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(71) Applicants (*for all designated States except US*): **ELI LILLY AND COMPANY** [US/US]; Lilly Corporate Center, Indianapolis, IN 46285 (US). **MILES, Rebecca, Ruth** [US/US]; 1604 North Thorntree, Muncie, IN 47304 (US). **ONYIA, Jude, Emeka** [NG/US]; 3401 North Kessler Boulevard, Indianapolis, IN 46222 (US). **THIRUNAVUKKARASU, Kannan** [IN/US]; 8367 Glenside Court, #C, Indianapolis, IN 46256 (US).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): **CHANDRASEKHAR, Srinivasan** [IN/US]; 10225 Coral Reef Way, Indianapolis, IN 46256 (US). **HALLADAY, David, Lloyd** [US/US]; 7804 Hardwick Place, Fishers, IN 46038 (US). **MARTIN, Thomas, John** [AU/AU]; 6 Stoke Avenue, Kew, Victoria 3101 (AU).

(74) Agents: **COHEN, Charles, E.** et al.; Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285 (US).

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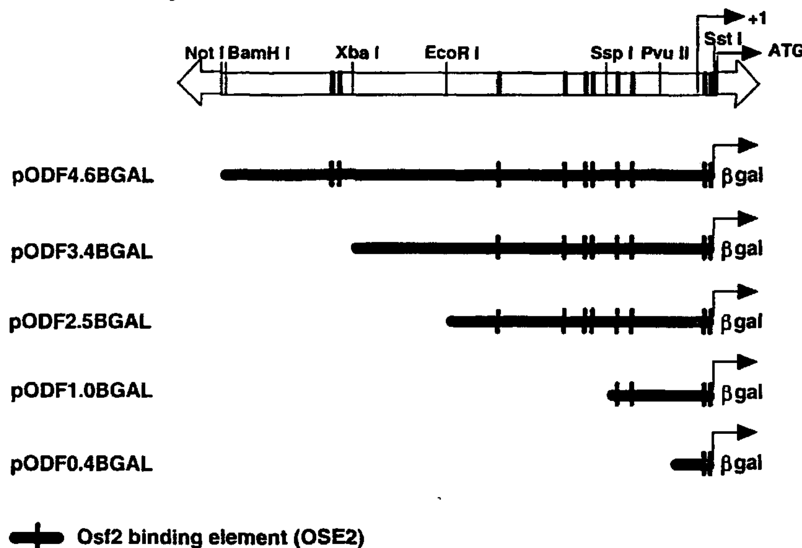

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(57) Abstract: The present invention provides the complete transcriptional regulatory region of the human *odf* gene. The disclosed sequence, fragments thereof, and functional variants thereof, can be used in methods for regulating osteoclastogenesis, and treating bone diseases and other diseases caused by over- or under-expression of osteoclast differentiation factor. The disclosed sequences are also useful in diagnosing patient susceptibility to developing ODF-related bone, cartilage, immune, and arterial diseases, and for diagnosing patient receptivity to treatment with drugs for such diseases. Methods for identifying compounds that modulate osteoclast formation, bone resorption, and other ODF-related bone, cartilage, immune, and arterial diseases are also provided.

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OSTEOCLAST DIFFERENTIATION FACTOR REGULATORY REGION

5 This application claims the benefit of priority of United States Provisional Application Serial No. 60/155,785, filed September 27, 1999, the entire contents of which are herein incorporated by reference.

10 BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to the fields of medical therapeutics and diagnostics. More particularly, the present 15 invention relates to the development of therapeutic drugs, treatment methods, and diagnostic methods in the area of skeletal and other diseases, such as arterial and immune diseases, associated with the over- or under-expression of osteoclast differentiation factor. Among its many aspects, 20 the present invention provides assay methods useful in developing therapeutic drugs for the treatment of such diseases.

Description of Related Art

25 Bone growth, development, and maintenance in mammals is a highly regulated process. The level of bone mass is dependent on the balance of bone formation and resorption. At the cellular level, this balance involves the coordinate regulation and interaction of its component cell types: bone 30 forming cells, called osteoblasts, and bone-resorbing cells, called osteoclasts. Osteoblasts are derived from mesenchymal stem cells, and produce bone matrix during development, after bone injury, and during bone remodeling. Osteoclasts, the only cells that resorb bone, are derived from hematopoietic 35 precursors, most likely of the monocyte/macrophage series

(CFU-GM). Activity of these cell types is tightly regulated by a variety of hormones, growth factors, and cytokines in order to control the integrity of, as well as the amount of, bone during normal bone remodeling.

5 Osteoclast formation has been studied extensively since 1988 in *in vitro* systems and in transgenic animal models. These efforts have identified genes that act as key determinants in the formation of osteoclasts and the regulation of bone mass. Recently, members of the TNF
10 receptor and TNF ligand families have been shown to influence osteoclast formation. These proteins have therapeutic value for inhibiting bone loss in patients.

Osteoprotegerin (OPG), a member of the TNF receptor family, inhibits osteoclast formation at an early stage of
15 development. See Tsuda et al., *Biochem. Biophys. Res. Comm.*, 234:137-142 (1998); Simonet et al., *Cell*, 89:309-319 (1997); and Morinaga et al., *Eur. J. Biochem.*, 254:685-691 (1998). Overexpression of OPG in transgenic mice inhibits osteoclast formation, causing osteopetrosis. Similarly, treatment of
20 ovarectomized (OVX) rats with OPG prevents bone loss. See Simonet et al., *Cell*, 89:309-319 (1997). Targeted deletion of the OPG gene causes severe, early-onset osteoporosis and calcification of the aorta and renal artery smooth muscle. See Bucay et al., *Genes and Development*, 12:1260-1268 (1998).
25 Osteoprotegerin was identified independently by a second group and called osteoclast inhibitory factor (OCIF). See Tsuda et al., *Biochem. Biophys. Res. Comm.*, 234:137-142 (1998).

The ligand for OPG, called osteoclast differentiation factor (ODF), is a member of the TNF ligand family, and
30 promotes osteoclast formation. See Yasuda et al., *Proc. Natl. Acad. Sci. USA*, 95:3597-3602 (1998), and Matsuzaki et al., *Biochem. Biophys. Res. Comm.*, 246:199-204 (1998). ODF is expressed in a limited number of tissues, for example lymph node (restricted to T-cells), lung, and bone
35 (osteoblast/stromal cells and chondrocytes, more specifically hypertrophic chondrocytes). ODF expression is regulated in

osteoblast/stromal cells by factors that increase osteoclast formation. See Tsukii et al., *Biochem. Biophys. Res. Comm.*, 246:337-341 (1998). Three other research groups independently cloned a molecule identical to ODF. The first group named the molecule TRANCE (TNF-related activation induced cytokine), and demonstrated that it regulated T-cell-dependent immune responses and activated c-jun N-terminal protein kinases (JNK). See Wong et al., *J. Exper. Med.*, 186:2075-2080 (1997). The second group identified the molecule in T-cells as a ligand for receptor activation of NF-KB (RANK), a TNFR family protein expressed in dendritic cells. See Anderson et al., *Nature*, 390:175-179 (1997), and Wong et al., *J. Biol. Chem.*, 272:25190-25194 (1997). A third group cloned the same molecule and called it OPG ligand (OPGL). See Lacey et al., *Cell*, 93:165-176 (1998). Recent studies suggest that soluble OPG can block ODF/TRANCE promotion of osteoclast formation. See Yasuda et al., *Proc. Natl. Acad. Sci. USA*, 7:3597-3602 (1998).

ODF is a critical regulator of osteoclast formation, function, and, therefore, bone resorption. Consequently, compounds that alter ODF expression have significant therapeutic value. Therapeutic compounds can be identified via *in vitro* screening assays. While the regulatory region of murine ODF has been partially characterized (Kodaira et al., *Gene* 230:121-127 (1999); Kitazawa et al., *Biochimica et Biophysica Acta*, 1445:134-141 (1999)), this sequence is undesirable for use in screening assays used to identify compounds that alter human ODF expression. Other publications disclosing aspects of ODF gene structure/expression include Lacey et al., *Cell*, 93(2):165-176 (1998); Anderson et al., *Nature*, 390(6656):175-179 (1997); PCT International Publications WO 97/00317, WO 97/00318, and WO 99/00496; and JP10146189 and JP11009269. Effective screening assays evaluate test compounds by comparing: (1) a compound's effect on expression and (2) a baseline that represents a normal level of expression. Screening for compounds that affect a

level of expression that is not the norm, such as that stimulated by a partial regulatory region derived from another species, is of little value in human pharmaceutical development. In this regard, an effective screening assay for identifying physiologically-relevant ligands preferably requires a gene's complete regulatory region. Therefore, there exists a need to fully characterize the transcriptional and translational regulatory region of the human *odf* gene.

SUMMARY OF THE INVENTION

Accordingly, in one aspect, the present invention provides the nucleotide sequence of the complete regulatory region for the human *odf* gene.

In another aspect, the present invention provides a method of identifying compounds that affect osteoclast formation, bone resorption, or immune responsiveness.

In another aspect, the present invention provides methods of diagnosing bone, immune, and arterial diseases in a patient.

In accomplishing these and other aspects of the present invention, there is provided, in accordance with one aspect of the present invention, a DNA sequence that represents the complete regulatory region of the human *odf* gene. DNA constructs containing the ODF regulatory region also are provided.

In another aspect, there is provided a method for identifying a compound that affects osteoclast formation and/or bone resorption, comprising:

(a) contacting a host cell with one or more test compounds, wherein said host cell comprises a DNA construct of the present invention, and wherein said construct comprises the human *odf* regulatory region and a reporter polynucleotide; and

(b) assaying for evidence of expression of said reporter polynucleotide.

In yet another aspect, there is provided a method for identifying a compound that affects osteoclast formation and/or bone resorption, comprising:

(a) contacting a cell-free translation system with one or more test compounds, wherein said system comprises a polynucleotide construct of the present invention comprising the human *odf* regulatory region and a reporter polynucleotide; and

(b) assaying for evidence of expression of said reporter polynucleotide.

In yet another aspect, there is a provided a method for diagnosing bone disease in a patient, comprising comparing the DNA from bone cells of the patient with the presently disclosed DNA sequence. Similarly, there are also provided methods for diagnosing immune and arterial diseases in a patient, comprising comparing the DNA from bone cells of the patient with the presently disclosed DNA sequence.

In still another aspect, the present invention provides a method of identifying in a patient susceptibility, receptiveness, or responsiveness to drug therapy, comprising comparing the DNA from bone cells of said patient with the presently disclosed DNA sequence.

In other aspects, there are provided methods of identifying in a patient a predisposition to developing bone or immune disease, comprising comparing the DNA from bone cells of said patient with the presently disclosed DNA sequence.

In still other aspects, there are provided methods of modulating bone resorption or immune responsiveness in a patient, comprising administering to the patient a DNA construct of the present invention, wherein the construct comprises a polynucleotide encoding osteoclast differentiation factor.

In other aspects, there are provided methods of modulating bone resorption or immune responsiveness in a patient, comprising administering to the patient one or more

compounds identified using the methods of the presently disclosed invention. There are also provided methods of modulating ODF expression in a cell, *in vitro* or *in vivo*, using one or more compounds identified using a screening assay of the present invention. Pharmaceutically effective amounts of compounds for *in vivo* use can be determined by routine methods well known the pharmaceutical arts, such as by establishing dose-response relationships in subjects.

More specifically, in a first aspect, the present invention provides an isolated nucleic acid fragment comprising the transcriptional regulatory region of the human *odf* gene, a subfragment thereof, or a functional variant of either, exhibiting human *odf* gene transcriptional regulatory activity, excluding the *odf* protein coding region. The isolated nucleic acid fragment or subfragment thereof can comprise a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15, or the complement of any one of said nucleotide sequences.

In a second aspect, the present invention provides an isolated nucleic acid fragment that hybridizes to the complement of a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15 in 1X phosphate buffer comprising 0.1M Na_2HPO_4, 0.5M NaCl, 0.0052 M EDTA, pH 7.0, and 1% Sarkosyl, at 45-65°C for 2 hours to overnight, followed by washing in 1mM Tris-HCl, pH 8.0, 1% sarkosyl at room temperature for 10 to 15 minutes, wherein said fragment exhibits human *odf* gene regulatory region transcriptional regulatory activity, with the *proviso* that said fragment comprises a novel nucleotide sequence, previously unknown at the time of filing of this application.

In a third aspect, the present invention provides an isolated nucleic acid fragment having a sequence identity in the range of from about 85% to about 99% compared to a nucleotide sequence selected from the group consisting of SEQ

ID NO:1, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15, wherein said fragment exhibits human *odf* gene regulatory region transcriptional regulatory activity, with the *proviso* that said fragment comprises a novel nucleotide sequence, previously unknown at the time of filing of this application.

In another aspect, the present invention provides a recombinant DNA construct comprising any of the preceding isolated nucleic acid fragments, subfragments, or functional variants of either. The recombinant DNA construct can further comprise a polynucleotide encoding a protein of interest, and, optionally, at least one translational regulatory region required for expression of said polynucleotide, wherein said polynucleotide encoding said protein of interest is operably linked for expression to said isolated nucleic acid fragment, subfragment, or functional variant, and to said translational regulatory region. The recombinant DNA construct can be an expression cassette or an expression vector.

In another aspect, the present invention provides a cultured host cell comprising any one of the foregoing recombinant DNA constructs.

In another aspect, the present invention provides the use of any of the foregoing isolated nucleic acid fragments, subfragments, or functional variants thereof, in an assay to identify an agonist or antagonist of osteoclast differentiation factor expression.

In another aspect, the present invention provides the use of any one of the foregoing isolated nucleic acid fragments, subfragments, or functional variants thereof for the manufacture of a composition for the diagnosis of a human susceptible to, predisposed to, or at increased risk for developing a symptom, condition, or disease caused by over- or under-expression of osteoclast differentiation factor.

In another aspect, the present invention provides a composition, comprising any of the foregoing isolated nucleic acid fragments or subfragments, or functional variants

thereof, recombinant DNA constructs, or host cells, and a carrier, diluent, or excipient.

In another aspect, the present invention provides a pharmaceutical composition, comprising any of the foregoing
5 isolated nucleic acid fragments or subfragments, or functional variants thereof, recombinant DNA constructs, or host cells, and a pharmaceutically acceptable carrier, diluent, or excipient.

In another aspect, the present invention provides a
10 method of identifying a compound that modulates expression of osteoclast differentiation factor, comprising:

(a) contacting:

(i) a host cell in which osteoclast differentiation factor is normally
15 expressed, and

(ii) a test compound,

wherein said host cell comprises a DNA expression construct comprising a nucleic acid fragment or subfragment selected from the group consisting of SEQ ID NO:1, SEQ ID
20 NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15, or a functional variant thereof, and a reporter polynucleotide operably linked thereto, and wherein said reporter polynucleotide is expressed;

(b) determining the level of expression of said
25 reporter polynucleotide in said host cell of step (a);

(c) determining the level of expression of said reporter polynucleotide in a host cell identical to said host cell of step (a),

wherein said identical host cell is not contacted
30 with said test compound; and

(d) comparing the level of expression of said reporter polynucleotide in step (b) with the level of expression of said reporter polynucleotide in step (c),

wherein an increase or decrease in the level of
35 expression of said reporter polynucleotide in step (b) compared to the level of expression of said reporter

polynucleotide in step (c) identifies said test compound as a compound that modulates the expression of osteoclast differentiation factor. In this method, the host cell can be selected from the group consisting of an osteoclast progenitor cell, an osteoclast, an osteoblast, a stromal cell, a chrondrocyte, a T-cell, and a fibroblast.

In another aspect, the present invention provides a method of identifying a compound that modulates expression of osteoclast differentiation factor, comprising:

(a) contacting a test compound, and
a host cell comprising:
(i) a plasmid comprising a nucleic acid fragment or subfragment selected from the group consisting of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15, or a functional variant thereof, and a reporter polynucleotide operably linked for expression thereto, and
(ii) an effector plasmid comprising a nucleotide sequence that codes on expression for a factor required for osteoclast differentiation factor expression,
wherein both said reporter polynucleotide and said factor required for osteoclast differentiation factor expression are expressed;

(b) determining the level of expression of said reporter polynucleotide in said host cell of step (a);

(c) determining the level of expression of said reporter polynucleotide in a host cell identical to said host cell of step (a),
wherein said identical host cell is not contacted with said test compound; and

(d) comparing the level of expression of said reporter polynucleotide in step (b) with the level of expression of said reporter polynucleotide in step (c),
wherein an increase or decrease in the level of expression of said reporter polynucleotide in step (b)

compared to the level of expression of said reporter polynucleotide in step (c) identifies said test compound as a compound that modulates osteoclast differentiation factor expression.

5 In the foregoing method, the factor required for osteoclast differentiation factor expression can be osteoblast specific transcription factor 2, and the effector plasmid can be pEF/Cbfa1/myc/cyto, encoding Cbfa1 (osteoblast specific transcription factor 2). Furthermore, the host cell can be 10 selected from the group consisting of CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, and WI38 cell lines.

In any of the foregoing methods, expression of the reporter polynucleotide can be determined by measuring activity of the expressed reporter polynucleotide product, 15 which can be beta-galactosidase. Furthermore, in any of the foregoing methods, an increase in expression of the reporter polynucleotide in step (b) compared to that in step (c) identifies the test compound as an agonist of osteoclast differentiation factor expression; a decrease in expression of 20 the reporter polynucleotide in step (b) compared to that in step (c) identifies the test compound as an antagonist of osteoclast differentiation factor expression.

In another aspect, the present invention provides an agonist or antagonist of osteoclast differentiation factor 25 expression identified by any of the foregoing methods.

In another aspect, the present invention provides the use of an agonist or antagonist identified by any of the foregoing methods in the manufacture of a medicament for the treatment of a disease in a human caused by under-expression or over-30 expression, respectively, of osteoclast differentiation factor.

In another aspect, the present invention provides the use of a compound that modulates expression of osteoclast differentiation factor in the manufacture of a medicament for 35 the treatment of a disease in a human caused by abnormal expression of osteoclast differentiation factor. Such disease

can be bone disease, arthritis, arterial disease, abnormal immune function, abnormal lymph node development, or abnormal T- or B-cell function caused by abnormal expression of osteoclast differentiation factor. The bone disease can be malignant bone disease, rheumatoid arthritis, osteoarthritis, elevated bone resorption, osteoporosis, Paget's disease of bone, hypercalcemia of malignancy, expansile osteolysis, or periodontal disease, and the compound can be an antagonist of osteoclast differentiation factor expression. The arterial disease can be arterial calcification, and the compound can be an antagonist of osteoclast differentiation factor expression. When the bone disease is osteopetrosis, the compound can be an agonist of osteoclast differentiation factor expression. Furthermore, in the use according to any one of these indications, the compound can be identified by any of the methods discussed above. Additionally, in the use according to any one of these indications, the human can be diagnosed as having a polymorphism or mutation at one or more nucleotide positions in the osteoclast differentiation factor regulatory region in DNA thereof.

In another aspect, the present invention provides a composition, comprising an agonist or antagonist of osteoclast differentiation factor expression, and a carrier, diluent, or excipient. Such agonist or antagonist is preferably a novel compound unknown prior to the time of filing of this application, and one other than a hormone, growth factor, or cytokine such as bone morphogenetic protein 2, 1α,25-dihydroxy vitamin D_3, dibutyryl cyclic AMP, dexamethasone, IL-11, IL-17, prostaglandin E_2, parathyroid hormone, or the molecules disclosed in PCT International Publication WO 00/15807. The agonist or antagonist can be identified by any one of the methods discussed above.

In another aspect, the present invention provides a pharmaceutical composition or pharmaceutical pack, comprising an agonist or antagonist of osteoclast differentiation factor expression, and a pharmaceutically acceptable carrier,

diluent, or excipient. Such agonist or antagonist is preferably a novel compound unknown prior to the time of filing of this application, and one other than a hormone, growth factor, or cytokine such as bone morphogenetic protein 2, 1α,25-dihydroxy vitamin D_3, dibutyryl cyclic AMP, dexamethasone, IL-11, IL-17, prostaglandin E_2, parathyroid hormone, or the molecules disclosed in PCT International Publication WO 00/15807. The agonist or antagonist can be identified by any one of the methods discussed above. The pharmaceutical pack can comprise instructions for administration of the agonist or antagonist to a human. In either the pharmaceutical composition or pharmaceutical pack, the agonist or antagonist can be identified by any of the methods discussed above. Furthermore, in either case, the human can be diagnostically tested for a polymorphism or mutation at one or more nucleotide positions in the osteoclast differentiation factor regulatory region in DNA thereof.

