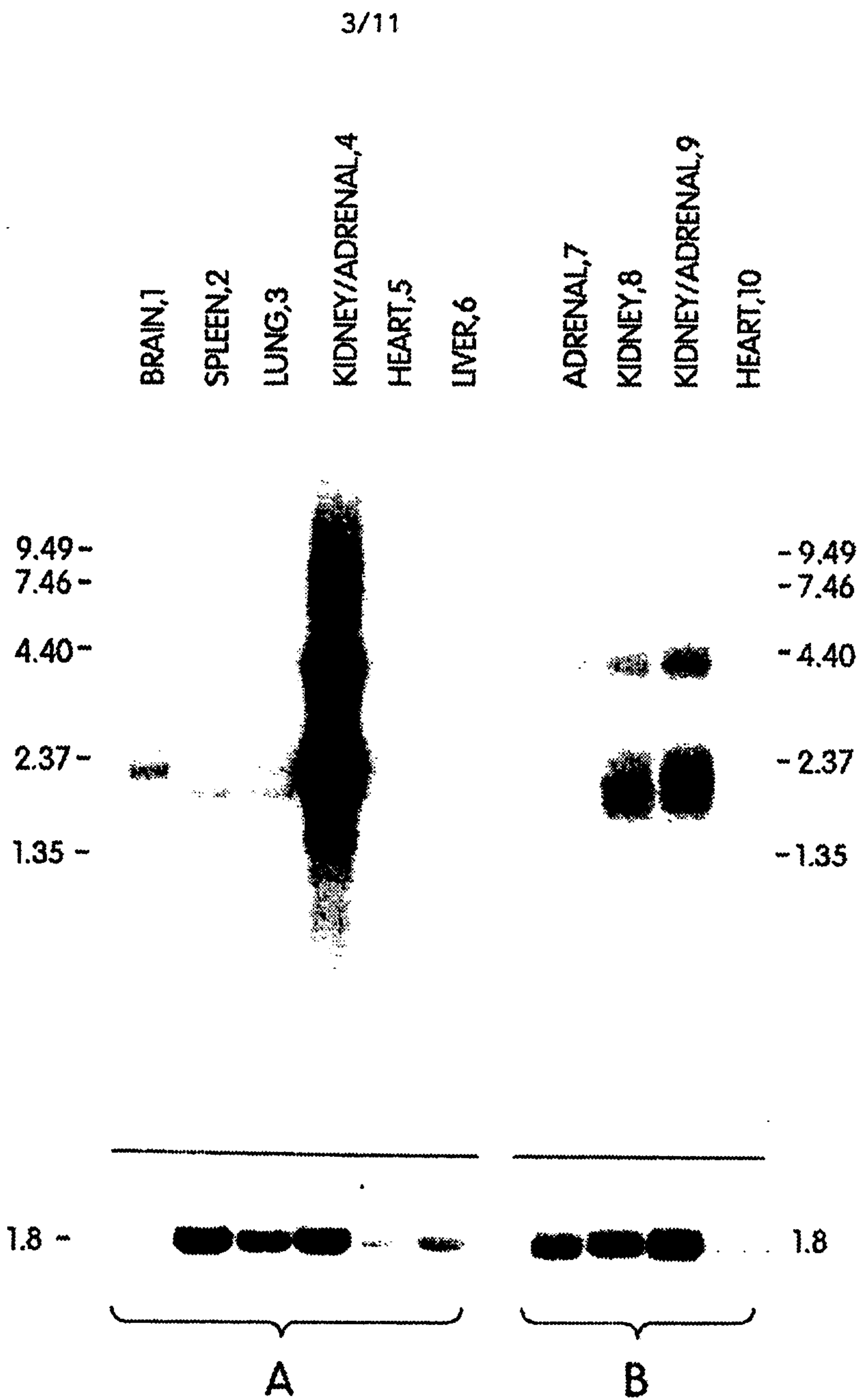


Fig. 3

SUBSTITUTE SHEET



Fig. 7

SUBSTITUTE SHEET

Application number / numéro de demande: 2363965

Figures: 4A,B, 5A,B, 6A,B,C,D 8A,B,e

Pages: _____

Unscannable items
received with this application
(Request original documents in File Prep. Section on the 10th floor)

Documents reçu avec cette demande ne pouvant être balayés
(Commander les documents originaux dans la section de préparation des dossiers au
10^{ème} étage)

1 2 3 4 5 6 7 8 9 10 11 12



4.40

2.37

1.35

0.24