0013-7227/07/$15.00/0
Printed in U.S.A.

Endocrinology 148(6):2635–2643

doi: 10.1210/en.2007-0270

Minireview: Targeting the Wnt/β-Catenin Pathway to Regulate Bone Formation in the Adult Skeleton

Roland Baron and Georges Rawadi

Yale University School of Medicine (R.B.), New Haven, Connecticut 06520; and Galapagos (G.R.), 93230 Romainville, France

The recent identification of a link between bone mass in humans and gain- or loss-of-function mutations in the Wnt coreceptor low-density lipoprotein receptor-related protein 5 (osteoporosis pseudoglioma syndrome, high bone mass trait) or in the Wnt antagonist sclerostin (sclerosteosis, van Buchem syndrome) has called the attention of academic and industry scientists and clinicians to the importance of this signaling pathway in skeletal biology and disease. Multiple genetic and pharmacological manipulations of Wnt signaling in mice have since then confirmed the central role of this pathway in regulating bone formation. (*Endocrinology* 148: 2635–2643, 2007)

THE SKELETON IS continuously remodeled through a regulated process that involves a complex network of systemic and local factors, activating multiple signaling pathways. Indeed, all skeletal diseases can be linked to the local or systemic dysregulation of this process. Two cell lineages are involved in bone remodeling: the hematopoietic bone-resorbing osteoclasts and the bone-forming mesenchymal osteoblasts and osteocytes (1). Two important mesenchymal cell gene products are required for osteoclast differentiation, macrophage colony-stimulating factor (Csf1/M-CSF) and receptor activator of nuclear factor-κB ligand (Tnfsf11/RANKL) and are secreted by the osteoblast (2, 3). RANKL stimulates osteoclast maturation and function through interaction with its receptor, RANK, expressed at the surface of osteoclasts. In addition, osteoblast-secreted osteoprotegerin (OPG) acts as a decoy receptor inhibiting the interaction between RANKL and RANK (4). Conversely, gene products derived from the osteoclast and/or from the bone matrix during bone resorption can modulate osteoblasts (5–7).

In the adult, bone formation by mesenchymal stem cell-derived osteoblasts occurs in the context of bone remodeling and is counterbalanced by the bone-resorbing activity of osteoclasts. It is the overall balance between these two activities that allows bone mass to accrue during establishment of peak bone mass in children and young adults and to be maintained throughout life in adults. In contrast, it is an imbalance between bone resorption and bone formation that leads to bone loss and osteoporosis in older patients.

If current therapies can prevent bone loss through antiresorptive activity, they cannot activate bone formation, with the exception of PTH. Thus, understanding the mechanisms that regulate bone formation is one of the highest priorities in both academic and pharmaceutical research on the skeleton and a new frontier in the treatment of osteoporosis, possibly allowing treatment to rebuild bone mass and architecture to levels where the biomechanical function of the skeleton can safely be performed.

The exploration of the mechanisms by which the formation of bone is regulated during embryogenesis has clearly established a role for members of the hedgehog family of proteins and their receptors (smoothened and patch) as well as for bone morphogenetic proteins (BMPs) and their receptors (8–11). Several marker genes of the osteoblast lineage are now identified, and the picture that emerges is that of a cascade of signaling pathways each of which activates the expression of a few genes characteristic of the osteoblast lineage as well as the expression of the ligand for the following signaling cascade, ultimately leading to the expression of the full set of genes characteristic of a mature, bone matrix-secreting osteoblast. In several instances, loss-of-function mutations in humans lead to a characteristic genetic skeletal syndrome mimicked by gene deletion in mice. Deletion of Runx2 leads to a complete lack of bone formation in mice and to craniofacial dysostosis in heterozygous humans (12–14); deletion of osterix leads to an arrest along the osteoblast differentiation pathway and several skeletal abnormalities in mice (15). Although in humans, RUNX2 mutations are clearly linked to cleidocranial dysplasia (14, 16), the link between these genes and the regulation of bone mass and/or the susceptibility to osteoporosis is still unclear, despite some recent reports (17, 18).

In what constitutes the most important breakthrough in this field in recent years, a clear link has now been established between low-density lipoprotein receptor (LDLR)-related protein 5 (LRP5), a coreceptor for Wnts, and bone mass in humans and in mice. Loss of function in LRP5 leads to the osteoporosis pseudo-glioma syndrome (OPPG), with extremely low bone mass, whereas gain of function leads to the

First Published Online March 29, 2007

Abbreviations: BMD, Bone mineral density; BMP, bone morphogenetic protein; Dkk, dickkopf; Fz, frizzled; GSK-3β, glycogen synthase kinase 3β; HBM, high bone mass; Krm1, Kremen-1; LDLR, low-density lipoprotein receptor; LRP5, LDLR-related protein 5; OPG, osteoprotegerin; OPPG, osteoporosis pseudo-glioma syndrome; RANKL, receptor activator of nuclear factor-κB ligand.

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

high bone mass (HBM) phenotype in humans (19–21). In addition, deletion mutations in the gene encoding sclerostin (Sost), an endogenous inhibitor of the Wnt pathway, also lead to osteosclerotic phenotypes (sclerosteosis, van Buchem syndrome) (22–24). Before these findings, aberrant Wnt signaling in adult life was mainly linked to tumor progression (25). These important observations have opened a whole new field of investigation both in terms of understanding the mechanism that regulate osteoblasts and their activity and in terms of drug discovery, in the hope to target one component of the Wnt signaling pathway and increase bone mass in osteoporotic patients.

The goal of this review is to briefly discuss our current understanding of Wnt signaling in bone and how it affects drug discovery.

Wnt Signaling: Canonical Pathway

The Wnt signaling cascade is triggered upon binding of members of the Wnt family proteins (over a dozen distinct Wnts have been identified; see http://www.stanford.edu/~rnusse/wntwindow.html) to a coreceptor complex, including frizzled (Fz, a G protein-coupled receptor-like protein) and LRP5 or -6. The signal is transmitted through recruitment of several proteins to the C-terminal intracellular moieties of the activated Fz and LRP5/6 coreceptors. From this point, disheveled (Dvl) is recruited and posttranslationally modified, and depending on the specific nature of the Wnt and of the Fz that are complexed with LRP5/6, three independent pathways can be activated: canonical, noncanonical, or Ca^{2+}. In this review, and because all the data generated so far indicate that this is the pathway that regulates bone formation downstream of LRP5/6, we will focus our discussion only on the canonical pathway (for more details see review, Ref. 25).

The Wnt canonical signaling pathway relies mainly on the stabilization of cytosolic β-catenin. In the absence of Wnt proteins, β-catenin is phosphorylated by several kinases, mainly glycogen synthase kinase 3β (GSK-3β) but also casein kinase 1, and targeted to ubiquitination and degradation by the proteasomal machinery. Wnt binding to its receptor complex results in the inhibition of GSK-3β activity. This inhibition mediates the prevention of β-catenin degradation, leading to an accumulation of β-catenin in the cytoplasm. Upon reaching a certain concentration level, β-catenin translocates to the nucleus where it associates with the Tcf/Lef family of transcription factors to regulate the expression of canonical Wnt target genes (Fig. 1).