In another aspect, the present invention provides a process for making an agonist or antagonist of osteoclast differentiation factor expression, comprising:

(a) carrying out any of the methods discussed above to identify an agonist or antagonist of osteoclast differentiation factor expression; and

(b) manufacturing said agonist or antagonist.

In another aspect, the present invention provides a method of preparing a medicament for the treatment of a bone disease, arthritis, arterial disease, abnormal immune function, abnormal lymph node development, abnormal T- or B-cell function, or other disease in a human caused by abnormal osteoclast differentiation factor expression, comprising:

(a) identifying an agonist or antagonist of osteoclast differentiation factor expression by any of the methods discussed above; and

(b) formulating said agonist or antagonist as a medicament.

In another aspect, the present invention provides a method of identifying a mutation or polymorphism in the osteoclast differentiation factor regulatory region of a human subject's or patient's *odf* gene, comprising comparing the nucleotide sequence of the osteoclast differentiation factor regulatory region of the *odf* gene in DNA from said subject or patient with a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15, wherein any difference in nucleotide sequence between said osteoclast differentiation factor regulatory region DNA and said nucleotide sequence identifies a mutation or polymorphism in the osteoclast differentiation factor regulatory region of said subject's or patient's DNA. In this method, the comparison can be conducted using nucleotide sequence analysis or nucleic acid hybridization analysis.

In another aspect, the present invention provides a method of identifying a human subject or patient at increased risk for having an altered susceptibility or predisposition to developing a bone disease, cartilage disease, immune disease, arterial disease, or other disease caused by abnormal osteoclast differentiation factor expression, comprising comparing the nucleotide sequence of the osteoclast differentiation factor regulatory region of the *odf* gene in DNA from said subject or patient with a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15, wherein any difference in nucleotide sequence between said osteoclast differentiation factor regulatory region DNA and said nucleotide sequence identifies a mutation or polymorphism in the osteoclast differentiation factor regulatory region of said subject's or patient's DNA that places said subject or patient at increased risk for having an altered susceptibility or predisposition to developing said bone disease, cartilage disease, arterial disease, immune disease, or other disease.

In another aspect, the present invention provides a method of identifying a human patient or subject at increased risk for having an altered susceptibility or receptiveness to treatment of a disease caused by abnormal osteoclast differentiation factor expression with a compound that affects osteoclast differentiation factor expression through an interaction with the osteoclast differentiation factor gene regulatory region, comprising comparing the nucleotide sequence of the osteoclast differentiation factor regulatory region of the *odf* gene from DNA of said subject or patient with a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15, wherein any difference in nucleotide sequence between said osteoclast differentiation factor regulatory region DNA and said nucleotide sequence identifies a mutation or polymorphism in the osteoclast differentiation factor regulatory region of said subject's or patient's DNA that places said subject or patient at increased risk for having an altered susceptibility or receptiveness to said treatment.

In another aspect, the present invention provides a method of treating a human suffering from a symptom, condition, or disease caused by over-expression of osteoclast differentiation factor, comprising administering to said human a pharmaceutically effective amount of an antagonist of osteoclast differentiation factor expression. In this method, the antagonist can be identified by any of the methods discussed above.

In another aspect, the present invention provides a method of treating a human suffering from a symptom, condition, or disease caused by under-expression of osteoclast differentiation factor, comprising administering to said human a pharmaceutically effective amount of an agonist of osteoclast differentiation factor expression. In this method, the agonist can be identified by any of the methods discussed above.

In another aspect, the present invention provides a method of treating a human in need of treatment with an agonist of osteoclast differentiation factor expression, comprising:

(a) determining whether a polymorphism or mutation exists at one or more nucleotide sites in the osteoclast differentiation factor regulatory region in DNA of said human; and

(b) if a polymorphism or mutation exists, administering to said human a pharmaceutically effective amount of an agonist of osteoclast differentiation factor expression.

In another aspect, the present invention provides a method of treating a human in need of treatment with an antagonist of osteoclast differentiation factor expression, comprising:

(a) determining whether a polymorphism or mutation exists at one or more nucleotide sites in the osteoclast differentiation factor regulatory region in DNA of said human; and

(b) if a polymorphism or mutation exists, administering to said human a pharmaceutically effective amount of an antagonist of osteoclast differentiation factor expression.

In either of the two foregoing methods, the human can be suffering from a symptom, condition, or disease caused by an abnormal level of expression of osteoclast differentiation factor.

In another aspect, the present invention provides a method of modulating bone resorption in a patient in need thereof, comprising administering to said patient a pharmaceutically effective amount of a DNA construct as discussed above, wherein the protein of interest is osteoclast differentiation factor.

In another aspect, the present invention provides a method of modulating bone resorption in a patient in need

thereof, comprising administering to said patient a pharmaceutically effective amount of a compound identified by any of the methods discussed above.

In another aspect, the present invention provides a method of modulating immune responsiveness in a patient in need thereof, comprising administering to said patient a pharmaceutically effective amount of a DNA construct as discussed above, wherein the protein of interest is osteoclast differentiation factor.

In another aspect, the present invention provides a method of modulating immune responsiveness in a patient in need thereof, comprising administering to said patient a pharmaceutically effective amount of a compound identified by any of the methods discussed above.

In another aspect, the present invention provides a kit or package, comprising an isolated nucleic acid fragment comprising the transcriptional regulatory region of the human *odf* gene, a subfragment thereof, or functional variant of either exhibiting human *odf* gene transcriptional regulatory activity, wherein said fragment, subfragment, or functional variant thereof excludes the *odf* protein coding region. The isolated nucleic acid fragment or subfragment thereof can comprise a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15. The isolated nucleic acid fragment, subfragment thereof, or functional variant of either can be contained within an expression cassette. Furthermore, the isolated nucleic acid fragment, subfragment thereof, or functional variant of either can be (a) operatively linked within a vector to a polynucleotide encoding human osteoclast differentiation factor, or (b) operatively linked within a vector to a polynucleotide encoding a heterologous reporter molecule. The vector can be contained within a vector-releasing cell. Furthermore, the vector of (a) can further comprise, operably linked to said polynucleotide encoding said human osteoclast differentiation

factor, at least one translational regulatory region required for expression of said human osteoclast differentiation factor in said vector-releasing cell. The vector of (b) can further comprise, operably linked to said polynucleotide encoding said heterologous reporter molecule, at least one translational regulatory region required for expression of said heterologous reporter molecule in said vector-releasing cell.

In another aspect, the present invention provides a computer readable medium having stored thereon the nucleotide sequence of a nucleic acid fragment encoding the transcriptional regulatory region of the human *odf* gene, a subfragment thereof, or a functional variant of either, exhibiting osteoclast differentiation factor transcriptional regulatory region activity, wherein said fragment, subfragment thereof, or functional variant thereof excludes the *odf* protein coding region. The nucleotide sequence can be selected from the group consisting of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15.

In another aspect, the present invention provides a diagnostic method, comprising determining the nucleotide sequence of the osteoclast differentiation factor transcriptional regulatory region in DNA from a human, or a diagnostically useful fragment thereof, and comparing said nucleotide sequence to a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15 provided in a computer readable medium, thereby identifying any polymorphism or mutation in said osteoclast differentiation factor transcriptional regulatory region in said DNA from said human.

Further scope of the applicability of the present invention will become apparent from the detailed description provided below. However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the present invention, are given by way of illustration only since various changes and

modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

5 The above and other aspects, features, and advantages of the present invention will be better understood from the following detailed description taken in conjunction with the accompanying drawings, all of which are given by way of illustration only, and are not limitative of the present 10 invention, in which:

Figure 1 provides the nucleotide sequence of the human ODF regulatory region. The entire sequence shown in Figure 1 is 4.628 kilobases, and starts proximally at the ATG translational start site (in bold face) at the 3′ end of the 15 sequence. The initial 31 nucleotides shown in bold at the 5′ end of the nucleotide sequence shown in Figure 1 are from the P1 vector (pAd10SacBII) component of the human P1 library, discussed below. Thus, the nucleotide sequence of the human ODF regulatory region *per se* is 4.597 kilobases, and has the 20 following sequence (SEQ ID NO:1):

SEQ ID NO:1

GGATCCTCTCCGGAGTTCAGCAAAGTGAAACGTCTGTCATAATAATAACGAATGACTTCCTT
TTTCATTTTCATTCATATAGTGAAGTTTTCTAAAGGCTGCATCATGTGCAATATTGTAACAG
25 AGTAAGTGCAGGACTGAATGTGACTCTATCAGGCCAATTGTAGAGATGTGAAAAAATGTAAA
ACAGTGGCACTTTTCTCCCTACTTTTTTTTGAAAATCTGTTTTTTTAAATAAAATAATTTTA
TAAAAGTATTATGAATTATTTATAAAATTATATTAACATTATGTTAACATGCTAATATGGTA
AAATTTTCTGCTTGGAGTTTGAATACACCAAATATTTATAAATATAACTCACACAAATAAAA
CCTCTTTGGTGTTCTCAAAATTTTGAAGAATGTAAAAGGTTTGAAAATTGCTGATCTAGCAA
30 ATGACTGAACATGAACAGCTATAGTATTTGTACCTGCCCAGCAGTGCAGCAATTCCTTATCC
TTCTCATATCTGCACTTTAATTTTCCTTTGACAAATATCTCTCCCTCCTCTCAGCCCATGAC
ATGAGGTTCACATGGGGTTAACTTAATTCCCTGGCTCAAAGGAAAGGTATTAAATTCAGACT
TGTATCCAACCATTCCTGAAGCTAGACTTAGCCCTATTTTTCAATAACATGAACCAATCAAT
TTTCACATGAGTCCAAAATAATTCTATGTTAATACACTAAGGTACTAGGAAATATAGTTTGA
35 GAAATGTTGATCCAAACATTGTGTTATTTACAGTGGAGTATTGACATAAACTTTGAATCTTC

AAATATGTTCTGGTGTCTTGGCATCTCTTAATACCTATTAGCTTACAAGGCTTTCACTCAAC
TATTTTATAATTTTGATAATGACTTAATTGATTAGTTGATATATTGTTAAAATAAATATATT
AATGAATTTATGATAAATAAGGCAGATAAATAAGACATGCAATTAGGAAGACATGTTAAACA
AATTGTTATAATAATACAATCACTCTCAGCTTAGGATAGCTCCTGGCCACTTTCTCTCTGGG
5 TGGTTTTTACTCTGGGAGTAGTTTAAATCATTATCTAGTAGTAGTTTAAAGCATTATCTTTG
CCTAAGAGCTTTCGCTGACTCCCCACATTTGCATTGTACTAAGAGTTTTCTCTGACTCCCCA
CATAGGTCTAGACCCTAGTATTATAAGATTCTCATTGTACTTGCACTTTGCCTTCAAAGTAC
TAATCACGGTTTTGTTAGTGATTTGTGTGATGATTTGTTGAATCTTTTTTTTTTCCCACTA
GGGTGTAAGCCCCATGTTCCATCTTGATCACCATGTTTCTAGCCCAGTGCTGGCATATAGTG
10 GGTTCTCACTAATATATCTGTAGAGTAAATGAAGAAATGCATGAGTGACATGACAGGAGAAT
TTAAGGATGCCATGGGAGCATAAAACAGAGGGAGCCACCTGGGTGAGGAGAGCTGAGAAAGA
CTTCTGGAGAGGCGACATTTGAGCTGAGAAAGGAAAGACAAGTGGGAGAGTCCTCCAGGTGT
AGAAGTTGGAGAGATGAGCGCTCCAGTTAGGTAGTATTTGAAGCTGATGTAGAAAAGGAGTC
TTGAGCCAGCTTGTGAAGGACTATTGGAGAGTTTTATTTTTATTTTTATCTTTTTTTTAATT
15 TTTGAGACAGAATCTTGCTTTGTCTCCCAGGCTGGAGTGCAGTGGCATGATTGTAGCTTACT
GCAGCTTCGACCTCCTGGGCTCAAACAATCCACCTATCTCAGCCTTCTGAGTAACTGGGACC
AGAGATGTGCACCAAAATGCCTGGCTAATTTGTTCATTTTTTGTAAAGATAGGGTCTCCCTA
TGTTCCCCAGGCTATTCTCCATCTCCTGGGCTCCAGTGATCCTCACGCCTCGGCCACCCAAA
GTGCTGGGATTATAGAAGTGAACCACTGCGCCTGGCCTATTGAAGGTTTTTAATCTTCAGAG
20 TTTCGACTTTATCAACAACACTTAGAAGCCACCAAAGAATTGCAGGTATGGAAATGACATAT
ACTTTTGCTTTTAGAAGAAAATCCTGATCAGTGTGCACAGAATTCTTCAGGGGGCAAGTGTG
ATTCATTCTGATAAGATATAGCATGGCTTAGACTGGGAGACTGGCAGAGGCTTTGAAGATTT
CTTTGCTCAAATTTTATTCAGCAAGTATTTACCATGCACCTACTATAGCAGGCAACATTTTT
AGGAAATGGTGAATGTTACAGAGGTGAATAATACAGCAAGAGTCGTTGAACATATGGAGTTT
25 ATCTATTAGTTGGGGAGTGAATGTTGACAAAGGAATAAGTAAATACATAGGCAAGAAAGATA
CATTACCTGTGAAACAGCAGCAGGTAGACTGACAGTGGAGTATCTAATACAGCCTATGGAAG
CCAGAAGATAGTGGGATGACATTTTTGGAGTACTAGTAGAAATGTCATATGAAGAACTCTGT
AGGAATGTAACATACGGTCCCATATATGAAGCTCCTGGGTCAAGTATACCTGAACATAATTC
AGGGATTTGAGGGACTTTCTTGTAACCTGAGGATCAAGATGTCAAGGAATTAAAAACATGTA
30 TAAAACATTGTTGTATAAAAACCCATTAAAAAGAATGGAAGACACTATAGTAAAATCATTGT
GGGTTTAGTTGTTATAACACATTTTAAAAATCTTTGATCCCAATCAATATTTATAAGAAAGA
AGAAATATGGAATTATTTCCTGAGTCAAGGAGCAGGGAGAGAATGAGGAAGAAGAGGAGGAG
GAGGAGGGGGAGGAGGAGACAATAAACCTACTTCCCAAAGTTAACAAACAAAAAGTGGGAAG
AGGTCAAAGACTACAAGGAGTAGAATTAACGTCAATTGTTTCTATGTTTGAGTCTGAAAATT
35 TTTTGTCCCTTCTCCACCAACCTATATATTGATACACATATTAATGCTAAAGGCATTTTTGT
ATTTGAACAGATCATTTTCTTTGTATGGCTGCCTTTAAAAAAAATTCAACCTGGTCACTCTT

CCTCAACATTTACTGAGGTCTAAGTGTTCAATTTAGAACACATGCTTTAATAACTCAGAGAC
CTGTCATTTGTCACAAATCTTGCCTAGAGAAATACTCATTAGCGAATTAGGCAGAAAGAGGA
TGCAAAATAAAAAGGCACAGTAGTCCCCTGATATCCATGGAAGACTGGTTCCAGGACACCAC
CAAACCCCTCCCCGCAAATACCAAAATCCATGGATGTTCAAGTTTCTTAACATATCATGGCA
5 TAGTATTTGCATTTAACCTACACACATCCTCTTGTACACTTGAAATTATCTTTAGATTATTT
ATAATACTTAATAGAATGTAAATGCTATGTAACTAGTTGTGTATCATTTAGGAAATGATCAC
AAGAAAAAAAGTCTACAGATGTTAGTCCAGACACAGCCATCCTTTTTTTTTTTTCAAATAT
TTTTGATCTGTGGTTCATTGCATCCACAGATGTGGAACCCATGGATACTGTGGCTAACTGT
ATTAATAAAAAAGTGGAAACATCCTAAGTTTCATGGGTGTTTAAATTGGTCAGCAACTTCCT
10 TCTGAAGAAGTATCAGAATTTGTGAGCAATGTTAATATTTTTGTTTCTCACTAAGAGCCAC
AGTTCTGAATAGAGGTTTTTAAAAAGCCCTAGCAAGGTTTCTTTAGCAATGAAACTAACATT
TAACTGTATCATCAGCTTCGTGTTACATCTCTTTCCTGACTGTTGGGTGAGCCCTCCTCGGA
TGCTTGCTTCTGGCTACACGCCCCTTTACCCTTTTCTCTGCACTGTTTTCATCTTTATAAAG
TCAGAGTTGGTGTCTATAGGCTCTCTACTGCCACATTCAAGACCTGCCTCGCTCAATGTCAC
15 CTTCAAGATGCAGAAATAGGGATTTGGGAAGGGGATTGTGAAATTTTCGAAGTCTTCCAAAA
TACTTTGAGAAACTATATTTGGAAGCACTTTGGGGGGAGAGGTTGGACAGGAAGGGTCTTCA
GAGATCATCAAATTTAACTTTCTAAATCCTAAGGAGGAAACCGAGACTCCAGGATGTGAAGT
CCCTTCTCTACCAAACTAGAATGGATGCAGGAGGAATGTCTGAGGTGCAATCCTTATCCTTT
AGCAAAGGTGTCCTCTGCGTCTTCTTTAACCCATCTCTTGGACCTCCAGAAAGACAGCTGAG
20 GATGGCAAGGGGAGTCTGGAACCACTGGAGTAGCCCCCAGCCTCCTCCTTGGAGGGCCCCCA
TGAAGGAGGCCCTTCAGTGACAGAGATTGAGAGAGAGGGAGGGCGAAAGGAAGGAAGGGGAG
CCAGAGGTGGGAGTGGAAGAGGCAGCCTCGCCTGGGGCTGATTGGCTCCCGAGGCCAGGGCT
CTCCAAGCGGTTTATAAGAGTTGGGGCTGCCGGGCGCCCTGCCCGCTCGCCCGCGCGCCCCA
GGAGCCAAAGCCGGGCTCCAAGTCGGCGCCCCACGTCGAGGCTCCGCCGCAGCCTCCGGAGT
25 TGGCCGCAGACAAGAAGGGGAGGGAGCGGGAGAGGGAGGAGAGCTCCGAAGCGAGAGGGCCG
AGCGCCATG

Also highlighted in Figure 1 are the TATAA box (in bold face, italicized, and underlined); the +1 site (the G residue
30 49 nucleotides downstream of the TATAA box, shown in bold, and underlined); potential OSE-2 sites (binding sites for osteoblast specific factor 2, Osf2/CBFA1) (in bold face and underlined); and an *Sst*I restriction endonuclease site (underlined). The +1 site was arbitrarily assigned, based on
35 published information. For the studies described in Examples 1 and 2, the cloned ODF regulatory region sequence excluded

the sequence 5'-CGAAGCGAGAGGGCCGAGCGCCATG-3' (SEQ ID NO:2) after the *Sst*I site, containing the ODF ATG start codon.

Figure 2 schematically depicts the ODF regulatory region 5' deletion constructs of Example 1.

5 Figure 3 graphically depicts basal expression achieved by the ODF regulatory region deletion constructs of Example 1 in UMR106 cells.

Figure 4 shows the effect of Osf2 on ODF regulatory region expression in COS1 cells using the regulatory region 10 deletion constructs of Example 1.

DETAILED DESCRIPTION OF THE INVENTION

The following detailed description of the invention is provided to aid those skilled in the in practicing the present 15 invention. Even so, the following detailed description should not be construed to unduly limit the present invention as modifications and variations in the embodiments discussed herein can be made by those of ordinary skill in the art without departing from the spirit or scope of the present 20 inventive discovery.

The contents of each of the references cited herein are herein incorporated by reference in their entirety.

The present invention provides a nucleic acid fragment containing the complete transcription regulatory region of the 25 human *odf* gene. The novel 4.6 kb regulatory region contains several regulatory elements that are utilized by a variety of transcription factors to influence ODF expression. Potential OSE-2 sites (binding sites for osteoblast specific transcription factor 2 (Osf2; CBFA1)) are shown in bold face 30 and underlined in Figure 1. Table 1 lists these sequences.

35

Table 1.

Sequences of Putative OSE-2 and OSE-2-like Elements in the ODF Regulatory Region

5

5'-CCCACA-3' (SEQ ID NO:3)
5'-TGTGGG-3' (SEQ ID NO:4)
5'-CCCGCA-3' (SEQ ID NO:5)
5'-TGTGGTT-3' (SEQ ID NO:6)
10 5'-GCCACA-3' (SEQ ID NO:7)
5'-GCCGCA-3' (SEQ ID NO:8)

In addition, the presently disclosed ODF regulatory region may contain other transcriptional regulatory elements 15 and one or more translational regulatory elements.

As used herein, the terms "ODF regulatory region," "ODF transcriptional regulatory region," "transcriptional regulatory region of the human *odf* gene," "fragment thereof," or "subfragment thereof," or similar terms, refer to the DNA 20 region (or fragments thereof) upstream of, and which regulates the transcription of, the *odf* gene structural nucleic acid sequence that codes on expression for the ODF protein. This region can include the ATG start codon. Thus, these terms exclude the structural nucleic acid sequence (exons) encoding 25 the ODF protein, or fragments thereof, except for the ATG start codon. Also as used herein, the terms "nucleic acid fragment" or "fragment" exclude whole chromosomes or total chromosomal DNA from cells. Also for the purposes of the present invention, the presently disclosed and claimed nucleic 30 acid fragments can comprise, consist essentially of, or consist of the specific nucleotide sequences described herein. The phrase "consisting essentially of" includes, but is not limited to, allelic variants (polymorphs) of the disclosed sequence, as well as *in vitro* chemically or genetically 35 modified versions thereof. As is known in the art, an allelic variant is an alternate form of a polynucleotide sequence that

may contain an addition, deletion, or substitution of one or more nucleotides.

As used herein, the term "isolated nucleic acid fragment" refers to a nucleic acid fragment, for example DNA, that has been removed from its native or naturally occurring environment. As noted above, such nucleic acid fragments do not include whole chromosomes, or the entire chromosomal DNA of a cell. For example, recombinant nucleic acid fragments or molecules contained or generated in culture, in a vector, and/or in a host cell are considered isolated for the purposes of the present invention. Further examples of isolated nucleic acid fragments include recombinant nucleic acid molecules maintained in heterologous host cells, or purified (partially or substantially) nucleic acid molecules in solution. Isolated nucleic acid fragments according to the present invention further include nucleic acid molecules produced synthetically, or purified from or provided in cells containing such synthetic nucleic acids, where the nucleic acid exists in other than a naturally occurring form, quantitatively or qualitatively.

The presently disclosed ODF regulatory region, fragments thereof such as those described in Example 1 below, and functional variants of either, provide invaluable tools for regulating osteoclastogenesis. In one embodiment of the present invention, the disclosed ODF regulatory region, fragments thereof, or functional variants of either, can be used in screening assays to identify drugs that regulate bone balance, bone loss, or which can be used to treat metabolic bone diseases, such as osteoporosis, osteopetrosis, Paget's disease, rheumatoid arthritis, osteoarthritis, periodontal disease, bone tumors and hypercalcemia of malignancy, and arterial related diseases, such as vascular calcification, or to regulate immune function, lymphocyte development, lymph node development, or T- and B-cell formation (note Kong et al., *Nature*, 397:315-323, (1999)). Other diseases in which the presently disclosed ODF regulatory region, fragments

thereof, or functional variants of either are useful in drug screening assays include osteopenic conditions associated with diseases having immune system involvement, such as autoimmune diseases including rheumatoid arthritis, systemic lupus erythematosus, and the spondyloarthropathies; adult and childhood leukemias; and various viral infections, such as hepatitis and HIV. In another embodiment, mutations or polymorphisms in the ODF regulatory region, or fragments thereof, can be used as prognostic diagnostic markers for bone, cartilage, immune, arterial, and the other diseases mentioned above, and for determining a patient's susceptibility to therapy. In still another embodiment, the presently disclosed ODF regulatory region, fragments thereof, or functional variants of either, can be used in expression vectors to control the expression *in vitro* or *in vivo* of a protein or reporter of interest. In another embodiment, the disclosed regulatory element, fragments thereof, or functional variants of either, can be used to identify, isolate, and clone *cis*-elements and interacting *trans*-factors. This can be accomplished by using the presently disclosed ODF regulatory region, or fragments or functional variants, in (a) foot printing, (b) gelshift/electrophoretic mobility shift assays (GMSA), (c) crosslinking, (d) affinity purification of proteins, (e) Southwestern analyses, or other approaches designed to delineate *cis*-elements and *trans*-factors. Such methods are well known to those skilled in the art. Note, for example, chapters 1-32 in *Methods in Molecular Biology,* Volume 30, *DNA-Protein Interactions, Principles and Protocols,* G. Geoff Kneale, Ed., Humana Press, Totowa, NJ (1994), and chapters 30-34 in *Methods in Molecular Biology,* Volume 31, *Protocols for Gene Analysis*, A.J. Harwood, Ed., Humana Press, Totowa, NJ (1994).

The terms "transcription regulatory region" and "regulatory region" refer to the section of DNA located upstream of the sequence encoding a protein and which regulates gene transcription. A regulatory region may include

a variety of *cis*-acting elements, including, but not limited to, promoters, enhancers, and hormone response elements.

In the present case, two approaches were combined to identify and isolate an approximately 4.6 kb genomic fragment located immediately 5′ to the coding region of the human *odf* gene. The first approach utilized the GenomeWalker kit (Clonetech, Palo Alto, CA). In this approach, the polymerase chain reaction (PCR) was used to "walk" upstream of known DNA sequences. The following gene-specific primers were designed from the published ODF cDNA sequence (GenBank #AF019047):

outside primer

5′ cca tct cct ccg agc cac gca ggt act tg 3′ (SEQ ID NO:9)

nested primer

5′ ctt ggt gta gtc tct gct ggc gcg gcg 3′ (SEQ ID NO:10)

These primers were used in combination with primers specific to the GenomeWalker library adaptor to amplify a 1 kb fragment of DNA upstream from the human *odf* gene. This fragment was subcloned into a pCR2.1 vector (Invitrogen, Carlsbad, CA), and designated pODF5.

The second approach involved screening a conventional genomic library. A human P1 library in pAd10SacBII vector (Genome Systems, Inc., St. Louis, MO) was screened using the full length ODF cDNA (#AF019047). Two positive clones, each containing 70-100 kb of genomic DNA, were identified. To identify the clone containing the ODF regulatory region, each positive clone was digested with a panel of restriction enzymes and then Southern blotted with the 1 kb regulatory region fragment described above. The 1 kb ODF regulatory region fragment hybridized to a 4603 bp *Not*I/*Sst*I fragment. The 4603 bp fragment was gel-purified, cloned, and sequenced using standard procedures (see Sambrook et al., *Molecular Cloning, A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press (1989) and Ausubel et al., *Current*

Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1987 and updates)). The 4603 bp fragment was cloned into the pSPORT1 vector (Life Technologies, Inc., Rockville, MD). The nucleotide sequence of the 4603 bp *Not* I/*Sst*I fragment is provided in Figure 1 (SEQ ID NO:1).

One skilled in the art will recognize that modest changes to the composition of the ODF regulatory region will not disrupt its regulatory function. Since transcription regulation is limited to a few discrete sequences within the regulatory region, base changes in non-critical sequences will produce minimal changes in gene expression. Functional variants of the presently disclosed ODF regulatory region and fragments thereof, disclosed in Example 1, below, are encompassed by the present invention, and can be identified using *in vitro* expression assays such as those described herein. Functional variants capable of achieving ODF gene expression at a level comparable to that exhibited by the sequences disclosed herein can be identified by comparing the variants' expression levels to that achieved by the presently disclosed 4.6 kb regulatory region, or fragments thereof. Any variant that drives expression of an operably linked gene or other peptide-, polypeptide-, or protein-encoding polynucleotide at a level greater than about 25%, more preferably greater than about 30%, more preferably greater than about 35%, more preferably greater than about 40%, more preferably greater than about 45%, more preferably greater than about 50%, more preferably greater than about 55%, more preferably greater than about 60%, more preferably greater than about 65%, more preferably greater than about 70%, more preferably greater than about 75%, more preferably greater than about about 80%, more preferably greater than about 85%, more preferably greater than about 90%, more preferably greater than about 95%, more preferably greater than about 98%, more preferably greater than about 99%, and even more preferably 100% or more of the gene expression level achieved using the presently disclosed human ODF regulatory region, or

fragment thereof, in any of the cells or assays disclosed herein is considered a functional variant encompassed within the scope of the present invention.

Alternatively, functional variants exhibiting the activities noted above can be identified using nucleic acid hybridization assays. Functional variants, for example fragments, analogs, or derivatives, can be identified by their ability to hybridize to the complement DNA sequence of the presently disclosed ODF regulatory region (SEQ ID NO:1), or the complement of fragments thereof, i.e., the complements of SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, or SEQ ID NO:15, under mild to stringent hybridization conditions. The following conditions illustrate one example of a mildly stringent hybridization condition:

Hybridization: 1X phosphate buffer, (comprising 0.1M Na_2HPO_4, 0.5M NaCl, 0.0052 M EDTA) pH 7.0, and 1% Sarkosyl, at 45-65°C, preferably 55-65 °C, more preferably 60-65°C, for approximately 2 hours to overnight;

First Wash: 1mM Tris-HCl, pH 8.0, 1% sarkosyl at room temperature for approximately 10-15 minutes;

Second-Fifth Washes (if needed): 1mM Tris-HCl, pH 8.0, for approximately 10-15 minutes each.

Functional variants of the nucleic acid sequences of the present invention identified by nucleic acid hybridization can hybridize fully, i.e., along their entire length, to SEQ ID NO:1 or fragments thereof, respectively. In such cases, they are fully (100%) complementary to SEQ ID NO:1 or fragments thereof, respectively. Functional variants can also hybridize only partially thereto. In these cases, they are partially complementary (less than fully or 100% complementary) to SEQ ID NO:1 and fragments thereof, respectively, e.g., ranging in single step increments of 1% each from 25% to 99% complementary. In any case, such fully or partially hybridizing, functional variants drive expression of an operably linked gene or other peptide, polypeptide-, or protein-encoding polynucleotide at a level as described above.

Functional variants can also be identified *in silico* by comparing their structural similarity, or sequence homology (sequence identity), to the presently disclosed ODF regulatory region or fragments thereof. A DNA fragment possessing about 25% or greater sequence identity, more preferably about 30% or greater sequence identity, more preferably about 35% or greater sequence identity, more preferably about 40% or greater sequence identity, more preferably about 45% or greater sequence identity, more preferably about 50% or greater sequence identity, more preferably about 55% or greater sequence identity, more preferably about 60% or greater sequence identity, more preferably about 65% or greater sequence identity, more preferably about 70% or greater sequence identity, more preferably about 75% or greater sequence identity, more preferably about 80% or greater sequence identity, more preferably about 85% or greater sequence identity, more preferably about 90% or greater sequence identity, more preferably about 95% or greater sequence identity, more preferably about 98% or greater sequence identity, more preferably about 99% or greater sequence identity, especially 85%-95% sequence identity, to the presently disclosed regulatory region (SEQ ID NO:1) or fragment thereof as disclosed herein is considered a functional variant encompassed by the present invention if it is capable of driving the expression of an operably linked peptide-, polypeptide-, or protein-encoding polynucleotide at any of the levels in the range of from about 25% to about 100% or more, as indicated above in connection with the identification of functional variants by *in vitro* expression assays, of the transcriptional control activity exhibited by SEQ ID NO:1 or fragment thereof disclosed herein, when measured in any of the cells, or by any of the assays, disclosed herein.

Mathematical algorithms, for example the Smith-Waterman algorithm, can be used to determine homology (sequence identity). See Smith and Waterman, *J. Mol. Biol.*, 147:195-197

(1981); Pearson, *Genomics*, 11:635-650 (1991). Although any sequence algorithm can be used to identify functional variants, the present invention defines functional variants with reference to the Smith-Waterman algorithm, where SEQ ID
5 NO:1 (or fragments thereof such as those discussed in Example 1) is used as the reference sequence to define the percentage of sequence identity of polynucleotide homologues over its length. The choice of parameter values for matches, mismatches, and inserts or deletions is arbitrary, although
10 some parameter values have been found to yield more biologically realistic results than others. One preferred set of parameter values for the Smith-Waterman algorithm is set forth in the "maximum similarity segments" approach, which uses values of 1 for a matched residue and -1/3 for a
15 mismatched residue (a residue being a either a single nucleotide or single amino acid) (Waterman, *Bulletin of Mathematical Biology* 46:473-500 (1984)). Insertions and deletions x, are weighted as

$$x_k = 1 + k/3,$$

20 where k is the number of residues in a given insert or deletion (*Id.*).

Preferred polynucleotides are those having at least about 50% sequence identity, more preferably at least about 55% sequence identity, more preferably at least about 60% sequence
25 identity, more preferably at least about 65% sequence identity, more preferably at least about 70 % sequence identity, and even more preferably about, or at least about, 75% sequence identity to SEQ ID NO:1 using the Smith-Waterman algorithm. More preferred variant polynucleotides have at
30 least about 80% sequence identity, more preferably at least about 85% sequence identity, more preferably at least about 90% sequence identity, more preferably at least about 95% sequence identity, more preferably at least, or at least about 98% sequence identity, and even more preferably at least about
35 99% sequence identity to SEQ ID NO:1, or fragments thereof disclosed herein.

In more specific embodiments, the polynucleotide comprises DNA having at least about 50% sequence identity, preferably at least about 51% sequence identity, more preferably at least about 52% sequence identity, yet more preferably at least about 53% sequence identity, yet more preferably at least about 54% sequence identity, yet more preferably at least about 55% sequence identity, yet more preferably at least about 56% sequence identity, yet more preferably at least about 57% sequence identity, yet more preferably at least about 58% sequence identity, yet more preferably at least about 59% sequence identity, yet more preferably at least about 60% sequence identity, yet more preferably at least about 61% sequence identity, yet more preferably at least about 62% sequence identity, yet more preferably at least about 63% sequence identity, yet more preferably at least about 64% sequence identity, yet more preferably at least about 65% sequence identity, yet more preferably at least about 66% sequence identity, yet more preferably at least about 67% sequence identity, yet more preferably at least about 68% sequence identity, yet more preferably at least about 69% sequence identity, yet more preferably at least about 70% sequence identity, yet more preferably at least about 71% sequence identity, yet more preferably at least about 72% sequence identity, yet more preferably at least about 73% sequence identity, yet more preferably at least about 74% sequence identity, yet more preferably at least about 75% sequence identity, yet more preferably at least about 76% sequence identity, yet more preferably at least about 77% sequence identity, yet more preferably at least about 78% sequence identity, yet more preferably at least about 79% sequence identity, yet more preferably at least about 80% sequence identity, yet more preferably at least about 81% sequence identity, yet more preferably at least about 82% sequence identity, yet more preferably at least about 83% sequence identity, yet more preferably at least about 84% sequence identity, yet more

preferably at least about 85% sequence identity, yet more preferably at least about 86% sequence identity, yet more preferably at least about 87% sequence identity, yet more preferably at least about 88% sequence identity, yet more preferably at least about 89% sequence identity, yet more preferably at least about 90% sequence identity, yet more preferably at least about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 93% sequence identity, yet more preferably at least about 94% sequence identity, yet more preferably at least about 95% sequence identity, yet more preferably at least about 96% sequence identity, yet more preferably at least about 97% sequence identity, yet more preferably at least about 98% sequence identity, yet more preferably at least about 99% sequence identity to the ODF DNA regulatory region (SEQ ID NO:1), or fragments thereof, disclosed herein.

In the case of each and every one of the individual DNA molecules discussed above in connection with the identification of functional variants of the presently disclosed ODF regulatory region, or fragment thereof, whether such functional variants are identified by *in vitro* expression assays, nucleic acid hybridization, *in silico* determination of sequence identity, or any other method conventional in the art, such as changes in gel electrophoretic mobility, e.g., single stranded conformational polymorphism (SSCP), restriction endonuclease fragment analysis, e.g., restriction fragment length polymorphism, etc., the *proviso* applies that said individual DNA molecule is not one known in the art at the time of filing of this application; is preferably of primate origin, more preferably of human origin; and further, exhibits ODF regulatory region transcriptional control activity at any of the levels in the range of from about 25% to about 100% or more, as indicated above in connection with the identification of functional variants by *in vitro* expression assays, of the transcriptional control activity

exhibited by SEQ ID NO:1 or fragments thereof, when measured in any of the cells, or by any of the assays, disclosed herein. For example, the ODF transcriptional regulatory sequences disclosed in Kodaira et al., *Gene* 230:121-127 (1999); Kitazawa et al., *Biochimica et Biophysica Acta*, 1445:134-141 (1999); Lacey et al., *Cell*, 93(2):165-176 (1998); Anderson et al., *Nature*, 390(6656):175-179 (1997); PCT International Publications WO 97/00317, WO 97/00318, and WO 99/00496; and JP10146189 and JP11009269 are specifically excluded from the functional variants or hybridizing nucleic acid fragments encompassed by the present invention.

A. SCREENING ASSAYS

In one embodiment of the present invention, the presently disclosed ODF regulatory region, or a fragment thereof such as those disclosed in Example 1, below, or a functional variant of either, is used in a method for identifying a compound that affects osteoclast formation, activity, or survival, or bone resorption. An example of such a method is a cell-based screening assay, wherein an expression cassette or vector comprising the presently disclosed ODF regulatory region, fragment thereof, or functional variant of either, and an operably linked polynucleotide encoding a protein of interest heterologous to the ODF regulatory region, e.g., a reporter polynucleotide encoding a reporter molecule, are transfected into an appropriate cell line. An appropriate cell line is one that is "competent," i.e., one in which ODF expression can or does occur naturally, for example one which naturally expresses factors, such as transcription factors, required for ODF expression, for example osteoblast specific transcription factor 2 (Osf2) or other bone-specific transcription factors. An example is a bone cell line. Non-limiting examples of appropriate bone cell lines include osteoblasts, for example UMR106 cells, osteoclasts, osteocytes, fibroblasts, stromal cells, chondrocytes, T-cells, and other cells of similar origin.

Screens can also be performed using non-competent cells that are engineered to be competent for ODF expression. This can be achieved by, for example, introducing an "effector plasmid," i.e., a plasmid that expresses a factor that binds to one or more expression regulatory elements necessary for ODF expression. An example of such an effector plasmid is one that comprises a nucleotide sequence which codes on expression for Osf2, operably linked to transcriptional and translational regulatory elements required for expression. Where an effector plasmid, such as pEF/Cbfa1/myc/cyto, encoding Cbfa1 (osteoblast specific transcription factor 2; Osf2), is used in conjunction with a vector comprising the presently disclosed ODF regulatory region, fragment thereof, or functional variant of either, and an operably linked reporter polynucleotide, a host cell strain that modulates the expression of the reporter molecule, or modifies and processes the reporter polynucleotide expression product in the specific fashion desired, can be used. Such modifications, for example, glycosylation, and processing, for example cleavage, of protein products may be important for the function of the expressed product. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and other gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of expressed foreign proteins. Accordingly, eukaryotic host cells that possess the cellular machinery for proper processing of the primary transcript, glycosylation, and/or phosphorylation of the expressed product can be used. Examples of appropriate mammalian host cells for this purpose include, but are not limited to, CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, and WI38 cell lines.

In either case, the transfected cells are treated with a myriad of compounds, and then monitored for deviations in the basal expression of the reporter protein. Active compounds can then be further assessed for their activity on expression

in vivo. Any convenient test compound or library of test compounds can be used in such assays. Test compounds include low molecular weight chemical compounds, for example having molecular weights less than about 1500 daltons, suitable as pharmaceutical or veterinary agents for human or animal use. Compounds may stimulate (agonists), inhibit (antagonists), or have no effect on, expression of the reporter polynucleotide operably linked to the ODF regulatory region, fragment thereof, or functional variant of either due to their effect on transcription, including transcription initiation. Cell-based methods of assaying for agonists and antagonists employing reporter genes are well known in the art. See, for example, Broach et al., *Nature*, 384(Supp.):14-16 (1996); Naylor, *Biochem. Pharmacol.*, 58:749-757 (1999); and U.S. Patent No. 5,908,609. In one design of such methods, reporter gene expression is measured in the presence of an agonist, with and without a second compound, which is the candidate antagonist. Increasing amounts (or concentrations) of the second compound can be used to assess its antagonistic effect, if any, on the expression induced by a given amount (or concentration) of agonist. In other designs, expression of the reporter gene is measured in cells exposed to a test compound, and compared to expression in identical control cells that have not been contacted with the test compound. However carried out, such screening assays are useful for identifying compounds that promote or inhibit the synthesis of ODF. Since ODF is known to promote osteoclast formation, a compound that increases ODF expression is expected to increase osteoclast formation, and lead to more bone resorption. Conversely, a compound that reduces ODF expression should decrease osteoclast formation, and therefore decrease the amount of bone resorption.