Given the importance of this signaling pathway in the control of numerous cellular functions, several fine-tuning regulatory systems have evolved at the extracellular, cytosolic as well as nuclear levels. Extracellular regulators of Wnt signaling include mainly two types of naturally occurring inhibitors, each targeting signaling from one of the Wnt coreceptors. The first group [secreted Fz-related-proteins (sfrp)] binds and neutralizes Wnt proteins, acting as soluble decoy Fz receptors and preventing binding of Wnt to Fz. The second group includes dickkopf (Dkk) and sclerostin (Sost) proteins that bind to and inactivate signaling from LRP5/6 receptors. Cytosolic Wnt signaling inhibitors are also found intracellularly, the most predominant ones being GSK-3β (described above), the scaffolding protein axin and the tumor suppressor adenomatous polyposis coli. All these proteins are included in the β-catenin phosphorylation complex, which leads to β-catenin degradation. Finally, in the nucleus, the β-catenin/Tcf complex is also subject to tight control of either its transcriptional activity or its nuclear localization. For instance, Ctnnbip1/Icat or Cby/Chibby have been shown bind to β-catenin, and either inhibit its interaction with Tcf or translocate it to the cytoplasm, respectively. Alternatively, transcriptional repressors such as Groucho are capable of binding to a specific domain of Tcf, thus neutralizing its activity, downstream of β-catenin.

Thus, the fact that Wnt canonical signaling requires LRP5/6 activity and that there are a number of extracellular agonists and antagonists as well as intracellular enzymatic regulators offers significant opportunities for therapeutic regulation. For the skeleton, the fact that LRP5 gain- or loss-of-function mutations have been shown to regulate bone mass in humans and that mutations in at least one LRP5 natural antagonist, sclerostin, is linked to two osteosclerotic phenotypes (sclerosteosis and van Buchem syndrome) has focused the attention on LRP5 and its signaling activity.

Canonical Wnt Receptor LRP5/6: Structure, Function, and Regulation

LRP5/6 belong to the LDLR family, which are cell surface proteins capable of binding and internalizing ligands through receptor-mediated endocytosis. Unlike other members of the LDLR family, LRP5/6 do not contain an internalization signal sequence required for endocytosis but have been shown to bind Wnt proteins and mediate canonical Wnt signaling (26).

Lrp5 and Lrp6 encode 1615- and 1613-amino-acid transmembrane proteins, with 71% homology between them. The extracellular domain of both receptors is mainly composed of N-terminal 6xYWTD repeats and (EGF)-like sequence, this repeated four times then followed by a LDLR-like ligand-binding domain. Crystal structure analysis has revealed that the YWTD repeats forms a six-bladed b-propeller module (27). The first module was shown to bind Wnt and Sost proteins, whereas the third one binds and mediates Dkk inhibition.

The cytoplasmic domain of LRP5/6 is also very distinct from other LDLRs. First, the classical NPXY motif found in LDLRs is absent in LRP5/6. Second, the LRP5/6 cytoplasmic tail is particularly rich in prolines and serines and several PPP(S/T)P motifs are essential for LRP5/6-mediated Wnt signaling. It has been shown that Wnt binding to LRP5/6 results in the dual phosphorylation of PPP(S/T)P motifs by CKIε and or GSK-3β, resulting in the recruitment of axin to the membrane, an event that is crucial to activate downstream signaling (28, 29). The cytoplasmic tail of LRP5/6 recruits not only axin but also other proteins such as frequently rearranged in advanced T-cell lymphomas (Frat1) and microtubule-actin cross-linking factor 1 (MACF1) (30, 31). These interactions are required to disrupt the GSK-3β/APC/axin/β-catenin protein complex and thereby prevent

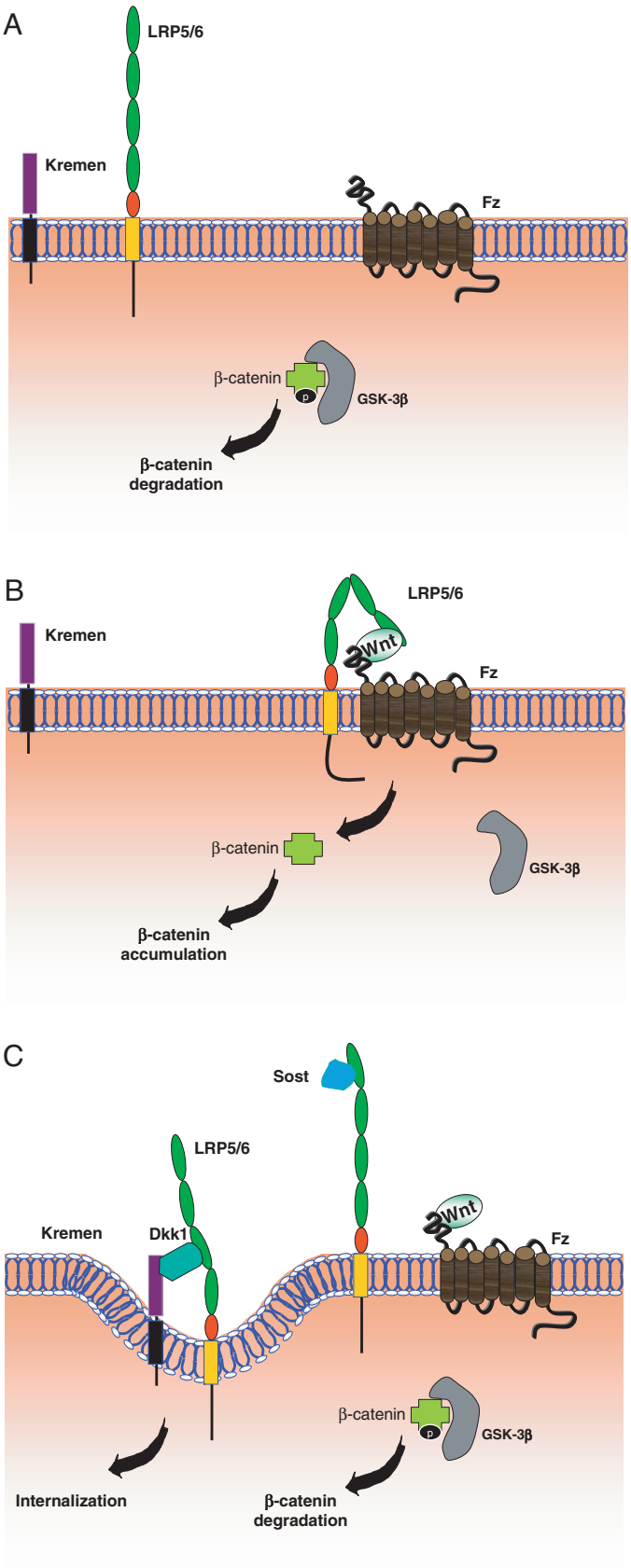

FIG. 1. Wnt/β-catenin signaling pathway. A, In the absence of Wnt ligand, β-catenin is phosphorylated by GSK-3β leading to its degradation and pathway signaling inactivation. B, After Wnt binding to its LRP5/6 and Fz coreceptors, GSK-3β is inactivated. β-Catenin is then stabilized and accumulates in the cytoplasm. β-Catenin will consequently translocate into the nucleus where it affects gene expression. C, The secreted Dkk proteins bridge LRP5/6 and the transmembrane protein Krm. This results in the LRP5/6 membrane depletion by internalizing the receptors. As a consequence, Wnt signaling is inhibited. Sclerostin (Sost) also inhibits Wnt signaling through binding to LRP5/6, but its activity is independent of Krm proteins.

the phosphorylation and degradation of β-catenin, leading to its cytoplasmic stabilization.