A variety of reporter polynucleotides and genes are known in the art. Non-limiting examples include acid phosphatase, alkaline phosphatase, chloramphenicol acetyltransferase, aequorin, firefly luciferase, and β-glucuronidase. Expression

of reporter polynucleotides can be carried out using techniques that are well-known in the art. See, for example, Alam *et al., Anal. Biochem.*, 188:245-254 (1990), Bronstein *et al., Anal. Biochem.*, 219:169-181 (1994), and Bronstein *et al., Clin. Chem.*, 42(9):1542-1546 (1996) for reviews. "Expression" can be assessed by measuring reporter polynucleotide or gene transcription, translation, or activity of the expressed product. For example, reporter mRNA, protein levels, or protein activity can be measured.

One example of a reporter polynucleotide is that which encodes the enzyme beta-galactosidase. An ODF regulatory region/beta-galactosidase construct can be created by excising the regulatory region fragment from the pSPORT1 vector, *supra*, with the restriction enzymes *SnaB*I and *Sal*I, and then subcloning it into the *Sma*I and *Xho*I sites of the pβGAL-Basic vector (CLONTECH, Palo Alto, CA). The ODF regulatory region/pβGAL-Basic vector construct can then be transiently transfected into osteoblast cells using Fugene™ 6 reagent (Boehringer Mannheim), as recommended by the manufacturer. After transfection, the cells are plated in 96 well plates (50,000 cells/well). Four hours after plating, the cells are transferred to medium containing 0.1% fetal bovine serum, and incubated overnight. The cells are then treated with a test compound. After a sufficient period of time, usually 4 to 24 hours, the cells are lysed in lysis buffer, and a portion of the extracts, for example 1/3, is assayed for beta-galactosidase activity using a luminometer. By comparing the levels of beta-galactosidase activity in those samples treated with the test compounds to those of a control sample, compounds that alter ODF expression can be identified. As noted above, agonists and antagonists can be identified in such screening assays.

Once a compound that affects ODF expression has been identified in a screening assay, a pharmaceutically effective amount of that compound can be determined using techniques

that are well-known to the skilled artisan. Note, for example, Benet *et al.*, in *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, Ninth Edition, Hardman *et al.*, eds., McGraw-Hill, New York, (1996), Chapter 1, pp. 3-27, and the references cited therein. Thus, the appropriate dose(s) range, and dosing regimens, of such a compound can be easily determined by routine methods.

The screening assays described above also can be used to identify compounds that are useful for treating arterial diseases caused by over- or under-expression of ODF, for example arterial calcification. Targeted deletion of OPG in mice results not only in severe osteoporosis, but also in calcification of the aorta and renal arteries. See Bucay *et al.*, *Genes & Development*, 12:1260-1268 (1998). Since ODF serves as a ligand to OPG (a non-membrane decoy receptor), compounds that control the expression of ODF can influence the calcification of arteries.

Similarly, the screening assays described above can be used to identify compounds that can be used to regulate cartilage function, immune function, lymph node development, and T- and B-cell formation. Since osteoclasts are derived from hematopoietic precursors, alterations in osteoclast precursor proliferation/differentiation can affect immune modulation. Note, in this regard, Green et al., *J. Exp. Med.*, 189(7):1017-1020 (1999), and Bachmann et al., *J. Exp. Med.*, 189(7):1025-1031 (1999). For example, a compound may preferentially promote differentiation of common precursors down the osteoclast lineage, depleting formation of other lineage cells involved in immune function, for example macrophages. In another example, inhibition of the *c-fos* gene by knock out blocks osteoclast formation and increases macrophage cell formation (Grigoriadis et al., *Science*, 266(5184):443-448 (1994). Also, ODF has been shown to be instrumental in lymph-node organogenesis, T- and B-cell maturation, T-cell activation, and formation of normal growth

plate (cartilage). See Kong et al., *Nature*, 397:315-323 (1999).

B. DIAGNOSTIC ASSAYS

5 In another embodiment of the present invention, the presently disclosed ODF regulatory region, fragment thereof, or variant of either, can be used in methods to diagnose the presence of, or in prognostic methods to assess the susceptibility to or predisposition to develop, bone, 10 cartilage, immune, arterial, etc., diseases in a human patient. Altered levels of ODF can result in a variety of bone, cartilage, and immune response diseases. Gain of function or activating mutations in the ODF regulatory region resulting in increased ODF expression or function, or loss of 15 function or inactivating mutations therein resulting in decreased ODF expression or loss of function, can induce or cause, or may be associated with, a variety of disease states, such as osteoporosis, osteopetrosis, expansile osteolysis, rheumatoid arthritis, osteoarthritis, metastatic bone disease, 20 hypercalcemia, humoral hypercalcemia of malignancy, and Paget's disease of bone, due to the effect of such mutations on the level of ODF expression. These mutations can therefore serve as diagnostic markers for patients at risk for developing bone or cartilage disease, arterial disease, 25 altered immune response, etc., due to abnormally elevated or depressed levels of ODF. Mutations or polymorphisms in genes related to ODF are associated with familial expansile osteolysis (Hughes et al., *Nature Genetics*, 24:45-48 (2000), while altered expression of ODF has been demonstrated in 30 cancer cells responsible for humoral hypercalcemia of malignancy (Nagai et al., *Biochem. Biophys. Res. Comm.*, 269:532-536 (2000).

A wide variety of methods can be used to detect genetic mutations and polymorphisms. Examples thereof, and the use of 35 such methods in medical diagnostics, are discussed in U.S. Patent 6,083,698 and PCT International Publication WO

00/06767, herein incorporated by reference in their entirety. "Mutation" refers to an altered genetic sequence that can result in altered gene expression or function. A deleterious mutation can be associated with pathology, or the potential
5 for pathology. "Polymorphism" refers to a sequence variation in a gene that is not necessarily associated with pathology. Genetic mutations and polymorphisms can be detected, for example, by nucleic acid hybridization and nucleic acid sequence analysis, which can be facilitated by PCR
10 amplification of the ODF regulatory region. In either case, the subject's ODF regulatory sequence can be compared to that disclosed herein to determine if any differences exist.

In the conventional hybridization method, the initial step is to generate target DNA by amplifying DNA extracted
15 from the cells of a clinical sample using the polymerase chain reaction (PCR). The amplified DNA is then bound to a filter, which is placed into a hybridization tube containing a radiolabeled probe complementary to the genetic mutation of interest. After hybridization and appropriate washing, the
20 filter is examined radiographically for binding of the probe to the target DNA. *See*, for example, Sambrook et al., *Molecular Cloning, A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) and Ausubel et al., *Current Protocols in Molecular*
25 *Biology*, John Wiley & Sons, N.Y. (1987 and updates).

Hybridization is usually performed in two stages. First, in the "binding" stage, the probe is bound to the target under conditions favoring hybridization. A representative hybridization solution comprises 6X SSC, 0.5% SDS, 5X
30 Denhardt's solution, and 100μg of non-specific carrier DNA. See Ausubel et al., 1989, *Current Protocols in Molecular Biology*, section 2.9, supplement 27 (1994), Green Publishing Associates and Wiley Interscience, N.Y.. A stock 20X SSC solution contains 3M sodium chloride, 0.3M sodium citrate, pH
35 7.0. Of course many different, yet functionally equivalent, buffer conditions are known. For high stringency, the

temperature is between about 65°C and 70°C in a hybridization solution of 6X SSC, 0.5% SDS, 5X Denhardt's solution and 100μg of non-specific carrier DNA.

Second, in the "washing" stage, excess probe is removed. This is a critical step in determining relatedness via hybridization. Washing solutions typically contain lower salt concentrations. A medium stringency wash solution contains the equivalent ionic strength of 2X SSC and 0.1% SDS. A high stringency wash solution contains the equivalent ionic strength of less than about 0.2X SSC, with a preferred stringent solution containing about 0.1X SSC. The temperatures associated with various stringencies are the same as discussed above for "binding." Typically, the washing solution is replaced a number of times during washing. For example, typical high stringency washing conditions comprise washing twice for 30 minutes at 55°C, and three times for 15 minutes at 60°C.

In a preferred embodiment, clinical samples are evaluated for genetic mutations using a so-called "gene chip" diagnostic platform. Such platforms have been developed by, *inter alia*, Synteni and Affymetrix. Briefly, mutant-specific probes can be immobilized on a chip surface and used to identify mutations in targeted DNA via hybridization to the surface of the chip. Suitable chip technology is described, for example, in Wodicka et al., *Nature Biotechnology*, 15:1359 (1997), which is hereby incorporated by reference in its entirety, and references cited therein.

Alternatively, sequencing analysis can be used to detect genetic mutations or polymorphisms. This is accomplished by sequencing the DNA extracted from target cell populations. Diagnosis is accomplished by comparing the sequenced target sample with the disclosed sequence(s) of the present invention.

In a further diagnostic aspect of the present invention, the presence or absence of variant nucleotides in a patient's

or subject's ODF regulatory region can be detected by reference to the loss or gain of restriction endonuclease sites within the ODF regulatory region. Those of ordinary skill in the art will readily be able to design and implement diagnostic procedures based on the detection of restriction fragment length polymorphisms (RFLPs) due to the gain or loss of one or more restriction endonuclease sites.

In another embodiment, the presently disclosed ODF regulatory region, or fragment thereof, can be used in a method to diagnose a patient's predisposition, susceptibility, or risk of developing any of the bone, cartilage, arterial, immune, etc., diseases discussed herein. A predisposition, susceptibility, or increased risk of developing any of these diseases can be determined by comparing DNA from cells of a patient or subject, for example from the patient's or subject's bone cells, or from a sample of blood, bronchoalveolar lavage fluid, sputum, urine, or other body fluid or tissue obtained from an individual, with the ODF regulatory sequence(s) disclosed herein. All or part of the subject's ODF regulatory region can first be amplified using any convenient technique, for example PCR, prior to analysis of sequence variation. Using the techniques discussed above, DNA sequences can be compared by hybridization or sequencing analysis. Any deviation in sequence of a patient's ODF regulatory region compared to the sequence of the ODF regulatory region disclosed herein could result in alteration (either an increase or decrease) in the level of ODF expression, or ODF function, and can therefore result in a predisposition to developing any of the diseases or conditions discussed herein.

However mutations in the ODF regulatory region are detected, patients in whom such mutations are detected can be targeted for prevention programs designed to reduce the incidence or severity of those diseases related to ODF over- or under-expression.

In yet another embodiment of the present invention, the presently disclosed ODF regulatory region, or fragment thereof, can be used in a method to determine a patient's susceptibility, receptiveness, or responsiveness to a particular therapy for treating bone-, cartilage-, arterial-, or immune-related diseases related to ODF over- or under-expression. Evaluation of the nucleotide sequence of a patient's ODF regulatory region can indicate whether a proposed therapy with a compound that alters ODF expression (or function) by acting, directly or indirectly, at a discrete location(s) within the ODF regulatory region, will be effective. Deviations in ODF regulatory region sequence, especially in regions required for drug interaction/response, can be used as diagnostic or prognostic markers for susceptibility, receptiveness, or responsiveness to therapy in patients. A patient's ODF regulatory region, or fragment thereof, can be evaluated by comparing DNA from the patient's bone cells or other cells with the disclosed regulatory sequence of the present invention by, for example, hybridization or sequencing analysis, using the techniques described above. The degree of similarity (sequence identity) of a patient's ODF regulatory region, or fragment thereof, to the present ODF regulatory region, or fragment thereof, is expected to be indicative of patient's susceptibility, receptiveness, or responsiveness to therapy: any deviations may influence the effect of therapeutic drugs that act directly, or indirectly through another molecule, through an interaction, e.g., binding, with the ODF regulatory region, to affect ODF expression and therefore the level of ODF in cells. Individuals who carry particular allelic variants of the ODF regulatory region disclosed herein may therefore exhibit differences in ODF levels under different physiological conditions, and thus altered abilities to react to different diseases. In addition, differences in ODF level resulting from allelic variation may directly affect an individual's response to drug therapy. ODF polymorphism may therefore have

a significant effect on the efficacy of drugs designed to modulate the activity of ODF. The polymorphism(s) may also affect the response to agents acting on other biochemical pathways regulated by an ODF ligand. The diagnostic methods provided herein may therefore be useful both to predict the clinical response to such agents, and to determine therapeutic dose.

The use of knowledge of genetic polymorphisms or mutations as an aid in identifying patients most suited to therapy with particular pharmaceutical agents is often termed "pharmacogenetics". Pharmacogenetics can also be used in pharmaceutical research to assist the drug development process. As indicated above, polymorphisms can also be used in mapping the human genome to elucidate the genetic component of diseases. Clinical trials have shown that patient response to drugs can be heterogeneous, creating a necessity for improved approaches to pharmaceutical design and therapy. The following references provide further information on pharmacogenetics and other uses of polymorphism detection: Linder et al., *Clin. Chem.*, 43:254 (1997); Marshall, *Nature Biotechnology*, 15:1249 (1997); PCT International Patent Application WO 97/40462, Spectra Biomedical; Schafer et al., *Nature Biotechnology*, 16:33 (1998); and PCT International Patent Application WO 00/06767, Zeneca Limited.

Accordingly, in one aspect, the present invention provides a method for diagnosing at least one nucleotide polymorphism or mutation in the ODF regulatory region of a human, comprising determining the nucleotide sequence of the ODF regulatory region of the human, and determining the status of the human by referring to SEQ ID NO:1, or a fragment thereof as disclosed herein. The term "human" includes both a human having, or suspected of having, an ODF regulatory region-mediated disease, as well as an asymptomatic human who may be tested for predisposition, risk, or susceptibility to developing such disease. At each nucleotide position so-

identified, the human may be homozygous for an allele, or the human may be a heterozygote.

In another aspect, the present invention provides a method for diagnosing an ODF regulatory region-mediated disease, comprising:

(a) obtaining sample nucleic acid from an individual;

(b) detecting the presence or absence of a variant nucleotide at one or more positions within the ODF regulatory region therein; and

(c) determining the status of the individual by reference to polymorphism in the ODF regulatory region as compared to SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, or SEQ ID NO:15.

The utility and effectiveness of the diagnostic methods disclosed herein reside in the identification of the existence of different alleles a particular loci within the ODF regulatory region. The status of an individual can be determined by reference to allelic variation at one or more such loci. The "normal" nucleotide residue at a particular position is identified by it being the most common residue found at that position among the individuals tested, and is to some extent an arbitrary designation. As particular polymorphisms or mutations associated with certain clinical features, such as adverse or abnormal events, are likely to occur at low frequency within the population, low frequency single nucleotide polymorphisms ("SNPs") can be particularly useful in identifying these mutations. As examples, see De Stefano et al., *Ann. Hum. Genet.*, 62:481-490 (1998) and Keightley et al., *Blood*, 93:4277-4283 (1999).

The diagnostic methods of the present invention can be used in developing new drug therapies that selectively target one or more allelic variants of the ODF regulatory region. Identification of a link between a particular allelic variant and predisposition to disease development or response to drug therapy can significantly impact the design of drugs intended

for use in treating ODF-mediated diseases. Drugs can be specifically designed to regulate the expression of ODF driven by particular allelic variants in the ODF regulatory region, while minimizing effects on other variants or wild-type ODF regulatory regions.

The ODF regulatory region, SEQ ID NO:1, and fragments thereof disclosed herein represent valuable information that can be used to identify further similar sequences, and to characterize individuals in terms of, for example, their identity, haplotype, and other sub-groupings, such as susceptibility, risk, or predisposition to developing symptoms, conditions, or diseases associated with ODF over- or under-expression, and susceptibility to treatment with particular drugs. Such approaches are facilitated by storing the sequence information in a computer readable medium, and then using the information in standard macromolecular structure programs or to search sequence databases using search tools such as GCG (Genetics Computer Group), BlastX, BlastP, BlastN, FASTA (Altschul et al., *J. Mol. Biol.*, 215:403-410 (1990). Thus, the sequences provided herein are particularly useful as components in databases useful for searching for sequence identity, genome mapping, pharmacogenetics, and related search analyses. The sequence information disclosed herein can be reduced to, converted into, or stored in a tangible medium, such as a computer disk, preferably in computer readable form.

The present invention therefore also provides a computer readable medium having stored thereon a nucleotide sequence comprising, consisting essentially of, or consisting of the ODF regulatory region nucleic acid sequence shown in SEQ ID NO:1, or fragments thereof, useful for diagnostic purposes. The computer readable medium can be any composition of matter used to store information or data, including, for example, floppy disks, tapes, chips, compact disks, digital disks, video disks, punch cards, and hard drives.

Also provided is a computer based method for performing diagnosis, comprising determining the nucleotide sequence of the ODF regulatory region of DNA from a human subject, and comparing this sequence to a nucleotide sequence comprising, consisting essentially of, or consisting of the ODF regulatory region nucleic acid sequence shown in SEQ ID NO:1, or fragment thereof useful for diagnostic purposes, in a computer readable medium to identify any polymorphism or mutation that may be present in said human subject's DNA.

C. THERAPEUTIC METHODS

In another embodiment, the present invention provides methods of modulating osteoclast formation and function, bone resorption, and the other diseases, symptoms, and conditions discussed herein. As used herein, the term "modulate" or "affects" denotes an alteration, i.e., either an increase or a decrease. Thus, for example, a compound that modulates bone resorption is one that either increases or decreases bone resorption. A compound that "affects" reporter gene expression is one that stimulates or inhibits reporter gene transcription or expression. In the present context, compounds that stimulate or increase ODF gene expression are referred to as "agonists," while those that inhibit or decrease ODF gene expression are referred to as "antagonists." Osteoclast formation and function, and therefore bone resorption, can be modulated by administering to a patient one or more compounds identified by the methods described herein. For example, a compound that decreases reporter gene expression in a screening assay of the present invention employing a nucleic acid construct comprising the present ODF regulatory region, fragment thereof, or functional variant of either, is expected to be a candidate for treatment of abnormal bone resorption; osteoporosis; arterial disease; metastatic bone disease such as that resulting from prostate cancer, breast cancer, multiple myeloma, humoral hypercalcemia of malignancy, and lung cancer; rheumatoid arthritis;

osteoarthritis; Paget's disease of bone; hypercalcemia of malignancy; osteolysis; and periodontal disease. A compound that inhibits reporter gene expression, and hence ODF expression, would be expected to be effective, for example, for treating or preventing osteoporosis, a condition characterized by decrease in bone mass with decreased bone density, mineral content, and connectivity, producing porosity and fragility; tumor metastasis to bone; and rheumatoid arthritis. A compound that stimulates or increases reporter gene expression in a screening assay would be expected to increase ODF expression, and hence be effective for preventing or treating osteopetrosis, a condition characterized by abnormal thickening and hardening of bone. Similarly, immune responsiveness or function, including, for example, lymph node development, T- and B-cell development, T-cell activation, etc., can be regulated by administering to a patient one or more compounds identified in the methods described herein. As discussed above, since osteoclasts are derived from hematopoietic precursors, alterations in osteoclast precursor proliferation or differentiation can directly or indirectly affect immune modulation, lymph node development, and T- and B-cell formation.