Although LRP5 and LRP6 are undistinguishable when compared for their capacity to transduce Wnt signaling, genetic investigations demonstrate at least one difference between these two close receptors. LRP6, but not LRP5, is required during embryonic development. Actually, Lrp6-deficient mice die at birth, whereas Lrp5-deficient mice are viable, possibly due to redundant functions with Lrp6 (32). This clearly demonstrates that, although highly redundant, LRP5 and -6 have some distinct properties. More studies are required to determine whether LRP6 and LRP5 interact with a distinct subset of Wnt ligands or whether they act as receptors for other ligands engaging distinct signaling pathway. Interestingly, LRP6 has been recently shown to mediate the internalization and lethality of anthrax toxin, indicating that such receptors may be involved in distinct signaling pathways (33).

Noteworthy is the fact that if several families have been identified with mutations of LRP5 leading to skeletal alterations, whether HBM or OPPG, or with Sost mutations, leading to osteosclerosis, mutations in LRP6 have not yet been reported to be linked to skeletal defects. Thus, it seems to be that LRP5 and the canonical Wnt signaling are the keys to the regulation of bone mass in humans.

Involvement of Canonical Wnt Signaling in Bone Metabolism

Key data as to the importance of the Wnt signaling pathway in the control of bone mass were provided by the identification of mutations in the Wnt coreceptor LRP5 gene inducing either the OPPG or the hereditary HBM trait in humans (19–21). OPPG is a rare autosomal recessive disorder affecting bone and vision associated with loss-of function mutations in the LRP5 gene (20). OPPG patients display a very low bone mass and are prone to develop skeletal fractures and deformities. In contrast, gain-of-function mutations of LRP5 have been found to be associated with increased bone density in the autosomal dominant HBM trait (19, 21, 34). Additional independent epidemiological studies have linked sequence variants in the LRP5 gene with differences in bone mineral density (BMD) and/or fracture risk (35–39).

These findings were further supported by genetic manipulation of Lrp5 gene in mice. Indeed, knocking out Lrp5 or introducing gain-of-function mutations produced similar phenotypes to those observed, respectively, in OPPG and HBM (32, 40, 41). Several other mouse models support the notion that activating Wnt signaling leads to increased bone mass. For instance, overexpression of Wnt10b in adipose

tissue results in increased bone mass and strength and induces a resistance to aging or hormonal-related bone loss (42). Furthermore, inactivating or reducing the expression of Wnt antagonists such as Sfrp1, Apc, or Dkk1 markedly increased trabecular bone mass in adult mice (42–46). On the other hand, overexpressing Wnt antagonists such as Ctgf, Wif1, and Dkk1 decreases bone density (45, 47–49). Taken together, all these reports establish unequivocally the critical role of Wnt and LRP5 in the regulation of bone mass in humans and in rodents.

Interestingly, loss- or gain-of-function mutations affecting LRP5 either in humans or in mice alter bone formation without affecting the resorption parameters. This indicates that osteoblasts are indeed the main cellular targets of Wnt effects in bone. It was then demonstrated that β-catenin is essential in determining whether mesenchymal progenitors become osteoblasts or chondrocytes (50–52), indicating that Wnt signaling can affect osteoblast commitment. An additional mechanism by which Wnt may control osteoblast commitment is by blocking adipogenesis via the inhibition of the adipogenic transcription factors CCAAT/enhancer-binding protein-α (C/EBPα) and peroxisome proliferator-activated receptor-γ (PPARγ) as demonstrated *in vivo* in Wnt10b transgenic mice (42) or *in vitro* (53), although a direct inverse relationship between the pools of these two mesenchymal lineages has never been firmly established. Taken together, these results suggest that Wnt signaling can determine the cell fate of mesenchymal precursors (Fig. 2).

In addition to regulating osteoblast commitment, Wnt/β-catenin signaling is also suspected of affecting osteoblast proliferation. Lrp5$^{-/-}$ mice show reduced osteoblast proliferation (32) and, although osteoblast proliferation has not been directly measured, an Lrp5 (G171V) transgenic model displays a significant increase in osteoblast number and a reduced number of apoptotic osteoblasts and osteocytes in calvaria (41).

But the main effect of the canonical Wnt signaling may well be in osteoblast differentiation and function. First, the expression of several key components of Wnt signaling is regulated during osteoblast differentiation (54–56). Among those, Wnt signaling antagonists, including Sfrp2, Wif1, Dkk1, or FrzB, are strongly up-regulated during the late phase of osteoblast differentiation, suggesting that a negative Wnt feedback loop may control the last steps of osteoblast maturation. Second, Lrp5-deficient mice display a decrease in bone matrix deposition (32), and osteoblasts overexpressing a constitutively active mutant of β-catenin constitutive show an increase of collagens type Ia1 and -a2 gene expression (44). Thus, Wnt canonical signaling controls osteoblasts at different levels: commitment, proliferation/apoptosis, and function.

As discussed earlier, based on the data in Lrp5 mutants, one could conclude that the Wnt canonical pathway does not regulate osteoclasts. However, evidence *in vitro* and *in vivo* indicates that Wnt regulates the expression of osteoprotegerin, the decoy RANK receptor that inhibits osteoclast differentiation by interacting with RANKL. First, β-catenin-deficient osteoblasts exhibit an elevated expression of

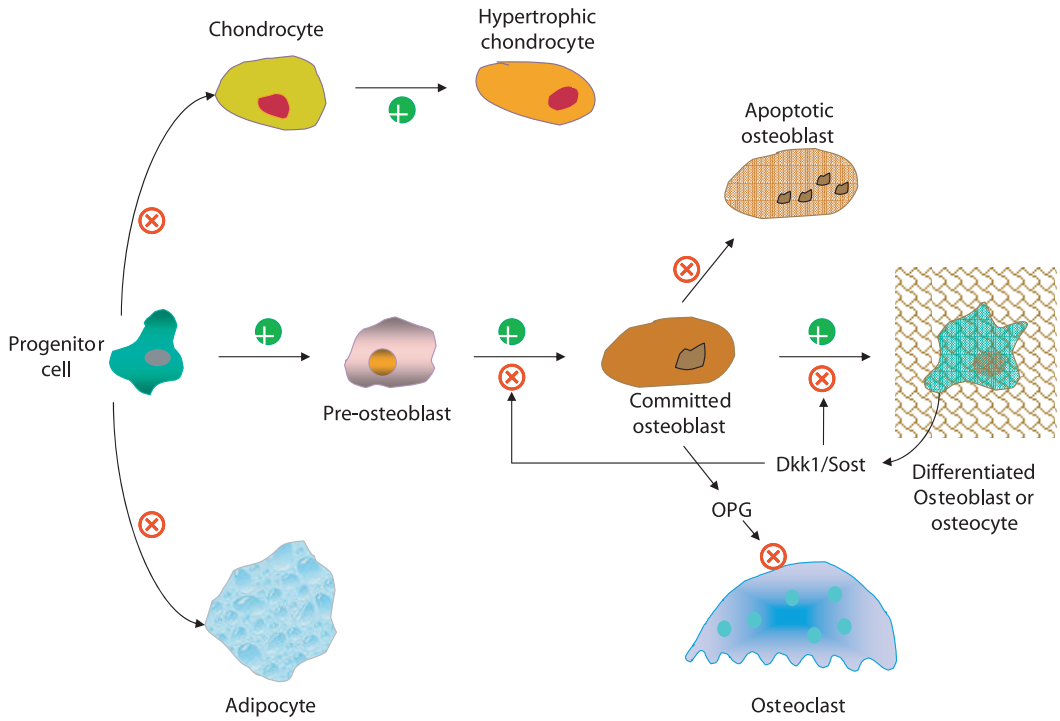

FIG. 2. Role of Wnt/β-catenin signaling in determining the cell fate from mesenchymal progenitor cells. Wnt signaling plays a dual role in regulating chrondrocytic differentiation. The Wnt canonical pathway represses chondrocyte differentiation from progenitor cells, whereas it is required for chondrocyte hypertrophy. Wnt pathway activation also inhibits adipocyte differentiation and promotes osteoblast cell lineages by controlling proliferation, maturation, and terminal differentiation. Differentiated osteoblasts or osteocytes produce Wnt inhibitors such as Dkk1 and sclerostin as a negative feedback control of osteoblast differentiation and/or function. Committed osteoblasts produce OPG to increase the OPG/RANKL ratio, thus decreasing osteoclast differentiation and activation.

RANKL and a diminished expression of OPG, whereas Apc-deficient osteoblasts display a decreased expression of RANKL and increased OPG (44, 45). This may explain why the osteoblast-selective deficiency of β-catenin in mice affects bone resorption rather than bone formation. Second, Dkk2 deficiency in mice results in osteopenia associated with reduced osteoblast differentiation and enhanced osteoclast function (57). The effect on osteoclasts in this model most likely results from an increase in osteoclast-activating cytokines, including macrophage colony-stimulating factor and RANKL. Finally, a recent report by Diarra *et al.* (58) clearly demonstrates a cross-talk between the bone-anabolic Wnt and the bone-catabolic RANKL pathway. All these data strongly suggest that Wnt signaling indirectly controls osteoclast differentiation via its effect on osteoblasts. These observations on the regulation of the RANKL/OPG ratio might be relevant not only to osteoporosis but also in the development of multiple myeloma osteolytic lesions and joint diseases such as rheumatoid arthritis and osteoarthritis (58, 59).

Targeting the LRP5/Wnt Signaling Pathway to Treat Bone Diseases

The logical consequence of the tremendous accumulation of knowledge on the role of the LRP5/Wnt signaling pathway in regulating bone formation, all generated in only the last 6 yr, has been to trigger a significant effort by the pharmaceutical and biotech industry to discover and develop therapeutic products that would be anabolic to bone, *i.e.* increase bone formation in osteoporotic and other osteopenic patients. Although there were, and still are, significant worries about activating the Wnt signaling pathway in a systemic manner, possibly leading to tumorigenesis (see Ref. 25 for review), the recent observations on the bone-specific distribution of two key natural inhibitors of LRP5/Wnt, specifically Dkk1 and Sost, have been reassuring for the targeting of these two antagonists to increase bone formation.

GSK-3β

Among the intracellular elements of the Wnt signaling cascade, one of the most amenable to drug targeting is the enzyme GSK-3β. It is a multifunctional serine/threonine kinase found in all eukaryotes. However, the enzyme is a key regulator of numerous signaling pathways, including cellular responses to Wnt, receptor tyrosine kinases, and G protein-coupled receptors and is involved in a wide range of cellular processes, ranging from glycogen metabolism to cell cycle regulation and proliferation (60), making it a far less desirable target than Dkk1 and Sost. GSK-3 is unusual in that it is normally active in cells and is primarily regulated through inhibition of its activity. Another peculiarity compared with other protein kinases is its preference for primed substrates, that is, substrates previously phosphorylated by another kinase (61).

Because increased GSK-3 activity may be linked to pathology in diseases such as Alzheimer's disease and non-insulin-dependent diabetes, several GSK-3 inhibitors have been developed. Treatment with GSK-3β inhibitors, including lithium and 6-bromo-indirubin-3′-oxime, enhance bone formation and increase bone mass in several mouse models (40). Lithium has been used safely and effectively for over a half-century in the treatment of bipolar disease. Interestingly, a separate study that addressed the effects of lithium on fracture risk using a case-control study design revealed that lithium treatment was associated with a decreased risk of fractures, thus potentially pointing at bone-anabolic properties in humans (62). In an independent study, an orally bioavailable dual GSK-3α/β inhibitor, LY603281-31-8, was tested for its activity in bone mass in ovariectomized rats. GSK-3 inhibitor increased expression of several bone-specific genes including collagen-α1 (I) and -α1 (V), biglycan, osteonectin, and runx-2 (63). Furthermore, significant increase in bone mineral content and BMD was observed in cancellous and cortical bone of ovariectomized rats treated with GSK-3 inhibitor. This was associated with improved mechanical properties of lumbar vertebrae (63). All these data clearly indicate that orally available small-molecule GSK-3 inhibitors induce osteoblast differentiation and bone formation, increasing bone mass and strength *in vivo*, consistent with a role for the canonical Wnt pathway in osteogenesis. The challenge will, however, be whether a specific molecule or dosage will allow the restriction of the effects of these inhibitors to the skeleton.

Targeting Endogenous Wnt Inhibitors in Bone

Indeed, the specific targeting of Wnt activation to the skeleton, extremely difficult to achieve with small molecules targeting LRP5/6, Fz, or the enzymes that are regulated intracellularly downstream of these receptors (see GSK-3β), may be achievable by targeting Dkk1 or Sost with humanized monoclonal antibodies. This prediction is based upon the fact that in the adult mouse skeleton, these two proteins are essentially restricted to osteoblasts and/or osteocytes (47, 64). Thus, systemic administration of antagonists to Dkk1 or Sost may possibly affect only the skeleton, favoring endogenous Wnt signaling and increasing bone formation without affecting Wnt signaling in other organs.

Dkk Proteins

Dkk genes include four evolutionary conserved members (Dkk1–4) that encode for a secreted glycoprotein of 255–235 amino acids. They share two conserved cysteine-rich domains at the N and C termini called, respectively, Cys-1 and Cys-2, and each displays a specific spacing sequence (Fig. 3). It is now well established that Dkk1, -2, and -4 either physically or functionally interact with LRP5 and LRP6 and affect Wnt signaling. Dkk1 binds to the C-terminal domains 3 and 4 of LRP5/6 receptors (65–70). The Cys-2 domain of Dkk proteins is sufficient to mediate binding to LRP6, and mutation of the conserved Cys220 residue abolishes the interaction (69). In addition, a 21-amino-acid synthetic peptide (L218-Ser204 or Cys233-Cys253) derived from the Dkk1 Cys-2 domain displays high binding affinity for LRP6 and is sufficient to inhibit Wnt signaling (71).