Alternatively, gene therapy can be utilized by administering to a patient a pharmaceutical composition comprising a recombinant DNA construct comprising the ODF regulatory region disclosed herein, a fragment thereof, or a functional variant thereof, operably linked to the *odf* gene. The literature teaches a variety of different methods for introducing exogenous genes into cells *ex vivo* and *in vivo*; vectors for delivering nucleic acids can be viral, non-viral, or physical. See, for example, Rosenberg et al., *Science*, 242:1575-1578 (1988), and Wolff et al., *Proc. Natl. Acad. Sci. USA*, 86:9011-9014 (1989). Recent reviews discussing methods and compositions for use in gene therapy include Eck *et al.*, in *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, Ninth Edition, Hardman *et al.*, eds., McGraw-

Hill, New York, (1996), Chapter 5, pp. 77-101; Wilson, *Clin. Exp. Immunol.* 107(Suppl. 1):31-32 (1997); Wivel et al., *Hematology/Oncology Clinics of North America, Gene Therapy*, S.L. Eck, ed., 12(3):483-501 (1998); Romano et al., *Stem Cells*, 18:19-39 (2000), and the references cited therein. U.S. Patent No. 6,080,728 also provides a discussion of a wide variety of gene delivery methods and compositions. The routes of delivery include, for example, systemic administration and administration *in situ*. Well-known viral delivery techniques include the use of adenovirus, retrovirus, lentivirus, foamy virus, herpes simplex virus, and adeno-associated virus vectors. Exemplary non-viral techniques include the use of naked DNA; DNA complexed with cationic lipids, alone or in combination with cationic polymers; anionic and cationic liposomes; DNA-protein complexes and particles comprising DNA condensed with cationic polymers such as heterogeneous polylysine, defined-length oligopeptides, and polyethylene imine, in some cases contained in liposomes; and the use of ternary complexes comprising a virus and polylysine-DNA. Physical methods include the use of needle-free injectors, such as "gene gun" devices and devices using liquid under high pressure for delivery into interstitial spaces, and electroporation.

Administration of pharmaceutical preparations comprising the present ODF regulatory region or fragments thereof disclosed herein, or functional variants thereof, can be systemic, such as with liposomes, by, for example, intravenous injection. Specific expression of constructs in target cells can occur predominantly from the specificity conferred by the cell type-specific expression due to the ODF regulatory sequence, or this regulatory sequence in combination with the nucleic acid delivery vehicle targeting particular cell types. Administra-tion can also be *in situ*, such as with viral vectors. Delivery of recombinant constructs can be limited by localized introduction, for example by catheter (see U.S. Patent No. 5,328,470), local injection, or by stereotactic

injection (Chen et al., *Proc. Natl. Acad. Sci. USA*, 91:3054-3057 (1994)).

Suitable vectors can be constructed by any of the methods well known in the art. See, for example, Sambrook et al., *Molecular Cloning, a Laboratory Manual*, Second Edition, Cold Spring Harbor Press (1989), and Ausubel et al., eds., *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1987 and updates). The use of cationic liposomes, such as the DC-Chol/DOPE liposome, has been widely documented as an appropriate vehicle to deliver DNA to a wide range of tissues through intravenous injection of DNA/cationic liposome complexes. See Caplen et al., *Nature Med.*, 1:39-46 (1995); Zhu et al., *Science*, 261:209-211 (1993). Liposomes transfer genes to target cells by fusing with the plasma membrane. Examples of the successful application of liposome complexes include those of Lesson-Wood et al., *Human Gene Therapy*, 6:395-405 (1995), and Xu et al., *Molecular Genetics and Metabolism*, 63:103-109 (1998).

Pharmaceutical compositions for gene therapy comprising ODF regulatory region constructs can comprise the desired nucleic acid delivery system, and a pharmaceutically acceptable carrier, diluent, or excipient. Such compositions can also be used in transfecting cells for *in vitro* assays such as those described herein. Slow release matrices containing the nucleic acid delivery vehicle can also be employed. Where desirable or necessary, the delivery system can comprise a pharmaceutical composition comprising recombinant cells, and a pharmaceutically acceptable carrier, diluent, or excipient.

For use in the assay, diagnostic, and therapeutic methods disclosed herein, the present invention also provides in one of its aspects a kit or package, in the form of a sterile-filled vial or ampoule, that contains a polynucleotide comprising SEQ ID NO:1, a fragment thereof, or a functional variant thereof, or a vector containing SEQ ID NO:1, etc., operatively linked to the *odf* gene or a heterologous coding

sequence such as a reporter gene or other polynucleotide, as well as instructions for use in these various methods. The vector can optionally be contained within a vector-releasing cell. In one embodiment, the kit contains a polynucleotide vector containing an ODF regulatory region, fragment thereof, or functional variant thereof, operatively linked to an *odf* coding region as an administration-ready formulation, in either unit dose or multi-dose amounts, wherein the package incorporates a label or manual with instructions for use of its contents for the treatment of one or more of the symptoms, conditions, or diseases discussed herein. In another embodiment, the package provides a sterile-filled vial or ampoule containing a vector-releasing cell or cell line. Such kits or packages can also contain media and reagents, such as reaction buffers, for carrying out appropriate methods as disclosed herein with the nucleic acids, recombinant constructs, vectors, or cells contained therein, as well as instructions therefor.

From a prophylactic or therapeutic point of view, any prevention or alleviation of an undesirable symptom, condition, or disease as noted herein would be desirable. Thus, the terms "treatment" or "therapeutic use" as used herein refer to any and all uses of the presently claimed compositions that remedy a disease state, condition, or symptoms, or which prevent, hinder, retard, or reverse the progression of symptoms, conditions, or diseases discussed herein.

Effective amounts of ODF regulatory region constructs, delivery vehicles containing such constructs, agonists, and antagonists, and treatment protocols, can be determined by conventional means. For example, the medical practitioner can commence treatment with a low dose in a subject or patient in need thereof, and then increase the dosage, or systematically vary the dosage regimen, monitor the effects thereof on the patient or subject, and adjust the dosage or treatment regimen to maximize the desired therapeutic effect. Further discussion

of optimization of dosage and treatment regimens can be found in Benet et al., in *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, Ninth Edition, Hardman *et al.*, Eds., McGraw-Hill, New York, (1996), Chapter 1, pp. 3-27, and L.A.
5 Bauer, in *Pharmacotherapy, A Pathophysiologic Approach*, Fourth Edition, DiPiro et al., Eds., Appleton & Lange, Stamford, Connecticut, (1999), Chapter 3, pp.21-43, and the references cited therein, to which the reader is referred.

Viral vector-mediated gene transfer has been used
10 successfully in mouse models and human clinical trials. See Fujiwara et al., *Cancer Research*, 54:2287-2291 (1994), and Roth et al., *Nature Medicine*, 2:985-991 (1996). Mountain, *TIBTECH*, 18:119-128 (2000) discusses recent examples of gene therapy with clinical benefit progressing to Phase II clinical
15 studies using cationic lipids, adenovirus, retrovirus, and adeno-associated virus vectors, as well as naked DNA. Cavazzana-Calvo et al., *Science*, 288:669-672 (2000) reported that gene therapy using a retroviral vector was able to provide full correction of human severe combined
20 immunodeficiency (SCID)-X1 disease phenotype, including clinical benefit. Ueki et al., *J. Clin. Invest.*, 105(10):1437-1445 (2000) reported the successful use of adenovirus-mediated gene therapy to restore insulin sensitivity in mice having a homozygous disruption of insulin receptor substrate-1.
25 Morishita et al., *Biochem. Biophys. Res. Commun.*, 273(2):666-674 (2000) reported that systemic administration of HVJ viral coat-liposome complex containing a human insulin vector decreased glucose levels in diabetic mice, accompanied by the detection of human insulin in liver and spleen. Anderson
30 (*Nature Medicine*, 6(8):862-863 (2000)) has noted that gene therapy has also recently achieved success in the treatment of hemophilia with an adeno-associated viral vector (Kay et al., *Nature Genetics*, 24:257-261 (2000); cardiovascular disease with naked plasmid DNA (Isner et al., *J. Clin. Invest.*,
35 103:1231-1266 (1999); and cancer therapy using an oncolytic adenovirus (Khuri et al., *Nature Medicine*, 6(8):879-885

(2000). Recent U.S. Patents claiming methods of gene therapy include Nos. 6,080,728 and 6,087,164.

The following examples illustrate various aspects of the present invention, but should not be construed to limit the same.

Example 1
Characterization of the Human ODF Regulatory Region Using 5′ Deletion Constructs

To determine which areas of the presently disclosed ODF regulatory region are required for basal expression, a series of ODF regulatory region 5′ deletion constructs was prepared. Each construct contained the reporter gene beta-galactosidase. See Figure 2. The 4603 bp ODF regulatory region fragment was excised from the pSPORT1 vector, *supra*, with *SnaB*I and *Sal*I, vector restriction sites which flank the *Not*I and *Sst*I sites of the insert. The fragment, SEQ ID NO:11, was then subcloned into the *Sma* I and *Xho* I sites of pβGAL-Basic (Clontech, Palo Alto, CA), and designated pODF4.6βGAL (-4467 to +105).

SEQ ID NO:11 (-4467 to +105)

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GGATCCTCTCCGGAGTTCAGCAAAGTGAAACGTCTGTCATAATAATAACGAATGACTTCCTT
TTTCATTTTCATTCATATAGTGAAGTTTTCTAAAGGCTGCATCATGTGCAATATTGTAACAG
AGTAAGTGCAGGACTGAATGTGACTCTATCAGGCCAATTGTAGAGATGTGAAAAAATGTAAA
ACAGTGGCACTTTTCTCCCTACTTTTTTTTGAAAATCTGTTTTTTTAAATAAAATAATTTTA
TAAAAGTATTATGAATTATTTATAAAATTATATTAACATTATGTTAACATGCTAATATGGTA
AAATTTTCTGCTTGGAGTTTGAATACACCAAATATTTATAAATATAACTCACACAAATAAAA
CCTCTTTGGTGTTCTCAAAATTTTGAAGAATGTAAAAGGTTTGAAAATTGCTGATCTAGCAA
ATGACTGAACATGAACAGCTATAGTATTTGTACCTGCCCAGCAGTGCAGCAATTCCTTATCC
TTCTCATATCTGCACTTTAATTTTCCTTTGACAAATATCTCTCCCTCCTCTCAGCCCATGAC
ATGAGGTTCACATGGGGTTAACTTAATTCCCTGGCTCAAAGGAAAGGTATTAAATTCAGACT
TGTATCCAACCATTCCTGAAGCTAGACTTAGCCCTATTTTTCAATAACATGAACCAATCAAT
TTTCACATGAGTCCAAAATAATTCTATGTTAATACACTAAGGTACTAGGAAATATAGTTTGA
GAAATGTTGATCCAAACATTGTGTTATTTACAGTGGAGTATTGACATAAACTTTGAATCTTC
AAATATGTTCTGGTGTCTTGGCATCTCTTAATACCTATTAGCTTACAAGGCTTTCACTCAAC
TATTTTATAATTTTGATAATGACTTAATTGATTAGTTGATATATTGTTAAAATAAATATATT
AATGAATTTATGATAAATAAGGCAGATAAATAAGACATGCAATTAGGAAGACATGTTAAACA
AATTGTTATAATAATACAATCACTCTCAGCTTAGGATAGCTCCTGGCCACTTTCTCTCTGGG
TGGTTTTTACTCTGGGAGTAGTTTAAATCATTATCTAGTAGTAGTTTAAAGCATTATCTTTG
CCTAAGAGCTTTCGCTGACTCCCCACATTTGCATTGTACTAAGAGTTTTCTCTGACTCCCCA
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CATAGGTCTAGACCCTAGTATTATAAGATTCTCATTGTACTTGCACTTTGCCTTCAAAGTAC
TAATCACGGTTTTGTTAGTGATTTGTGTGATGATTTGTTGAATCTTTTTTTTTTTCCCACTA
GGGTGTAAGCCCCATGTTCCATCTTGATCACCATGTTTCTAGCCCAGTGCTGGCATATAGTG
GGTTCTCACTAATATATCTGTAGAGTAAATGAAGAAATGCATGAGTGACATGACAGGAGAAT
5 TTAAGGATGCCATGGGAGCATAAAACAGAGGGAGCCACCTGGGTGAGGAGAGCTGAGAAAGA
CTTCTGGAGAGGCGACATTTGAGCTGAGAAAGGAAAGACAAGTGGGAGAGTCCTCCAGGTGT
AGAAGTTGGAGAGATGAGCGCTCCAGTTAGGTAGTATTTGAAGCTGATGTAGAAAAGGAGTC
TTGAGCCAGCTTGTGAAGGACTATTGGAGAGTTTTATTTTTATTTTTATCTTTTTTTTAATT
TTTGAGACAGAATCTTGCTTTGTCTCCCAGGCTGGAGTGCAGTGGCATGATTGTAGCTTACT
10 GCAGCTTCGACCTCCTGGGCTCAAACAATCCACCTATCTCAGCCTTCTGAGTAACTGGGACC
AGAGATGTGCACCAAAATGCCTGGCTAATTTGTTCATTTTTTGTAAAGATAGGGTCTCCCTA
TGTTCCCCAGGCTATTCTCCATCTCCTGGGCTCCAGTGATCCTCACGCCTCGGCCACCCAAA
GTGCTGGGATTATAGAAGTGAACCACTGCGCCTGGCCTATTGAAGGTTTTTAATCTTCAGAG
TTTCGACTTTATCAACAACACTTAGAAGCCACCAAAGAATTGCAGGTATGGAAATGACATAT
15 ACTTTTGCTTTTAGAAGAAAATCCTGATCAGTGTGCACAGAATTCTTCAGGGGGCAAGTGTG
ATTCATTCTGATAAGATATAGCATGGCTTAGACTGGGAGACTGGCAGAGGCTTTGAAGATTT
CTTTGCTCAAATTTTATTCAGCAAGTATTTACCATGCACCTACTATAGCAGGCAACATTTTT
AGGAAATGGTGAATGTTACAGAGGTGAATAATACAGCAAGAGTCGTTGAACATATGGAGTTT
ATCTATTAGTTGGGGAGTGAATGTTGACAAAGGAATAAGTAAATACATAGGCAAGAAAGATA
20 CATTACCTGTGAAACAGCAGCAGGTAGACTGACAGTGGAGTATCTAATACAGCCTATGGAAG
CCAGAAGATAGTGGGATGACATTTTTGGAGTACTAGTAGAAATGTCATATGAAGAACTCTGT
AGGAATGTAACATACGGTCCCATATATGAAGCTCCTGGGTCAAGTATACCTGAACATAATTC
AGGGATTTGAGGGACTTTCTTGTAACCTGAGGATCAAGATGTCAAGGAATTAAAAACATGTA
TAAAACATTGTTGTATAAAAACCCATTAAAAAGAATGGAAGACACTATAGTAAAATCATTGT
25 GGGTTTAGTTGTTATAACACATTTTAAAAATCTTTGATCCCAATCAATATTTATAAGAAAGA
AGAAATATGGAATTATTTCCTGAGTCAAGGAGCAGGGAGAGAATGAGGAAGAAGAGGAGGAG
GAGGAGGGGGAGGAGGAGACAATAAACCTACTTCCCAAAGTTAACAAACAAAAAGTGGGAAG
AGGTCAAAGACTACAAGGAGTAGAATTAACGTCAATTGTTTCTATGTTTGAGTCTGAAAATT
TTTTGTCCCTTCTCCACCAACCTATATATTGATACACATATTAATGCTAAAGGCATTTTTGT
30 ATTTGAACAGATCATTTTCTTTGTATGGCTGCCTTTAAAAAAAATTCAACCTGGTCACTCTT
CCTCAACATTTACTGAGGTCTAAGTGTTCAATTTAGAACACATGCTTTAATAACTCAGAGAC
CTGTCATTTGTCACAAATCTTGCCTAGAGAAATACTCATTAGCGAATTAGGCAGAAAGAGGA
TGCAAAATAAAAAGGCACAGTAGTCCCCTGATATCCATGGAAGACTGGTTCCAGGACACCAC
CAAACCCCTCCCCGCAAATACCAAAATCCATGGATGTTCAAGTTTCTTAACATATCATGGCA
35 TAGTATTTGCATTTAACCTACACACATCCTCTTGTACACTTGAAATTATCTTTAGATTATTT
ATAATACTTAATAGAATGTAAATGCTATGTAACTAGTTGTGTATCATTTAGGAAATGATCAC
AAGAAAAAAAGTCTACAGATGTTAGTCCAGACACAGCCATCCTTTTTTTTTTTTTCAAATAT
TTTTGATCTGTGGTTCATTGCATCCACAGATGTGGAACCCATGGATACTGTGGGCTAACTGT
ATTAATAAAAAAGTGGAAACATCCTAAGTTTCATGGGTGTTTAAATTGGTCAGCAACTTCCT
40 TCTGAAGAAGTATCAGAATTTGTGAGCAATGTTAATATTTTTGTTTTCTCACTAAGAGCCAC
AGTTCTGAATAGAGGTTTTTAAAAAGCCCTAGCAAGGTTTCTTTAGCAATGAAACTAACATT
TAACTGTATCATCAGCTTCGTGTTACATCTCTTTCCTGACTGTTGGGTGAGCCCTCCTCGGA
TGCTTGCTTCTGGCTACACGCCCCTTTACCCTTTTCTCTGCACTGTTTTCATCTTTATAAAG
TCAGAGTTGGTGTCTATAGGCTCTCTACTGCCACATTCAAGACCTGCCTCGCTCAATGTCAC
45 CTTCAAGATGCAGAAATAGGGATTTGGGAAGGGGATTGTGAAATTTTCGAAGTCTTCCAAAA
TACTTTGAGAAACTATATTTGGAAGCACTTTGGGGGGAGAGGTTGGACAGGAAGGGTCTTCA
GAGATCATCAAATTTAACTTTCTAAATCCTAAGGAGGAAACCGAGACTCCAGGATGTGAAGT
CCCTTCTCTACCAAACTAGAATGGATGCAGGAGGAATGTCTGAGGTGCAATCCTTATCCTTT
AGCAAAGGTGTCCTCTGCGTCTTCTTTAACCCATCTCTTGGACCTCCAGAAAGACAGCTGAG
50 GATGGCAAGGGGAGTCTGGAACCACTGGAGTAGCCCCCAGCCTCCTCCTTGGAGGGCCCCCA
TGAAGGAGGCCCTTCAGTGACAGAGATTGAGAGAGAGGGAGGGCGAAAGGAAGGAAGGGGAG
CCAGAGGTGGGAGTGGAAGAGGCAGCCTCGCCTGGGGCTGATTGGCTCCCGAGGCCAGGGCT
CTCCAAGCGGTTTATAAGAGTTGGGGCTGCCGGGCGCCCTGCCCGCTCGCCCGCGCGCCCCA

GGAGCCAAAGCCGGGCTCCAAGTCGGCGCCCCACGTCGAGGCTCCGCCGCAGCCTCCGGAGT
TGGCCGCAGACAAGAAGGGGAGGGAGCGGGAGAGGGAGGAGAGCTC

The pODF4.6βGAL vector was digested with *Xba*I (one site is in the vector), releasing 1.2 KB of the 5' end of the ODF regulatory region, SEQ ID NO:12, and religated with T4 DNA ligase, forming pODF3.4βGAL (-3283 to +105).