In addition to LRP5/6, Dkk proteins bind to another single-pass transmembrane receptor family, Kremen-1 (Krm1) and Krm2 (72–74). Both Krms bind Dkk1 and Dkk2 with high affinity (nanomolar range). Like for LRP5/6, it is the Cys-2

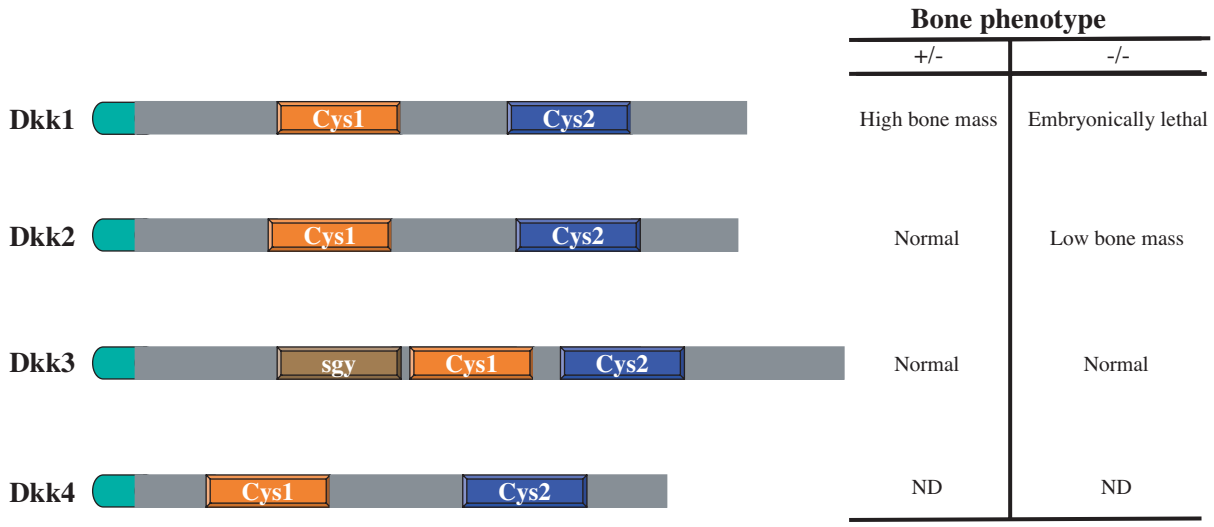

FIG. 3. Polypeptide structure of Dkk proteins. Cysteine-rich domain 1 (Cys1) and Cys2 as well as sogy (sgy) domains are shown, and the *right panel* describes the bone phenotypes of Dkk-deficient mice (46, 47, 57, 76, 77). ND, Not done.

domain that mediates binding to Krm1/2 (74). Although Dkk1, -2, and -4 are capable of binding LRP5/6 in the absence of Krm, Krm1/2 potentiate the capacity of Dkks to inhibit Wnt signaling (73). In fact, in the presence of Krms, a Dkk/LRP/Krm protein complex forms and is rapidly endocytosed, resulting in the removal of LRP5/6 receptors from the cell's membrane. Thus, Dkk binding to LRP5/6 inhibits canonical Wnt signaling and Dkk/LRP5/6 complexes with Krms, depleting the cell membrane of LRP receptors and resulting in a greater and more prolonged inhibition of Wnt signaling (Fig. 1).

Dkk3 functionally falls apart from the other Dkk proteins. In fact, Dkk3 is unable to inhibit Wnt signaling (75), and unlike Dkk1- or Dkk2-deficient mice (see bellow), Dkk3 knockout animals do not display bone phenotype (76). Dkk proteins play a distinct role during embryogenesis; for instance, Dkk1-deficient mice are not viable because of lack of lack head structures anterior of the midbrain, whereas Dkk2 and Dkk3 knockouts are viable and fertile (57, 76, 77).

Several lines of evidence demonstrate the involvement of Dkk1 in the regulation of bone formation and establish the validity of this target for therapeutic intervention for bone anabolism. Whereas reduced expression of Dkk1 in mice haploinsufficient for the Dkk1 gene results in a HBM phenotype, increased expression in transgenic mice leads to osteopenia (46, 47). This suggests that controlling Dkk1 levels or binding capacity could be used as a therapeutic strategy to increase bone mass in various bone diseases, such as postmenopausal or aging osteoporosis, osteogenesis imperfecta, or cancer-induced bone loss (this review and Refs. 59 and 78). As a matter of fact, Dkk1 antisense oligonucleotide in rats has been shown to prevent the effects of ovariectomy on mineral content, mineral density, and peak load of femurs (79). Dkk1 antisense oligonucleotide treatment affected bone metabolism by increasing osteoblast numbers and also by reducing RANKL expression, ultimately decreasing osteoclastogenesis.

Numerous tumors involve terminally differentiated plasma cells that home to the bone marrow, where they proliferate, leading to an osteolytic bone lesion (80). The expression of Dkk1 in plasma cells and the serum level of Dkk1 are positively correlated with the presence of bone lesions in patients with multiple myeloma (59). Dkk1 activity was also found to be associated with the osteolytic potential of other cancer cells such as the prostate cancer line PC-3 (81). A recent report from Shaughnessy and colleagues (82) provided the proof of concept that anti-Dkk1 therapy may be an effective adjunct in the clinical management of multiple myeloma-associated bone disease. Blocking Dkk1 activity by means of monoclonal neutralizing antibody in myelomatous bones reduces osteolytic bone resorption, increases bone formation, and helps control multiple myeloma growth. The bone-anabolic effect of anti-Dkk1 was associated with reduced multiple myeloma burden ($P < 0.04$). Interestingly, anti-Dkk1 also displayed a bone-anabolic activity as shown by a significant increase in BMD in the femur of nonmyelomatous mice (82). Thus, these studies confirmed that anti-Dkk1 treatment affects osteoblast activity and indirectly reduces osteoclast activity.

Wnt signaling is also essential in determining whether mesenchymal progenitors will become osteoblasts or chondrocytes (for review see Ref. 83). Until recently, it was thought that these mechanisms were involved only in development. However, a very recent study has demonstrated the involvement of Dkk1 in the destructive joint process in TNF-induced arthritis (58), suggesting that inflammation induces Dkk1 expression and that inhibition of Wnt signaling contributes to joint destruction.

Dkks are therefore a very attractive target for the pharmaceutical industry with applications in osteoporosis and cancer-induced bone loss as well as joint diseases. As discussed below, the key issue will remain the possibility of undesired effects outside of the skeleton.

Sclerostin (Sost)

The Sost gene product sclerostin is also a secreted Wnt antagonist, which shares homology with another Wnt an-

tagonist, Wise. Wise and sclerostin are members of the CCN family of proteins (for CTGF, Cyr6I, Nov), including Dan and Wif, which have the ability to bind BMPs and inhibit BMP signaling. However, several studies have now clearly demonstrated that sclerostin interacts with LRP5 and LRP6 to inhibit the canonical Wnt pathway (84, 85). Most importantly, the HBM LRP5 variant (LRP5G171V) and a homologous change in LRP6 (LRP6G158V) abolish the binding of sclerostin to LRP5/6 (86), strongly suggesting that the increased bone formation observed in HBM patients may be the result of decreased inhibition by endogenous sclerostin. Thus, agents that would alter the ability of sclerostin to bind to LRP5 would be expected to mimic the HBM phenotype in osteoporotic patients.