SEQ ID NO:12 (-3283 to +105)

TCTAGACCCTAGTATTATAAGATTCTCATTGTACTTGCACTTTGCCTTCAAAGTACTAATCA
CGGTTTTGTTAGTGATTTGTGTGATGATTTGTTGAATCTTTTTTTTTTTCCCACTAGGGTGT
AAGCCCCATGTTCCATCTTGATCACCATGTTTCTAGCCCAGTGCTGGCATATAGTGGGTTCT
CACTAATATATCTGTAGAGTAAATGAAGAAATGCATGAGTGACATGACAGGAGAATTTAAGG
ATGCCATGGGAGCATAAAACAGAGGGAGCCACCTGGGTGAGGAGAGCTGAGAAAGACTTCTG
GAGAGGCGACATTTGAGCTGAGAAAGGAAAGACAAGTGGGAGAGTCCTCCAGGTGTAGAAGT
TGGAGAGATGAGCGCTCCAGTTAGGTAGTATTTGAAGCTGATGTAGAAAAGGAGTCTTGAGC
CAGCTTGTGAAGGACTATTGGAGAGTTTTATTTTTATTTTTATCTTTTTTTTAATTTTTGAG
ACAGAATCTTGCTTTGTCTCCCAGGCTGGAGTGCAGTGGCATGATTGTAGCTTACTGCAGCT
TCGACCTCCTGGGCTCAAACAATCCACCTATCTCAGCCTTCTGAGTAACTGGGACCAGAGAT
GTGCACCAAAATGCCTGGCTAATTTGTTCATTTTTTGTAAAGATAGGGTCTCCCTATGTTCC
CCAGGCTATTCTCCATCTCCTGGGCTCCAGTGATCCTCACGCCTCGGCCACCCAAAGTGCTG
GGATTATAGAAGTGAACCACTGCGCCTGGCCTATTGAAGGTTTTTAATCTTCAGAGTTTCGA
CTTTATCAACAACACTTAGAAGCCACCAAAGAATTGCAGGTATGGAAATGACATATACTTTT
GCTTTTAGAAGAAAATCCTGATCAGTGTGCACAGAATTCTTCAGGGGGCAAGTGTGATTCAT
TCTGATAAGATATAGCATGGCTTAGACTGGGAGACTGGCAGAGGCTTTGAAGATTTCTTTGC
TCAAATTTTATTCAGCAAGTATTTACCATGCACCTACTATAGCAGGCAACATTTTTAGGAAA
TGGTGAATGTTACAGAGGTGAATAATACAGCAAGAGTCGTTGAACATATGGAGTTTATCTAT
TAGTTGGGGAGTGAATGTTGACAAAGGAATAAGTAAATACATAGGCAAGAAAGATACATTAC
CTGTGAAACAGCAGCAGGTAGACTGACAGTGGAGTATCTAATACAGCCTATGGAAGCCAGAA
GATAGTGGGATGACATTTTTGGAGTACTAGTAGAAATGTCATATGAAGAACTCTGTAGGAAT
GTAACATACGGTCCCATATATGAAGCTCCTGGGTCAAGTATACCTGAACATAATTCAGGGAT
TTGAGGGACTTTCTTGTAACCTGAGGATCAAGATGTCAAGGAATTAAAAACATGTATAAAAC
ATTGTTGTATAAAAACCCATTAAAAAGAATGGAAGACACTATAGTAAAATCATTGTGGGTTT
AGTTGTTATAACACATTTTAAAAATCTTTGATCCCAATCAATATTTATAAGAAAGAAGAAAT
ATGGAATTATTTCCTGAGTCAAGGAGCAGGGAGAGAATGAGGAAGAAGAGGAGGAGGAGGAG
GGGGAGGAGGAGACAATAAACCTACTTCCCAAAGTTAACAAACAAAAAGTGGGAAGAGGTCA
AAGACTACAAGGAGTAGAATTAACGTCAATTGTTTCTATGTTTGAGTCTGAAAATTTTTTGT
CCCTTCTCCACCAACCTATATATTGATACACATATTAATGCTAAAGGCATTTTTGTATTTGA
ACAGATCATTTTCTTTGTATGGCTGCCTTTAAAAAAAATTCAACCTGGTCACTCTTCCTCAA
CATTTACTGAGGTCTAAGTGTTCAATTTAGAACACATGCTTTAATAACTCAGAGACCTGTCA
TTTGTCACAAATCTTGCCTAGAGAAATACTCATTAGCGAATTAGGCAGAAAGAGGATGCAAA
ATAAAAAGGCACAGTAGTCCCCTGATATCCATGGAAGACTGGTTCCAGGACACCACCAAACC
CCTCCCCGCAAATACCAAAATCCATGGATGTTCAAGTTTCTTAACATATCATGGCATAGTAT
TTGCATTTAACCTACACACATCCTCTTGTACACTTGAAATTATCTTTAGATTATTTATAATA
CTTAATAGAATGTAAATGCTATGTAACTAGTTGTGTATCATTTAGGAAATGATCACAAGAAA
AAAAGTCTACAGATGTTAGTCCAGACACAGCCATCCTTTTTTTTTTTTTCAAATATTTTTGA
TCTGTGGTTCATTGCATCCACAGATGTGGAACCCATGGATACTGTGGGCTAACTGTATTAAT
AAAAAAGTGGAAACATCCTAAGTTTCATGGGTGTTTAAATTGGTCAGCAACTTCCTTCTGAA
GAAGTATCAGAATTTGTGAGCAATGTTAATATTTTTGTTTTCTCACTAAGAGCCACAGTTCT

GAATAGAGGTTTTTAAAAAGCCCTAGCAAGGTTTCTTTAGCAATGAAACTAACATTTAACTG
TATCATCAGCTTCGTGTTACATCTCTTTCCTGACTGTTGGGTGAGCCCTCCTCGGATGCTTG
CTTCTGGCTACACGCCCCTTTACCCTTTTCTCTGCACTGTTTTCATCTTTATAAAGTCAGAG
TTGGTGTCTATAGGCTCTCTACTGCCACATTCAAGACCTGCCTCGCTCAATGTCACCTTCAA
GATGCAGAAATAGGGATTTGGGAAGGGGATTGTGAAATTTTCGAAGTCTTCCAAAATACTTT
GAGAAACTATATTTGGAAGCACTTTGGGGGGAGAGGTTGGACAGGAAGGGTCTTCAGAGATC
ATCAAATTTAACTTTCTAAATCCTAAGGAGGAAACCGAGACTCCAGGATGTGAAGTCCCTTC
TCTACCAAACTAGAATGGATGCAGGAGGAATGTCTGAGGTGCAATCCTTATCCTTTAGCAAA
GGTGTCCTCTGCGTCTTCTTTAACCCATCTCTTGGACCTCCAGAAAGACAGCTGAGGATGGC
AAGGGGAGTCTGGAACCACTGGAGTAGCCCCCAGCCTCCTCCTTGGAGGGCCCCCATGAAGG
AGGCCCTTCAGTGACAGAGATTGAGAGAGAGGGAGGGCGAAAGGAAGGAAGGGGAGCCAGAG
GTGGGAGTGGAAGAGGCAGCCTCGCCTGGGGCTGATTGGCTCCCGAGGCCAGGGCTCTCCAA
GCGGTTTATAAGAGTTGGGGCTGCCGGGCGCCCTGCCCGCTCGCCCGCGCGCCCCAGGAGCC
AAAGCCGGGCTCCAAGTCGGCGCCCCACGTCGAGGCTCCGCCGCAGCCTCCGGAGTTGGCCG
CAGACAAGAAGGGGAGGGAGCGGGAGAGGGAGGAGAGCTC

The pODF4.6βGAL vector was also digested with *Xba*I and *EcoRI*, releasing 2.1 kb of the 5′ end of the ODF regulatory region, SEQ ID NO:13. The fragment was treated with T4 DNA polymerase to blunt the ends and religated with T4 DNA ligase to form the vector pODF2.5βGAL (-2382 to +105).

SEQ ID NO:13 (-2382 to +105)

GAATTCTTCAGGGGGCAAGTGTGATTCATTCTGATAAGATATAGCATGGCTTAGACTGGGAG
ACTGGCAGAGGCTTTGAAGATTTCTTTGCTCAAATTTTATTCAGCAAGTATTTACCATGCAC
CTACTATAGCAGGCAACATTTTTAGGAAATGGTGAATGTTACAGAGGTGAATAATACAGCAA
GAGTCGTTGAACATATGGAGTTTATCTATTAGTTGGGGAGTGAATGTTGACAAAGGAATAAG
TAAATACATAGGCAAGAAAGATACATTACCTGTGAAACAGCAGCAGGTAGACTGACAGTGGA
GTATCTAATACAGCCTATGGAAGCCAGAAGATAGTGGGATGACATTTTTGGAGTACTAGTAG
AAATGTCATATGAAGAACTCTGTAGGAATGTAACATACGGTCCCATATATGAAGCTCCTGGG
TCAAGTATACCTGAACATAATTCAGGGATTTGAGGGACTTTCTTGTAACCTGAGGATCAAGA
TGTCAAGGAATTAAAAACATGTATAAAACATTGTTGTATAAAAACCCATTAAAAAGAATGGA
AGACACTATAGTAAAATCATTGTGGGTTTAGTTGTTATAACACATTTTAAAAATCTTTGATC
CCAATCAATATTTATAAGAAAGAAGAAATATGGAATTATTTCCTGAGTCAAGGAGCAGGGAG
AGAATGAGGAAGAAGAGGAGGAGGAGGAGGGGGAGGAGGAGACAATAAACCTACTTCCCAAA
GTTAACAAACAAAAAGTGGGAAGAGGTCAAAGACTACAAGGAGTAGAATTAACGTCAATTGT
TTCTATGTTTGAGTCTGAAAATTTTTTGTCCCTTCTCCACCAACCTATATATTGATACACAT
ATTAATGCTAAAGGCATTTTTGTATTTGAACAGATCATTTTCTTTGTATGGCTGCCTTTAAA
AAAAATTCAACCTGGTCACTCTTCCTCAACATTTACTGAGGTCTAAGTGTTCAATTTAGAAC
ACATGCTTTAATAACTCAGAGACCTGTCATTTGTCACAAATCTTGCCTAGAGAAATACTCAT
TAGCGAATTAGGCAGAAAGAGGATGCAAAATAAAAAGGCACAGTAGTCCCCTGATATCCATG
GAAGACTGGTTCCAGGACACCACCAAACCCCTCCCCGCAAATACCAAAATCCATGGATGTTC
AAGTTTCTTAACATATCATGGCATAGTATTTGCATTTAACCTACACACATCCTCTTGTACAC
TTGAAATTATCTTTAGATTATTTATAATACTTAATAGAATGTAAATGCTATGTAACTAGTTG
TGTATCATTTAGGAAATGATCACAAGAAAAAAAGTCTACAGATGTTAGTCCAGACACAGCCA
TCCTTTTTTTTTTTTTCAAATATTTTTGATCTGTGGTTCATTGCATCCACAGATGTGGAACC
CATGGATACTGTGGGCTAACTGTATTAATAAAAAAGTGGAAACATCCTAAGTTTCATGGGTG
TTTAAATTGGTCAGCAACTTCCTTCTGAAGAAGTATCAGAATTTGTGAGCAATGTTAATATT

TTTGTTTTCTCACTAAGAGCCACAGTTCTGAATAGAGGTTTTAAAAAGCCCTAGCAAGGTT
TCTTTAGCAATGAAACTAACATTTAACTGTATCATCAGCTTCGTGTTACATCTCTTTCCTGA
CTGTTGGGTGAGCCCTCCTCGGATGCTTGCTTCTGGCTACACGCCCCTTTACCCTTTTCTCT
GCACTGTTTTCATCTTTATAAAGTCAGAGTTGGTGTCTATAGGCTCTCTACTGCCACATTCA
5 AGACCTGCCTCGCTCAATGTCACCTTCAAGATGCAGAAATAGGGATTTGGGAAGGGGATTGT
GAAATTTTCGAAGTCTTCCAAAATACTTTGAGAAACTATATTTGGAAGCACTTTGGGGGGAG
AGGTTGGACAGGAAGGGTCTTCAGAGATCATCAAATTTAACTTTCTAAATCCTAAGGAGGAA
ACCGAGACTCCAGGATGTGAAGTCCCTTCTCTACCAAACTAGAATGGATGCAGGAGGAATGT
CTGAGGTGCAATCCTTATCCTTTAGCAAAGGTGTCCTCTGCGTCTTCTTTAACCCATCTCTT
10 GGACCTCCAGAAAGACAGCTGAGGATGGCAAGGGGAGTCTGGAACCACTGGAGTAGCCCCCA
GCCTCCTCCTTGGAGGGCCCCCATGAAGGAGGCCCTTCAGTGACAGAGATTGAGAGAGAGGG
AGGGCGAAAGGAAGGAAGGGGAGCCAGAGGTGGGAGTGGAAGAGGCAGCCTCGCCTGGGGCT
GATTGGCTCCCGAGGCCAGGGCTCTCCAAGCGGTTTATAAGAGTTGGGGCTGCCGGGCGCCC
TGCCCGCTCGCCCGCGCGCCCCAGGAGCCAAAGCCGGGCTCCAAGTCGGCGCCCCACGTCGA
15 GGCTCCGCCGCAGCCTCCGGAGTTGGCCGCAGACAAGAAGGGGAGGGAGCGGGAGAGGGAGG
AGAGCTC

The vector pODF1.0βGAL (-835 to +100) was derived from the pODF5 vector. As described above, pODF5 was obtained by ligating the 1 kb fragment of the ODF regulatory region
20 obtained via the Genome Walker Kit into a pCR2.1 vector. The pODF5 vector was digested with *Sma*I and *Sst*I, releasing the 1 kb insert, SEQ ID NO:14, treated with T4 DNA polymerase, blunting the *Sst*I site on the 3′ end, and subcloned into the *Sma*I site of pβGAL-Basic.

25

SEQ ID NO:14 (-835 to +100)

ATTTTTGTTTTCTCACTAAGAGCCACAGTTCTGAATAGAGGTTTTAAAAAGCCCTAGCAAG
GTTTCTTTAGCAATGAAACTAACATTTAACTGTATCATCAGCTTCGTGTTACATCTCTTTCC
30 TGACTGTTGGGTGAGCCCTCCTCGGATGCTTGCTTCTGGCTACACGCCCCTTTACCCTTTTC
TCTGCACTGTTTTCATCTTTATAAAGTCAGAGTTGGTGTCTATAGGCTCTCTACTGCCACAT
TCAAGACCTGCCTCGCTCAATGTCACCTTCAAGATGCAGAAATAGGGATTTGGGAAGGGGAT
TGTGAAATTTTCGAAGTCTTCCAAAATACTTTGAGAAACTATATTTGGAAGCACTTTGGGGG
GAGAGGTTGGACAGGAAGGGTCTTCAGAGATCATCAAATTTAACTTTCTAAATCCTAAGGAG
35 GAAACCGAGACTCCAGGATGTGAAGTCCCTTCTCTACCAAACTAGAATGGATGCAGGAGGAA
TGTCTGAGGTGCAATCCTTATCCTTTAGCAAAGGTGTCCTCTGCGTCTTCTTTAACCCATCT
CTTGGACCTCCAGAAAGACAGCTGAGGATGGCAAGGGGAGTCTGGAACCACTGGAGTAGCCC
CCAGCCTCCTCCTTGGAGGGCCCCCATGAAGGAGGCCCTTCAGTGACAGAGATTGAGAGAGA
GGGAGGGCGAAAGGAAGGAAGGGGAGCCAGAGGTGGGAGTGGAAGAGGCAGCCTCGCCTGGG
40 GCTGATTGGCTCCCGAGGCCAGGGCTCTCCAAGCGGTTTATAAGAGTTGGGGCTGCCGGGCG
CCCTGCCCGCTCGCCCGCGCGCCCCAGGAGCCAAAGCCGGGCTCCAAGTCGGCGCCCCACGT
CGAGGCTCCGCCGCAGCCTCCGGAGTTGGCCGCAGACAAGAAGGGGAGGGAGCGGGAGAGGG
AGGAG

The pODF1.0βGAL vector was digested with *Pvu*II and *Bgl*II
45 to release a fragment containing 356 bp of the 3′ end of the ODF regulatory region, SEQ ID NO:15. The fragment was then

subcloned into the *Sma*I/*Bgl*II site of pβGAL-Basic to form pODF0.4βGAL (-256 to +100).

SEQ ID NO:15 (-256 to +100)

CTGAGGATGGCAAGGGGAGTCTGGAACCACTGGAGTAGCCCCCAGCCTCCTCCTTGGAGGGC
CCCCATGAAGGAGGCCCTTCAGTGACAGAGATTGAGAGAGAGGGAGGGCGAAAGGAAGGAAG
GGGAGCCAGAGGTGGGAGTGGAAGAGGCAGCCTCGCCTGGGGCTGATTGGCTCCCGAGGCCA
GGGCTCTCCAAGCGGTTTATAAGAGTTGGGGCTGCCGGGCGCCCTGCCCGCTCGCCCGCGCG
CCCCAGGAGCCAAAGCCGGGCTCCAAGTCGGCGCCCCACGTCGAGGCTCCGCCGCAGCCTCC
GGAGTTGGCCGCAGACAAGAAGGGGAGGGAGCGGGAGAGGGAGGAG

SEQ ID Nos:11-15 are shown in the 5′ to 3′ direction.

All constructs were verified by restriction mapping and DNA sequencing, via the dideoxy-chain termination method. The deletion constructs were transiently transfected into the UMR106 osteoblast cell line (Partridge et al., *Endocrinology* 108:213-219 (1981); Partridge et al., *Cancer Research* 43:4308-4314 (1983); Forrest et al., *Calcified Tissue International* 37:51-56 (1985); Drake et al., *Endocrinology* 134: 1733-1737 (1994); Scott et al., *Molecular Endocrinology* 6: 2153-2159 (1992); Onishi et al., *Endocrinology* 138: 1995-2003 (1997); Verheijen et al., *Endocrinology* 136: 3331-3337 (1995)).

The ODF regulatory region deletion constructs, positive control plasmid pcDNA3.1/V5-HIS/lacz (Invitrogen, Carlsbad, CA) containing the beta-galactosidase gene under the control of the CMV promoter, and negative control plasmid pβGAL-basic, were transiently transfected into a confluent T150 flask of UMR 106 cells using Fugene™ 6 reagent (Boehringer Mannheim, Indianapolis IN) as recommended by the manufacturer. After transfection, the cells were plated in 96 well plates (Becton Dickinson Labware, Franklin Lakes, NJ)(50,000 cells/well). The UMR 106 cells were maintained in DMEM/Ham's F-12 (3:1) (GIBCO BRL, Grand Island, NY) containing 10% fetal bovine serum (FBS) and glutamine (GIBCO BRL), and incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Four hours after plating, the cells were transferred to medium containing 0.1%

fetal bovine serum and incubated overnight at 37°C in a humidified atmosphere of 95% air and 5% CO_2. After a 24 hour recovery period, the cells were lysed in 60 μl of lysis buffer provided by the manufacturer, and beta-galactosidase activity was assayed in a fixed amount of the extracts (1/3 of the extracts) using the beta-galactosidase reporter gene assay kit (Boehringer Mannheim). Twenty microliters of the supernatant were transferred to white, opaque microtiter plates (Dynex, Franklin, MA), and beta-galactosidase activity was measured using an automated injection MLX Luminometer (Dynex Corporation, Chantilly, VA) according to the manufacturer's instructions. Beta-galactosidase enzyme activity was determined and expressed as relative light units. The results, shown graphically in Figure 3, represent the mean ± SEM of 4-12 separate wells.

As shown in Figure 3, sequential deletions of the ODF regulatory region led to a progressive decrease in regulatory region activity. The largest regulatory region construct, pODF4.6βGAL, showed 2-3 times the activity of constructs containing smaller portions of the proximal ODF regulatory region.

These results suggest the presence of functionally important elements in the distal regulatory region of ODF that are absent in the published sequence of the mouse ODF regulatory region (Kodaira et al., *Gene* 230: 121-127 (1999); Kitazawa et al., *Biochimica et Biophysica Acta*, 1445:134-141 (1999)).

Example 2

Evaluation of Osf2 on ODF Expression

Since the ODF regulatory region of the present invention contains approximately 10 osteoblast specific element (OSE_2) motifs, shown in bold face and underlined in Figure 1, that function as binding sites for osteoblast specific transcription factor 2 (Osf2), the role of Osf2 in ODF

expression was evaluated. The ability of Osf2 to transactivate regulatory region constructs was evaluated in COS1 cells, a monkey kidney cell line lacking endogenous Osf2 protein. The COS-1 cell line was obtained from the American Type Culture Collection, Bethesda, MD (ATCC CRL 1650), and was grown in DMEM, supplemented with 10% fetal bovine serum (FBS) and antibiotics. All cultures were maintained at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Experiments were initiated when cells were approximately 70-80% confluent. The cells were seeded in a 6 well plate (Becton Dickinson Labware, Franklin Lakes, NJ) at 2×10^5 cells/well and transfected 24 hours later, as in Example 1, with 1 μg each of the reporter plasmid (OPG regulatory region deletion constructs linked to β-gal or pβgal-Basic) and the effector plasmid pEF/Cbfa1/myc/cyto (encoding Cbfa1 (Osf2) under the control of the human translation elongation factor EF-1a), or the control vector pEF/myc/cyto (Invitrogen, Carlsbad, CA), using Fugene™6 transfection reagent (Boehringer Mannheim). The Cbfa1 coding sequence was from mouse (Ducy et al., *Cell*, 89(5):747-754 (1997); GenBank accession no. AF010284). The constructs (1 μg each in a total volume of 20 μl in T.E. buffer, pH 8.0) were mixed with diluted Fugene™6 reagent (194 μl serum-free medium + 6 μl Fugene) and incubated for 15 minutes at room temperature. The DNA-Fugene mix was then added drop-wise to the plates, and the cells were incubated for an additional 36-48 hours in DMEM supplemented with 10% FBS. Following transfection, the plates were washed twice with phosphate-buffered saline (PBS) (Gibco, BRL), and then lysed with 100 μl of lysis buffer provided with the beta-galactosidase reporter gene assay kit (Boehringer Mannheim). The cell extracts were centrifuged for 2 minutes at 14,000 rpm in a microfuge to precipitate cellular debris. Twenty microliters of the supernatant were transferred to white, opaque microtiter plates (Dynex, Franklin, MA), and beta-galactosidase activity was measured using an automated injection MLX Luminometer

(Dynex Corporation, Chantilly, VA) according to the manufacturer's instructions. The beta-galactosidase activity values represent the integral value of light emitted over a period of two seconds, and are expressed as fold induction over basal (control vector transfected) levels. The results are shown in Figure 4.