Within the group of HBM mutations are van Buchem's disease and sclerosteosis. The increase in bone density is far greater than in the HBM families, leading to significant clinical abnormalities, such as increased intracranial pressure and deafness. Genetic analysis demonstrated that sclerosteosis results from loss of function of the Sost gene product (23, 87), whereas van Buchem disease is associated with a down-regulation of the expression of the Sost gene due to a partial deletion of the regulatory region downstream of Sost (22, 24).

The most important recent finding is that sclerostin is almost exclusively expressed in osteocytes (64). It is thought that these cells, embedded in the mineralized matrix of the bone, are the main mechanosensors in bone, participating in the regulation of bone formation and the determination of bone mass and shape through bone remodeling. Although the precise physiological role of sclerostin in osteocytes is not fully elucidated, recent data have indicated that sclerostin expression decreases in the presence of mechanical loading, and also upon PTH treatment, possibly locally relieving endogenous Wnt inhibition and activating bone formation (88–91).

Sclerostin-neutralizing monoclonal antibodies have been developed and show bone-anabolic activity in mice and rats (92). In nonhuman primates, this neutralizing antibody showed a favorable pharmacokinetic profile and displayed significant anabolic activity (93), making it a serious candidate to enter clinical studies in the near future.

Summary and Future Challenges

One important conclusion of the observations made with all the different *in vivo* models described above, is that activating Wnt/β-catenin signaling positively affects bone formation, whereas deletion or down-regulation of genes encoding for agonists of the Wnt cascade results in osteopenia. Better understanding of this pathway makes it amenable to pharmacological intervention at many levels. Current therapies for osteoporosis are almost exclusively based on an antiresorptive approach, but there is a real medical need for alternative therapies based on the stimulation of the anabolic pathways in bone, the only available anabolic agent being injectable PTH. As molecules emerge from current drug discovery activities targeting the Wnt signaling pathway at different levels, investigating the tumor potential and toxicity to other tissues will be crucial. Treatment of chronic disorder such as osteoporosis requires a very stringent safety profile, and given the known involvement of Wnt signaling in certain cancer, the challenge for molecules modulating the Wnt pathway is to target bone as specifically as possible. The most appropriate way to prevent systemic stimulation of the Wnt pathway by pharmacological agents is to target Wnt bone-specific genes. From this perspective, sclerostin, and possibly Dkk1, seem to fulfill the criterion of bone-specific antagonists. Of note, however, are the facts that HBM and lithium-treated patients have not been reported to have an increased frequency of tumors, and Dkk1$^{+/-}$ mice, in which the Wnt pathway is activated and bone formation increased, have not exhibited tumors over the 2 yr since they have been generated (unpublished observation).

The formidable progress made in the past 5 yr in understanding the role of Wnt signaling in bone will undoubtedly lead to the development of therapeutics activating the Wnt pathway, possibly only in the skeleton. Indeed, several compounds are in early-stage discovery or just reaching phase I clinical trials. Inhibiting sclerostin by means of neutralizing antibodies is probably the most advanced program, but Dkk1 antibodies are not far behind.

Acknowledgments

Received February 27, 2007. Accepted March 21, 2007.

Address all correspondence and requests for reprints to: Dr. Roland Baron, Yale University School of Medicine, 333 Cedar Street, P.O. Box 208044, New Haven, Connecticut 06520-8044. E-mail: roland.baron@yale.edu.

Author Disclosure Summary: The authors have nothing to disclose.

References

1. **Aubin JE, Triffitt JT** 2002 Mesenchymal stem cells and osteoblast differentiation. In: Bilezikian JP, Raisz LG, Rodan G, eds. Principles of bone biology. Chap 4. New York: Academic Press; 59–82
2. **Khosla S** 2001 The OPG/RANKL/RANK system. Endocrinology 142:5050–5055
3. **Suda T, Takahashi N, Udagawa N, Jimi E, Gillespie MT, Martin TJ** 1999 Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. Endocr Rev 20:345–357
4. **Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL, Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P, Sander S, Van G, Tarpley J, Derby P, Lee R, Boyle WJ** 1997 Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 89:309–319
5. **Deaton DN, Tavares FX** 2005 Design of cathepsin K inhibitors for osteoporosis. Curr Top Med Chem 5:1639–1675
6. **Zaidi M, Troen B, Moonga BS, Abe E** 2001 Cathepsin K, osteoclastic resorption, and osteoporosis therapy. J Bone Miner Res 16:1747–1749
7. **Zhao C, Irie N, Takada Y, Shimoda K, Miyamoto T, Nishiwaki T, Suda T, Matsuo K** 2006 Bidirectional ephrinB2-EphB4 signaling controls bone homeostasis. Cell Metab 4:111–121
8. **Chen D, Zhao M, Mundy GR** 2004 Bone morphogenetic proteins. Growth Factors 22:233–241
9. **Chung U-i, Schipani E, McMahon AP, Kronenberg HM** 2001 Indian hedgehog couples chondrogenesis to osteogenesis in endochondral bone development. J Clin Invest 107:295–304
10. **Long F, Chung U-i, Ohba S, McMahon J, Kronenberg HM, McMahon AP** 2004 Ihh signaling is directly required for the osteoblast lineage in the endochondral skeleton. Development 131:1309–1318
11. **Wan M, Cao X** 2005 BMP signaling in skeletal development. Biochem Biophys Res Commun 328:651–657
12. **Ducy P, Zhang R, Geoffroy V, Ridall AL, Karsenty G** 1997 Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. Cell 89:747–754
13. **Otto F, Thornell AP, Crompton T, Denzel A, Gilmour KC, Rosewell IR, Stamp GW, Beddington RS, Mundlos S, Olsen BR, Selby PB, Owen MJ** 1997 Cbfa1, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development. Cell 89:765–771
14. **Mundlos S, Otto F, Mundlos C, Mulliken JB, Aylsworth AS, Albright S, Lindhout D, Cole WG, Henn W, Knoll JH, Owen MJ, Mertelsmann R, Zabel**