As shown in Figure 4, cotransfection of an Osf2 expression construct (plasmid pEF/Cbfa1/myc/cyto) with the ODF/reporter constructs led to a 3-to-7-fold increase in beta-galactosidase activity compared to that in control vector transfected cells (pEF/myc/cyto). Sequential deletions, pODF4.6βGAL to pODF3.4βGAL and pODF2.5βGAL, led to an increase in regulatory region activity (6-to-7-fold). Additional deletion to pODF1.0βGal decreased transactivation to only 3-to-4-fold that of the control level, while further deletion to pODF0.4βGAL resulted in an increase in expression (6-to-7-fold).

These results suggest that the interaction of both distal and proximal OSE_2 elements is required to tightly regulate ODF regulatory region expression by Osf2. These data suggest that Osf2 can effectively regulate the expression of ODF and, therefore, the resorption process, and could be important in normal and pathologic states characterized by altered bone resorption.

The invention being thus described, it is obvious that the same can be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the present invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

WHAT IS CLAIMED IS:

1. An isolated nucleic acid fragment comprising the transcriptional regulatory region of the human *odf* gene, or subfragment thereof exhibiting human *odf* gene transcriptional regulatory activity, excluding the *odf* protein coding region.

2. The isolated nucleic acid fragment or subfragment of claim 1, comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15, or the complement of any one of said nucleotide sequences.

3. An isolated nucleic acid fragment that hybridizes to said complement nucleotide sequence of claim 2 in 1X phosphate buffer comprising 0.1M Na_2HPO_4, 0.5M NaCl, 0.0052 M EDTA, pH 7.0, and 1% Sarkosyl, at 45-65°C for 2 hours to overnight, followed by washing in 1mM Tris-HCl, pH 8.0, 1% sarkosyl at room temperature for 10 to 15 minutes,

wherein said fragment exhibits human *odf* gene regulatory region transcriptional regulatory activity.

4. An isolated nucleic acid fragment having a sequence identity in the range of from about 85% to about 99% compared to said nucleotide sequence of claim 2,

wherein said fragment exhibits human *odf* gene regulatory region transcriptional regulatory activity.

5. A recombinant DNA construct comprising the isolated nucleic acid fragment or subfragment of any one claims 1-4.

6. The recombinant DNA construct of claim 5, further comprising a polynucleotide encoding a protein of interest, and, optionally, at least one translational regulatory region required for expression of said polynucleotide, wherein said

polynucleotide encoding said protein of interest is operably linked for expression to said isolated nucleic acid fragment or subfragment and to said translational regulatory region.

7. The recombinant DNA construct of claim 6, which is an expression cassette or an expression vector.

8. A cultured host cell comprising said recombinant DNA construct of any one of claims 5-7.

9. Use of the isolated nucleic acid fragment or subfragment according to any one of claims 1-4 in an assay to identify an agonist or antagonist of osteoclast differentiation factor expression.

10. Use of the isolated nucleic acid fragment or subfragment according to any one of claims 1-4 for the manufacture of a composition for the diagnosis of a human susceptible to, predisposed to, or at increased risk for developing a symptom, condition, or disease caused by over- or under-expression of osteoclast differentiation factor.

11. A composition, comprising said isolated nucleic acid fragment or subfragment of any one of claims 1-4, said recombinant DNA construct of any one of claims 5-7, or said host cell of claim 8, and a carrier, diluent, or excipient.

12. A pharmaceutical composition, comprising said isolated nucleic acid fragment or subfragment of any one of claims 1-4, said recombinant DNA construct of any one of claims 5-7, or said host cell of claim 8, and a pharmaceutically acceptable carrier, diluent, or excipient.

13. A method of identifying a compound that modulates expression of osteoclast differentiation factor, comprising:

(a) contacting:

(i) a host cell in which osteoclast differentiation factor is normally expressed, and

(ii) a test compound,

wherein said host cell comprises a DNA expression construct comprising a nucleic acid fragment or subfragment of claim 2, and a reporter polynucleotide operably linked thereto, and wherein said reporter polynucleotide is expressed;

(b) determining the level of expression of said reporter polynucleotide in said host cell of step (a);

(c) determining the level of expression of said reporter polynucleotide in a host cell identical to said host cell of step (a),

wherein said identical host cell is not contacted with said test compound; and

(d) comparing the level of expression of said reporter polynucleotide in step (b) with the level of expression of said reporter polynucleotide in step (c),

wherein an increase or decrease in the level of expression of said reporter polynucleotide in step (b) compared to the level of expression of said reporter polynucleotide in step (c) identifies said test compound as a compound that modulates the expression of osteoclast differentiation factor.

14. The method of claim 13, wherein said host cell is selected from the group consisting of an osteoclast progenitor cell, an osteoclast, an osteoblast, a stromal cell, a chrondrocyte, a T-cell, and a fibroblast.

15. A method of identifying a compound that modulates expression of osteoclast differentiation factor, comprising:

(a) contacting a test compound, and
a host cell comprising:

(i) a plasmid comprising a nucleic acid fragment or subfragment of claim 2, and a reporter polynucleotide operably linked for expression thereto, and

(ii) an effector plasmid comprising a nucleotide sequence that codes on expression for a factor required for osteoclast differentiation factor expression,

wherein both said reporter polynucleotide and said factor required for osteoclast differentiation factor expression are expressed;

(b) determining the level of expression of said reporter polynucleotide in said host cell of step (a);

(c) determining the level of expression of said reporter polynucleotide in a host cell identical to said host cell of step (a),

wherein said identical host cell is not contacted with said test compound; and

(d) comparing the level of expression of said reporter polynucleotide in step (b) with the level of expression of said reporter polynucleotide in step (c),

wherein an increase or decrease in the level of expression of said reporter polynucleotide in step (b) compared to the level of expression of said reporter polynucleotide in step (c) identifies said test compound as a compound that modulates osteoclast differentiation factor expression.

16. The method of any one of claims 13 to 15, wherein expression of said reporter polynucleotide is determined by measuring activity of the expressed reporter polynucleotide product.

17. The method of claim 15, wherein said factor required for osteoclast differentiation factor expression is osteoblast specific transcription factor 2.

18. The method of claim 15, wherein said effector plasmid is pEF/Cbfa1/myc/cyto, encoding Cbfa1 (osteoblast specific transcription factor 2).

19. The method of claim 15, wherein said host cell is selected from the group consisting of CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, and WI38 cell lines.

20. The method of any one of claims 13 to 19, wherein said reporter polynucleotide encodes beta-galactosidase.

21. The method of any one of claims 13 to 20, wherein an increase in expression of said reporter polynucleotide in step (b) compared to that in step (c) identifies said test compound as an agonist of osteoclast differentiation factor expression.

22. The method of any one of claims 13 to 20, wherein a decrease in expression of said reporter polynucleotide in step (b) compared to that in step (c) identifies said test compound as an antagonist of osteoclast differentiation factor expression.

23. An agonist or antagonist of osteoclast differentiation factor expression identified by the method of any one of claims 13-22.

24. Use of an agonist according to claim 23 in the manufacture of a medicament for the treatment of a disease in a human caused by under-expression of osteoclast differentiation factor.

25. Use of an antagonist according to claim 23 in the manufacture of a medicament for the treatment of a disease in a human caused by over-expression of osteoclast differentiation factor.

26. Use of a compound that modulates expression of osteoclast differentiation factor in the manufacture of a medicament for the treatment of a disease in a human caused by abnormal expression of osteoclast differentiation factor.

27. The use according to claim 26, wherein said disease is bone disease, arthritis, arterial disease, abnormal immune function, abnormal lymph node development, or abnormal T- or B-cell function caused by abnormal expression of osteoclast differentiation factor.

28. The use according to claim 27, wherein said bone disease is malignant bone disease, rheumatoid arthritis, osteoarthritis, elevated bone resorption, osteoporosis, Paget's disease of bone, hypercalcemia of malignancy, expansile osteolysis, or periodontal disease, and said compound is an antagonist of osteoclast differentiation factor expression.

29. The use according to claim 27, wherein said arterial disease is arterial calcification, and said compound is an antagonist of osteoclast differentiation factor expression.

30. The use according to claim 27, wherein said bone disease is osteopetrosis, and said compound is an agonist of osteoclast differentiation factor expression.

31. The use according to any one of claims 24-30, wherein said compound is identified by the method of any one of claims 13-22.

32. The use according to any one claims of 24-31, wherein said human is diagnosed as having a polymorphism or mutation at one or more nucleotide positions in the osteoclast differentiation factor regulatory region in DNA thereof.

33. A composition, comprising:
an agonist or antagonist of osteoclast differentiation factor expression; and
a carrier, diluent, or excipient

34. The composition of claim 33, wherein said agonist or antagonist is identified by the method of any one of claims 13-22.

35. A pharmaceutical composition or pharmaceutical pack, comprising:
an agonist or antagonist of osteoclast differentiation factor expression; and
a pharmaceutically acceptable carrier, diluent, or excipient,
wherein said pharmaceutical pack comprises instructions for administration of said agonist or antagonist to a human.

36. The pharmaceutical composition or pharmaceutical pack of claim 35, wherein said agonist or antagonist is identified by the method of any one of claims 13-22.

37. The pharmaceutical pack of claim 35 or 36, wherein said human is diagnostically tested for a polymorphism or mutation at one or more nucleotide positions in the osteoclast differentiation factor regulatory region in DNA thereof.

38. A process for making an agonist or antagonist of osteoclast differentiation factor expression, comprising:
(a) carrying out the method of any one of claims 13-22 to identify an agonist or antagonist of osteoclast differentiation factor expression; and
(b) manufacturing said agonist or antagonist.

39. A method of preparing a medicament for the treatment of a bone disease, arthritis, arterial disease, abnormal immune function, abnormal lymph node development, abnormal T- or B-cell function, or other disease in a human caused by abnormal osteoclast differentiation factor expression, comprising:

(a) identifying an agonist or antagonist of osteoclast differentiation factor expression by the method of any one of claims 13-22; and

(b) formulating said agonist or antagonist as a medicament.

40. A method of identifying a mutation or polymorphism in the osteoclast differentiation factor regulatory region of a human subject's or patient's *odf* gene, comprising comparing the nucleotide sequence of the osteoclast differentiation factor regulatory region of the *odf* gene in DNA from said subject or patient with said nucleotide sequence of claim 2,

wherein any difference in nucleotide sequence between said osteoclast differentiation factor regulatory region DNA and said nucleotide sequence of claim 2 identifies a mutation or polymorphism in the osteoclast differentiation factor regulatory region of said subject's or patient's DNA.

41. The method of claim 40, wherein said comparing is conducted using nucleotide sequence analysis or nucleic acid hybridization analysis.

42. A method of identifying a human subject or patient at increased risk for having an altered susceptibility or predisposition to developing a bone disease, cartilage disease, immune disease, or arterial disease caused by abnormal osteoclast differentiation factor expression, comprising comparing the nucleotide sequence of the osteoclast differentiation factor regulatory region of the *odf* gene in

DNA from said subject or patient with said nucleotide sequence of claim 2,

wherein any difference in nucleotide sequence between said osteoclast differentiation factor regulatory region DNA and said nucleotide sequence of claim 2 identifies a mutation or polymorphism in the osteoclast differentiation factor regulatory region of said subject's or patient's DNA that places said subject or patient at increased risk for having an altered susceptibility or predisposition to developing said bone disease, cartilage disease, arterial disease, or immune disease.

43. A method of identifying a human patient or subject at increased risk for having an altered susceptibility or receptiveness to treatment of a disease caused by abnormal osteoclast differentiation factor expression with a compound that affects osteoclast differentiation factor expression through an interaction with the osteoclast differentiation factor gene regulatory region, comprising comparing the nucleotide sequence of the osteoclast differentiation factor regulatory region of the *odf* gene from DNA of said subject or patient with said nucleotide sequence of claim 2,

wherein any difference in nucleotide sequence between said osteoclast differentiation factor regulatory region DNA and said nucleotide sequence of claim 2 identifies a mutation or polymorphism in the osteoclast differentiation factor regulatory region of said subject's or patient's DNA that places said subject or patient at increased risk for having an altered susceptibility or receptiveness to said treatment.

44. A method of treating a human suffering from a symptom, condition, or disease caused by over-expression of osteoclast differentiation factor, comprising administering to said human a pharmaceutically effective amount of an antagonist of osteoclast differentiation factor expression.

45. The method of claim 44, wherein said antagonist is identified by a method according to any one of claims 13-22.

46. A method of treating a human suffering from a symptom, condition, or disease caused by under-expression of osteoclast differentiation factor, comprising administering to said human a pharmaceutically effective amount of an agonist of osteoclast differentiation factor expression.

47. The method of claim 46, wherein said agonist is identified by a method according to any one of claims 13-22.

48. A method of treating a human in need of treatment with an agonist of osteoclast differentiation factor expression, comprising:

(a) determining whether a polymorphism or mutation exists at one or more nucleotide sites in the osteoclast differentiation factor regulatory region in DNA of said human; and

(b) if a polymorphism or mutation exists, administering to said human a pharmaceutically effective amount of an agonist of osteoclast differentiation factor expression.

49. A method of treating a human in need of treatment with an antagonist of osteoclast differentiation factor expression, comprising:

(a) determining whether a polymorphism or mutation exists at one or more nucleotide sites in the osteoclast differentiation factor regulatory region in DNA of said human; and

(b) if a polymorphism or mutation exists, administering to said human a pharmaceutically effective amount of an antagonist of osteoclast differentiation factor expression.

50. The method of claim 48 or 49, wherein said human suffers from a symptom, condition, or disease caused by an abnormal level of expression of osteoclast differentiation factor.

51. A method of modulating bone resorption in a patient in need thereof, comprising administering to said patient a pharmaceutically effective amount of said DNA construct of claim 6 or 7, wherein said protein of interest is osteoclast differentiation factor.

52. A method of modulating bone resorption in a patient in need thereof, comprising administering to said patient a pharmaceutically effective amount of a compound identified by the method of any one of claims 13-22.

53. A method of modulating immune responsiveness in a patient in need thereof, comprising administering to said patient a pharmaceutically effective amount of said DNA construct of claim 6 or 7, wherein said protein of interest is osteoclast differentiation factor.

54. A method of modulating immune responsiveness in a patient in need thereof, comprising administering to said patient a pharmaceutically effective amount of a compound identified by the method of any one of claims 13-22.

55. A kit or package, comprising an isolated nucleic acid fragment comprising the transcriptional regulatory region of the human *odf* gene, or subfragment thereof exhibiting human *odf* gene transcriptional regulatory activity, wherein said fragment or subfragment thereof excludes the *odf* protein coding region.

56. The kit or package of claim 55, wherein said isolated nucleic acid fragment or subfragment thereof comprises said nucleotide sequence of claim 2.

57. The kit or package of claim 55 or 56, wherein said isolated nucleic acid fragment or subfragment thereof is contained within an expression cassette.

58. The kit or package of claim 55 or 56, wherein said isolated nucleic acid fragment or subfragment thereof is:

(a) operatively linked within a vector to a polynucleotide encoding human osteoclast differentiation factor; or

(b) operatively linked within a vector to a polynucleotide encoding a heterologous reporter molecule.

59. The kit or package of claim 58, wherein said vector of (a) or (b) is contained within a vector-releasing cell.

60. The kit or package of claim 59, wherein said vector of (a) further comprises, operably linked to said polynucleotide encoding said human osteoclast differentiation factor, at least one translational regulatory region required for expression of said human osteoclast differentiation factor in said vector-releasing cell.

61. The kit or package of claim 59, wherein said vector of (b) further comprises, operably linked to said polynucleotide encoding said heterologous reporter molecule, at least one translational regulatory region required for expression of said heterologous reporter molecule in said vector-releasing cell.

62. A computer readable medium having stored thereon the nucleotide sequence of a nucleic acid fragment encoding the transcriptional regulatory region of the human *odf* gene, or a

subfragment thereof exhibiting osteoclast differentiation factor transcriptional regulatory region activity, wherein said fragment or subfragment thereof excludes the *odf* protein coding region.

63. The computer readable medium of claim 62, comprising said nucleotide sequence of claim 2.

64. A diagnostic method, comprising determining the nucleotide sequence of the osteoclast differentiation factor transcriptional regulatory region in DNA from a human, or a diagnostically useful fragment thereof, and comparing said nucleotide sequence to said nucleotide sequence of claim 2 provided in a computer readable medium, thereby identifying any polymorphism or mutation in said osteoclast differentiation factor transcriptional regulatory region in said DNA from said human.

FIG. 1A

GCGGCCGCTAATACGACTCACTATAGGGAGAGGATCCTCTCCGGAGTTCAGCAAAGTGAAAC
GTCTGTCATAATAATAACGAATGACTTCCTTTTTCATTTTCATTCATATAGTGAAGTTTTCT
AAAGGCTGCATCATGTGCAATATTGTAACAGAGTAAGTGCAGGACTGAATGTGACTCTATCA
GGCCAATTGTAGAGATGTGAAAAAATGTAAAACAGTGGCACTTTTCTCCCTACTTTTTTTG
AAAATCTGTTTTTTTAAATAAAATAATTTTATAAAAGTATTATGAATTATTTATAAAATTAT
ATTAACATTATGTTAACATGCTAATATGGTAAAATTTTCTGCTTGGAGTTTGAATACACCAA
ATATTTATAAATATAACTCACACAAATAAAACCTCTTTGGTGTTCTCAAAATTTTGAAGAAT
GTAAAAGGTTTGAAAATTGCTGATCTAGCAAATGACTGAACATGAACAGCTATAGTATTTGT
ACCTGCCCAGCAGTGCAGCAATTCCTTATCCTTCTCATATCTGCACTTTAATTTTCCTTTGA
CAAATATCTCTCCCTCCTCTCAGCCCATGACATGAGGTTCACATGGGGTTAACTTAATTCCC
TGGCTCAAAGGAAAGGTATTAAATTCAGACTTGTATCCAACCATTCCTGAAGCTAGACTTAG
CCCTATTTTTCAATAACATGAACCAATCAATTTTCACATGAGTCCAAAATAATTCTATGTTA
ATACACTAAGGTACTAGGAAATATAGTTTGAGAAATGTTGATCCAAACATTGTGTTATTTAC
AGTGGAGTATTGACATAAACTTTGAATCTTCAAATATGTTCTGGTGTCTTGGCATCTCTTAA
TACCTATTAGCTTACAAGGCTTTCACTCAACTATTTTATAATTTTGATAATGACTTAATTGA
TTAGTTGATATATTGTTAAAATAAATATATTAATGAATTTATGATAAATAAGGCAGATAAAT
AAGACATGCAATTAGGAAGACATGTTAAACAAATTGTTATAATAATACAATCACTCTCAGCT
TAGGATAGCTCCTGGCCACTTTCTCTCTGGGTGGTTTTTACTCTGGGAGTAGTTTAAATCAT
TATCTAGTAGTAGTTTAAAGCATTATCTTTGCCTAAGAGCTTTCGCTGACTC**CCCACA**TTTG
CATTGTACTAAGAGTTTTCTCTGACTC**CCCACA**TAGGTCTAGACCCTAGTATTATAAGATTC
TCATTGTACTTGCACTTTGCCTTCAAAGTACTAATCACGGTTTTGTTAGTGATTTGTGTGAT
GATTTGTTGAATCTTTTTTTTTTTCCCACTAGGGTGTAAGCCCCATGTTCCATCTTGATCAC
CATGTTTCTAGCCCAGTGCTGGCATATAGTGGGTTCTCACTAATATATCTGTAGAGTAAATG
AAGAAATGCATGAGTGACATGACAGGAGAATTTAAGGATGCCATGGGAGCATAAAACAGAGG
GAGCCACCTGGGTGAGGAGAGCTGAGAAAGACTTCTGGAGAGGCGACATTTGAGCTGAGAAA
GGAAAGACAAGTGGGAGAGTCCTCCAGGTGTAGAAGTTGGAGAGATGAGCGCTCCAGTTAGG
TAGTATTTGAAGCTGATGTAGAAAAGGAGTCTTGAGCCAGCTTGTGAAGGACTATTGGAGAG
TTTTATTTTTATTTTTATCTTTTTTTTAATTTTTGAGACAGAATCTTGCTTTGTCTCCCAGG
CTGGAGTGCAGTGGCATGATTGTAGCTTACTGCAGCTTCGACCTCCTGGGCTCAAACAATCC
ACCTATCTCAGCCTTCTGAGTAACTGGGACCAGAGATGTGCACCAAAATGCCTGGCTAATTT
GTTCATTTTTTGTAAAGATAGGGTCTCCCTATGTTCCCCAGGCTATTCTCCATCTCCTGGGC
TCCAGTGATCCTCACGCCTCGGCCACCCAAAGTGCTGGGATTATAGAAGTGAACCACTGCGC
CTGGCCTATTGAAGGTTTTTAATCTTCAGAGTTTCGACTTTATCAACAACACTTAGAAGCCA
CCAAAGAATTGCAGGTATGGAAATGACATATACTTTTGCTTTTAGAAGAAAATCCTGATCAG