BU, Olsen BR 1997 Mutations involving the transcription factor CBFA1 cause cleidocranial dysplasia. Cell 89:773–779
15. **Nakashima K, Zhou X, Kunkel G, Zhang Z, Deng JM, Behringer RR, de Crombrugghe B** 2002 The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. Cell 108:17–29
16. **Mundlos S** 1999 Cleidocranial dysplasia: clinical and molecular genetics. J Med Genet 36:177–182
17. **Doecke JD, Day CJ, Stephens AS, Carter SL, van Daal A, Kotowicz MA, Nicholson GC, Morrison NA** 2006 Association of functionally different RUNX2 P2 promoter alleles with BMD. J Bone Miner Res 21:265–273
18. **Vaughan T, Pasco JA, Kotowicz MA, Nicholson GC, Morrison NA** 2002 Alleles of RUNX2/CBFA1 gene are associated with differences in bone mineral density and risk of fracture. J Bone Miner Res 17:1527–1534
19. **Boyden LM, Mao J, Belsky J, Mitzner L, Farhi A, Mitnick MA, Wu D, Insogna K, Lifton RP** 2002 High bone density due to a mutation in LDL-receptor-related protein 5. N Engl J Med 346:1513–1521
20. **Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Reginato AM, Wang H, Cundy T, Glorieux FH, Lev D, Zacharin M, Oexle K, Marcelino J, Suwairi W, Heeger S, Sabatakos G, Apte S, Adkins WN, Allgrove J, Arslan-Kirchner M, Batch JA, Beighton P, Black GC, Boles RG, Boon LM, Borrone C, Brunner HG, Carle GF, Dallapiccola B, De Paepe A, Floege B, Halfhide ML, Hall B, Hennekam RC, Hirose T, Jans A, Juppner H, Kim CA, Keppler-Noreuil K, Kohlschuetter A, LaCombe D, Lambert M, Lemyre E, Letteboer T, Peltonen L, Ramesar RS, Romanengo M, Somer H, Steichen-Gersdorf E, Steinmann B, Sullivan B, Superti-Furga A, Swoboda W, van den Boogaard MJ, Van Hul W, Vikkula M, Votruba M, Zabel B, Garcia T, Baron R, Olsen BR, Warman ML** 2001 LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. Cell 107:513–523
21. **Little RD, Carulli JP, Del Mastro RG, Dupuis J, Osborne M, Folz C, Manning SP, Swain PM, Zhao S, Eustace B, Lappe MM, Spitzer L, Zweier S, Braunschweiger K, Benchekroun Y, Hu X, Adair R, Chee L, FitzGerald MG, Tulig C, Caruso A, Tzellas N, Bawa A, Franklin B, McGuire S, Nogues X, Gong G, Allen KM, Anisowicz A, Morales AJ, Lomedico PT, Recker SM, Eerdewegh PV, Recker RR, Johnson ML** 2002 A mutation in the LDL receptor-related protein 5 gene results in the autosomal dominant high-bone-mass trait. Am J Hum Genet 70:11–19
22. **Staehling-Hampton K, Proll S, Paeper BW, Zhao L, Charmley P, Brown A, Gardner JC, Galas D, Schatzman RC, Beighton P, Papapoulos S, Hamersma H, Brunkow ME** 2002 A 52-kb deletion in the SOST-MEOX1 intergenic region on 17q12–q21 is associated with van Buchem disease in the Dutch population. Am J Med Genet 110:144–152
23. **Balemans W, Ebeling M, Patel N, Van Hul E, Olson P, Dioszegi M, Lacza C, Wuyts W, Van Den Ende J, Willems P, Paes-Alves AF, Hill S, Bueno M, Ramos FJ, Tacconi P, Dikkers FG, Stratakis C, Lindpaintner K, Vickery B, Foernzler D, Van Hul W** 2001 Increased bone density in sclerosteosis is due to the deficiency of a novel secreted protein (SOST). Hum Mol Genet 10:537–543
24. **Balemans W, Patel N, Ebeling M, Van Hul E, Wuyts W, Lacza C, Dioszegi M, Dikkers FG, Hildering P, Willems PJ, Verheij JB, Lindpaintner K, Vickery B, Foernzler D, Van Hul W** 2002 Identification of a 52 kb deletion downstream of the SOST gene in patients with van Buchem disease. J Med Genet 39:91–97
25. **Clevers H** 2006 Wnt/β-catenin signaling in development and disease. Cell 127:469–480
26. **Wehrli M, Dougan ST, Caldwell K, O'Keefe L, Schwartz S, Vaizel-Ohayon D, Schejter E, Tomlinson A, DiNardo S** 2000 *arrow* encodes an LDL-receptor-related protein essential for Wingless signalling. Nature [Erratum (2001) 410: 847] 407:527–530
27. **Jeon H, Meng W, Takagi J, Eck MJ, Springer TA, Blacklow SC** 2001 Implications for familial hypercholesterolemia from the structure of the LDL receptor YWTD-EGF domain pair. Nat Struct Biol 8:499–504
28. **Zeng X, Tamai K, Doble B, Li S, Huang H, Habas R, Okamura H, Woodgett J, He X** 2005 A dual-kinase mechanism for Wnt co-receptor phosphorylation and activation. Nature 438:873–877
29. **Davidson G, Wu W, Shen J, Bilic J, Fenger U, Stannek P, Glinka A, Niehrs C** 2005 Casein kinase 1γ couples Wnt receptor activation to cytoplasmic signal transduction. Nature 438:867–872
30. **Hay E, Faucheu C, Suc-Royer I, Touitou R, Stiot V, Vayssiere B, Baron R, Roman-Roman S, Rawadi G** 2005 Interaction between LRP5 and Frat1 mediates the activation of the Wnt canonical pathway. J Biol Chem 280:13616–13623
31. **Chen HJ, Lin CM, Lin CS, Perez-Olle R, Leung CL, Liem RK** 2006 The role of microtubule actin cross-linking factor 1 (MACF1) in the Wnt signaling pathway. Genes Dev 20:1933–1945
32. **Kato M, Patel MS, Levasseur R, Lobov I, Chang BH, Glass 2nd DA, Hartmann C, Li L, Hwang TH, Brayton CF, Lang RA, Karsenty G, Chan L** 2002 Cbfa1-independent decrease in osteoblast proliferation, osteopenia, and persistent embryonic eye vascularization in mice deficient in Lrp5, a Wnt coreceptor. J Cell Biol 157:303–314
33. **Wei W, Lu Q, Chaudry GJ, Leppla SH, Cohen SN** 2006 The LDL receptor-related protein LRP6 mediates internalization and lethality of anthrax toxin. Cell 124:1141–1154
34. **Van Wesenbeeck L, Cleiren E, Gram J, Beals RK, Benichou O, Scopelliti D, Key L, Renton T, Bartels C, Gong Y, Warman ML, De Vernejoul MC, Bollerslev J, Van Hul W** 2003 Six novel missense mutations in the LDL receptor-related protein 5 (LRP5) gene in different conditions with an increased bone density. Am J Hum Genet 72:763–771
35. **Kiel DP, Ferrari SL, Cupples LA, Karasik D, Manen D, Imamovic A, Herbert AG, Dupuis J** 2006 Genetic variation at the low-density lipoprotein receptor-related protein 5 (LRP5) locus modulates Wnt signaling and the relationship of physical activity with bone mineral density in men. Bone 40:587–596
36. **Koller DL, Ichikawa S, Johnson ML, Lai D, Xuei X, Edenberg HJ, Conneally PM, Hui SL, Johnston CC, Peacock M, Foroud T, Econs MJ** 2005 Contribution of the LRP5 gene to normal variation in peak BMD in women. J Bone Miner Res 20:75–80
37. **Ferrari SL, Deutsch S, Antonarakis SE** 2005 Pathogenic mutations and polymorphisms in the lipoprotein receptor-related protein 5 reveal a new biological pathway for the control of bone mass. Curr Opin Lipidol 16:207–214
38. **Hartikka H, Makitie O, Mannikko M, Doria AS, Daneman A, Cole WG, Ala-Kokko L, Sochett EB** 2005 Heterozygous mutations in the LDL receptor-related protein 5 (LRP5) gene are associated with primary osteoporosis in children. J Bone Miner Res 20:783–789
39. **Urano T, Shiraki M, Ezura Y, Fujita M, Sekine E, Hoshino S, Hosoi T, Orimo H, Emi M, Ouchi Y, Inoue S** 2004 Association of a single-nucleotide polymorphism in low-density lipoprotein receptor-related protein 5 gene with bone mineral density. J Bone Miner Metab 22:341–345
40. **Clement-Lacroix P, Ai M, Morvan F, Roman-Roman S, Vayssiere B, Belleville C, Estrera K, Warman ML, Baron R, Rawadi G** 2005 Lrp5-independent activation of Wnt signaling by lithium chloride increases bone formation and bone mass in mice. Proc Natl Acad Sci USA 102:17406–17411
41. **Babij P, Zhao W, Small C, Kharode Y, Yaworsky PJ, Bouxsein ML, Reddy PS, Bodine PV, Robinson JA, Bhat B, Marzolf J, Moran RA, Bex F** 2003 High bone mass in mice expressing a mutant LRP5 gene. J Bone Miner Res 18:960–974
42. **Bennett CN, Longo KA, Wright WS, Suva LJ, Lane TF, Hankenson KD, MacDougald OA** 2005 Regulation of osteoblastogenesis and bone mass by Wnt10b. Proc Natl Acad Sci USA 102:3324–3329
43. **Bodine PV, Zhao W, Kharode YP, Bex FJ, Lambert AJ, Goad MB, Gaur T, Stein GS, Lian JB, Komm BS** 2004 The Wnt antagonist secreted frizzled-related protein-1 is a negative regulator of trabecular bone formation in adult mice. Mol Endocrinol 18:1222–1237
44. **Glass 2nd DA, Bialek P, Ahn JD, Starbuck M, Patel MS, Clevers H, Taketo MM, Long F, McMahon AP, Lang RA, Karsenty G** 2005 Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. Dev Cell 8:751–764
45. **Holmen SL, Zylstra CR, Mukherjee A, Sigler RE, Faugere MC, Bouxsein ML, Deng L, Clemens TL, Williams BO** 2005 Essential role of β-catenin in postnatal bone acquisition. J Biol Chem 280:21162–21168
46. **Morvan F, Boulukos K, Clement-Lacroix P, Roman Roman S, Suc-Royer I, Vayssiere B, Ammann P, Martin P, Pinho S, Pognonec P, Mollat P, Niehrs C, Baron R, Rawadi G** 2006 Deletion of a single allele of the Dkk1 gene leads to an increase in bone formation and bone mass. J Bone Miner Res 21:934–945
47. **Li J, Sarosi I, Cattley RC, Pretorius J, Asuncion F, Grisanti M, Morony S, Adamu S, Geng Z, Qiu W, Kostenuik P, Lacey DL, Simonet WS, Bolon B, Qian X, Shalhoub V, Ominsky MS, Zhu Ke H, Li X, Richards WG** 2006 Dkk1-mediated inhibition of Wnt signaling in bone results in osteopenia. Bone 39:754–766
48. **Nakanishi T, Yamaai T, Asano M, Nawachi K, Suzuki M, Sugimoto T, Takigawa M** 2001 Overexpression of connective tissue growth factor/hypertrophic chondrocyte-specific gene product 24 decreases bone density in adult mice and induces dwarfism. Biochem Biophys Res Commun 281:678–681
49. **Cao J, Morony S, Warmington K, Pretorius J, McDorman K, Paszty C** 2004 Transgenic overexpression of WIF-1, a secreted Wnt antagonist expressed in bone, causes decreased bone mineral density and increased susceptibility to bone fracture in mice: a role for WIF-1 in bone biology. J Bone Miner Res 19:S55
50. **Hill TP, Taketo MM, Birchmeier W, Hartmann C** 2006 Multiple roles of mesenchymal β-catenin during murine limb patterning. Development 133: 1219–1229
51. **Hill TP, Spater D, Taketo MM, Birchmeier W, Hartmann C** 2005 Canonical Wnt/β-catenin signaling prevents osteoblasts from differentiating into chondrocytes. Dev Cell 8:727–738
52. **Day TF, Guo X, Garrett-Beal L, Yang Y** 2005 Wnt/β-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. Dev Cell 8:739–750
53. **Rawadi G, Vayssiere B, Dunn F, Baron R, Roman-Roman S** 2003 BMP-2 controls alkaline phosphatase expression and osteoblast mineralization by a Wnt autocrine loop. J Bone Miner Res 18:1842–1853
54. **Kalajzic I, Staal A, Yang WP, Wu Y, Johnson SE, Feyen JH, Krueger W, Maye P, Yu F, Zhao Y, Kuo L, Gupta RR, Achenie LE, Wang HW, Shin DG, Rowe DW** 2005 Expression profile of osteoblast lineage at defined stages of differentiation. J Biol Chem 280:24618–24626
55. **Roman-Roman S, Garcia T, Jackson A, Theilhaber J, Rawadi G, Connolly T,**