FIG. 1B

TGTGCACAGAATTCTTCAGGGGGCAAGTGTGATTCATTCTGATAAGATATAGCATGGCTTAG
ACTGGGAGACTGGCAGAGGCTTTGAAGATTTCTTTGCTCAAATTTTATTCAGCAAGTATTTA
CCATGCACCTACTATAGCAGGCAACATTTTTAGGAAATGGTGAATGTTACAGAGGTGAATAA
TACAGCAAGAGTCGTTGAACATATGGAGTTTATCTATTAGTTGGGGAGTGAATGTTGACAAA
GGAATAAGTAAATACATAGGCAAGAAAGATACATTACCTGTGAAACAGCAGCAGGTAGACTG
ACAGTGGAGTATCTAATACAGCCTATGGAAGCCAGAAGATAGTGGGATGACATTTTTGGAGT
ACTAGTAGAAATGTCATATGAAGAACTCTGTAGGAATGTAACATACGGTCCCATATATGAAG
CTCCTGGGTCAAGTATACCTGAACATAATTCAGGGATTTGAGGGACTTTCTTGTAACCTGAG
GATCAAGATGTCAAGGAATTAAAAACATGTATAAAACATTGTTGTATAAAAACCCATTAAAA
AGAATGGAAGACACTATAGTAAAATCAT**TGTGGG**TTTAGTTGTTATAACACATTTTAAAAAT
CTTTGATCCCAATCAATATTTATAAGAAAGAAGAAATATGGAATTATTTCCTGAGTCAAGGA
GCAGGGAGAGAATGAGGAAGAAGAGGAGGAGGAGGAGGGGGAGGAGGAGACAATAAACCTAC
TTCCCAAAGTTAACAAACAAAAAGTGGGAAGAGGTCAAAGACTACAAGGAGTAGAATTAACG
TCAATTGTTTCTATGTTTGAGTCTGAAAATTTTTTGTCCCTTCTCCACCAACCTATATATTG
ATACACATATTAATGCTAAAGGCATTTTTGTATTTGAACAGATCATTTTCTTTGTATGGCTG
CCTTTAAAAAAAATTCAACCTGGTCACTCTTCCTCAACATTTACTGAGGTCTAAGTGTTCAA
TTTAGAACACATGCTTTAATAACTCAGAGACCTGTCATTTGTCACAAATCTTGCCTAGAGAA
ATACTCATTAGCGAATTAGGCAGAAAGAGGATGCAAAATAAAAAGGCACAGTAGTCCCCTGA
TATCCATGGAAGACTGGTTCCAGGACACCACCAAACCCCTC**CCCGCA**AATACCAAAATCCAT
GGATGTTCAAGTTTCTTAACATATCATGGCATAGTATTTGCATTTAACCTACACACATCCTC
TTGTACACTTGAAATTATCTTTAGATTATTTATAATACTTAATAGAATGTAAATGCTATGTA
ACTAGTTGTGTATCATTTAGGAAATGATCACAAGAAAAAAAGTCTACAGATGTTAGTCCAGA
CACAGCCATCCTTTTTTTTTTTTTCAAATATTTTTGATC**TGTGGTT**CATTGCATCCACAGAT
GTGGAACCCATGGATAC**TGTGGG**CTAACTGTATTAATAAAAAAGTGGAAACATCCTAAGTTT
CATGGGTGTTTAAATTGGTCAGCAACTTCCTTCTGAAGAAGTATCAGAATTTGTGAGCAATG
TTAATATTTTTGTTTTCTCACTAAGA**GCCACA**GTTCTGAATAGAGGTTTTTAAAAAGCCCTA
GCAAGGTTTCTTTAGCAATGAAACTAACATTTAACTGTATCATCAGCTTCGTGTTACATCTC
TTTCCTGACTGTTGGGTGAGCCCTCCTCGGATGCTTGCTTCTGGCTACACGCCCCTTTACCC
TTTTCTCTGCACTGTTTTCATCTTTATAAAGTCAGAGTTGGTGTCTATAGGCTCTCTACT**GC**
CACATTCAAGACCTGCCTCGCTCAATGTCACCTTCAAGATGCAGAAATAGGGATTTGGGAAG
GGGATTGTGAAATTTTCGAAGTCTTCCAAAATACTTTGAGAAACTATATTTGGAAGCACTTT
GGGGGGAGAGGTTGGACAGGAAGGGTCTTCAGAGATCATCAAATTTAACTTTCTAAATCCTA
AGGAGGAAACCGAGACTCCAGGATGTGAAGTCCCTTCTCTACCAAACTAGAATGGATGCAGG
AGGAATGTCTGAGGTGCAATCCTTATCCTTTAGCAAAGGTGTCCTCTGCGTCTTCTTTAACC
CATCTCTTGGACCTCCAGAAAGACAGCTGAGGATGGCAAGGGGAGTCTGGAACCACTGGAGT
AGCCCCCAGCCTCCTCCTTGGAGGGCCCCCATGAAGGAGGCCCTTCAGTGACAGAGATTGAG

FIG. 1C

AGAGAGGGAGGGCGAAAGGAAGGAAGGGGAGCCAGAGGTGGGAGTGGAAGAGGCAGCCTCGC

CTGGGGCTGATTGGCTCCCGAGGCCAGGGCTCTCCAAGCGG***TTTATAA***GAGTTGGGGCTGCC

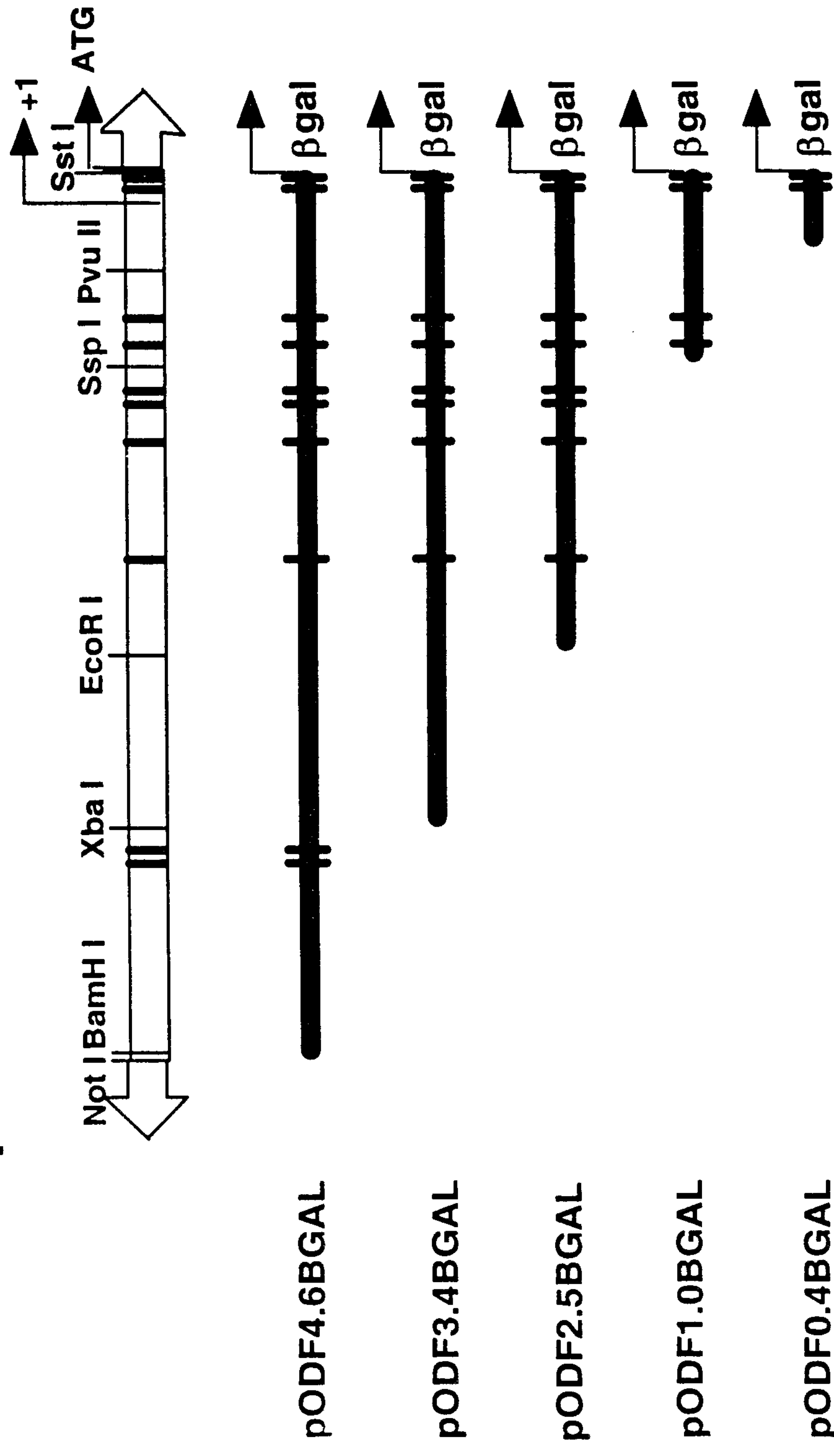
GGGCGCCCTGCCCGCTCGCCCGCGCGCCCCAGGA**G**CCAAAGCCGGGCTCCAAGTCGGCGCCC

CACGTCGAGGCTCC**GCCGCA**GCCTCCGGAGTTG**GCCGCA**GACAAGAAGGGGAGGGAGCGGGA

GAGGGAGGAGAGCTCCGAAGCGAGAGGGCCGAGCGCC**ATG**

FIG. 2

ODF promoter deletion constructs



Osf2 binding element (OSE2)

FIG. 3

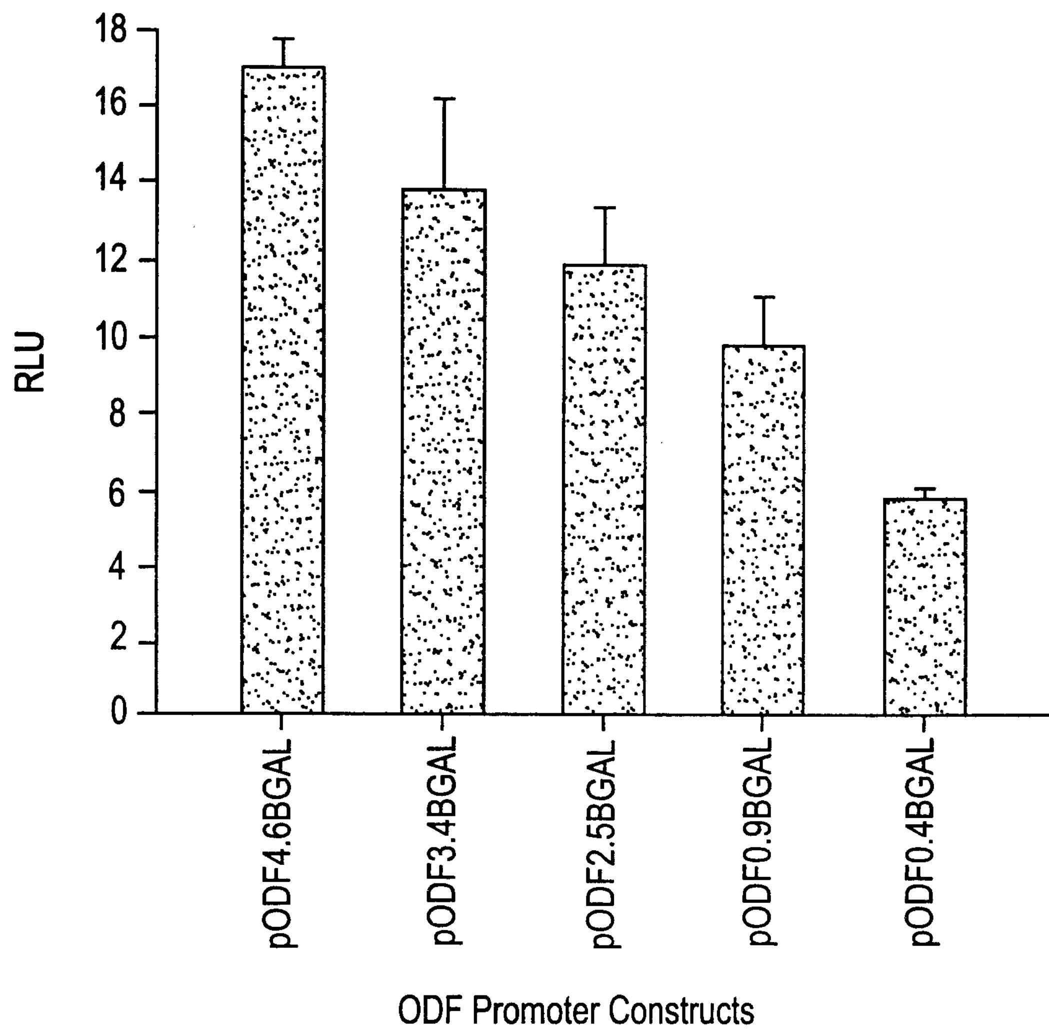
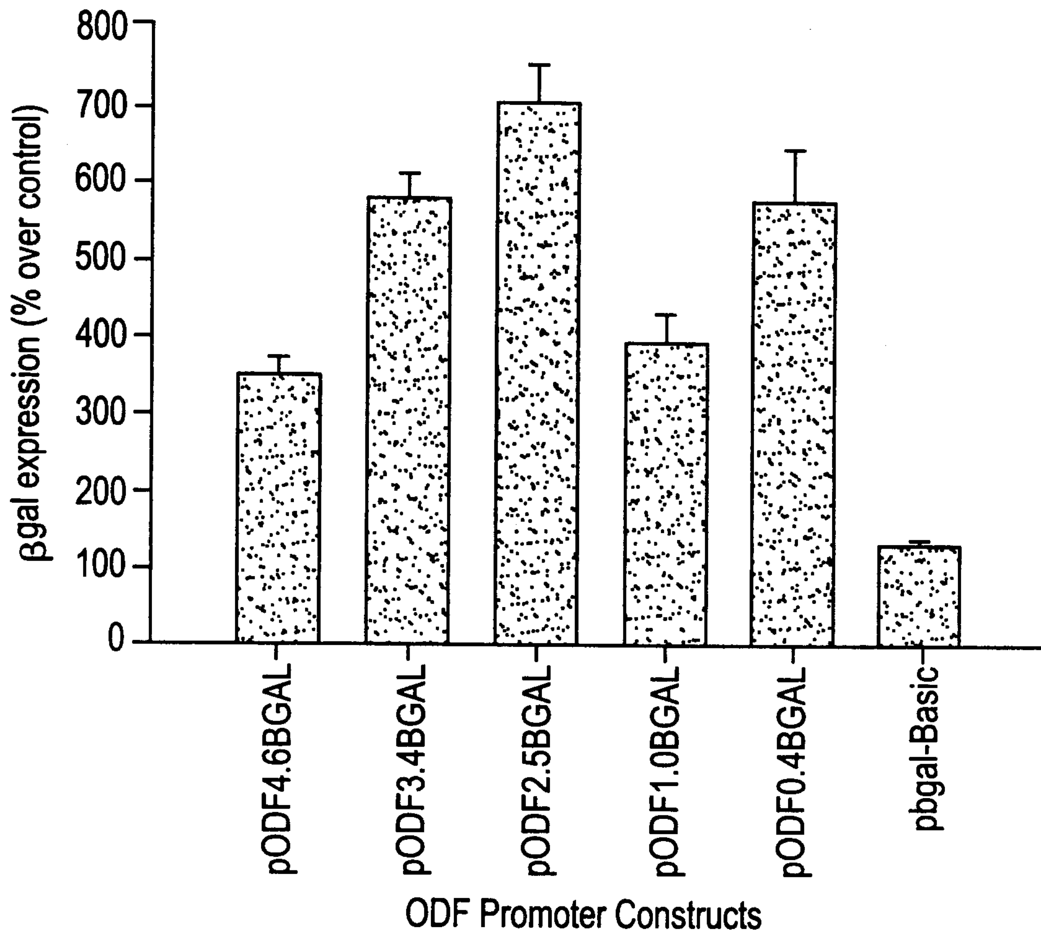


FIG. 4



INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/US 00/26407

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/705 C12Q1/68 A61K31/713

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K C12Q A61K

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

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

WPI Data, PAJ, CAB Data, STRAND, EMBL, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 'Online! Hinxton, UK; 23 November 1998 (1998-11-23) M.D. ADAMS ET AL.: "RPCI11-90G18.TV RPCI-11 Homo sapiens genomic clone RPCI-11-90G18, genomic survey sequence" XP002159270 EMBL EMGSS4.AQ282723, Sequence Accession no. AQ282723; abstract --- -/--	1-5

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 February 2001	14/02/2001
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Hornig, H

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 14 April 1999 (1999-04-14) KITAZAWA RIKO ET AL: "Promoter structure of mouse RANKL/TRANCE/OPGL/ODF gene." Database accession no. PREV199900256260 XP002159271 cited in the application abstract & BIOCHIMICA ET BIOPHYSICA ACTA, vol. 1445, no. 1, 14 April 1999 (1999-04-14), pages 134-141, ISSN: 0006-3002 ---	
A	KODAIRA K ET AL: "Cloning and characterization of the gene encoding mouse osteoclast differentiation factor" GENE,NL,ELSEVIER BIOMEDICAL PRESS. AMSTERDAM, vol. 230, no. 1, 1 April 1999 (1999-04-01), pages 121-127, XP004165540 ISSN: 0378-1119 cited in the application the whole document ---	
A	WO 98 46751 A (AMGEN INC ;BOYLE WILLIAM J (US)) 22 October 1998 (1998-10-22) the whole document ---	
P,A	WO 00 15807 A (M & E BIOTECH A S ;HAANING JESPER (DK); HALKIER TORBEN (DK)) 23 March 2000 (2000-03-23) the whole document -----	

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 44-54 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Remark: Although claims 62 and 63 could be, at least partially be considered as a mere presentation of information, Rule 39.1(v) PCT, and claim 64 at least partially as a computer program, Rule 39.1(v) PCT, the search has been carried out as far as possible in our systematic documentation.

Continuation of Box I.2

Claims Nos.: 23-39

Claims 23-39 refer to an antagonist and agonist of the polynucleotide reporter system without giving a true technical characterization. Moreover, no such compounds are defined in the application. In consequence, the scope of said claims is ambiguous and vague, and their subject-mater is not sufficiently disclosed and supported (Art.5 and 6 PCT). No search can be carried out for such purely speculative claims whose wording is, in fact, a mere recitation of the results to be achieved.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int(ional Application No
PCT/US 00/26407

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		AU 7120598 A	11-11-1998
		BG 103824 A	31-05-2000
		BR 9808545 A	23-05-2000
		CN 1264427 T	23-08-2000
		EP 0975754 A	02-02-2000
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		SK 141999 A	16-05-2000
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