Spinella-Jaegle S, Kawai S, Courtois B, Bushnell S, Auberval M, Call K, Baron R 2003 Identification of genes regulated during osteoblastic differentiation by genome-wide expression analysis of mouse calvaria primary osteoblasts in vitro. Bone 32:474–482
56. **Vaes BL, Dechering KJ, van Someren EP, Hendriks JM, van de Ven CJ, Feijen A, Mummery CL, Reinders MJ, Olijve W, van Zoelen EJ, Steegenga WT** 2005 Microarray analysis reveals expression regulation of Wnt antagonists in differentiating osteoblasts. Bone 36:803–811
57. **Li X, Liu P, Liu W, Maye P, Zhang J, Zhang Y, Hurley M, Guo C, Boskey A, Sun L, Harris SE, Rowe DW, Ke HZ, Wu D** 2005 Dkk2 has a role in terminal osteoblast differentiation and mineralized matrix formation. Nat Genet 37: 945–952
58. **Diarra D, Stolina M, Polzer K, Zwerina J, Ominsky MS, Dwyer D, Korb A, Smolen J, Hoffmann M, Scheinecker C, van der Heide D, Landewe R, Lacey D, Richards WG, Schett G** 2007 Dickkopf-1 is a master regulator of joint remodeling. Nat Med 13:156–163
59. **Tian E, Zhan F, Walker R, Rasmussen E, Ma Y, Barlogie B, Shaughnessy Jr JD** 2003 The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. N Engl J Med 349:2483–2494
60. **Harwood AJ** 2001 Regulation of GSK-3: a cellular multiprocessor. Cell 105: 821–824
61. **Doble BW, Woodgett JR** 2003 GSK-3: tricks of the trade for a multi-tasking kinase. J Cell Sci 116:1175–1186
62. **Vestergaard P, Rejnmark L, Mosekilde L** 2005 Reduced relative risk of fractures among users of lithium. Calcif Tissue Int 77:1–8
63. **Kulkarni NH, Onyia JE, Zeng Q, Tian X, Liu M, Halladay DL, Frolik CA, Engler T, Wei T, Kriauciunas A, Martin TJ, Sato M, Bryant HU, Ma YL** 2006 Orally bioavailable GSK-3α/β dual inhibitor increases markers of cellular differentiation in vitro and bone mass in vivo. J Bone Miner Res 21:910–920
64. **Poole KE, van Bezooijen RL, Loveridge N, Hamersma H, Papapoulos SE, Lowik CW, Reeve J** 2005 Sclerostin is a delayed secreted product of osteocytes that inhibits bone formation. FASEB J 19:1842–1844
65. **Holmen SL, Robertson SA, Zylstra CR, Williams BO** 2005 Wnt-independent activation of β-catenin mediated by a Dkk1-Fz5 fusion protein. Biochem Biophys Res Commun 328:533–539
66. **Bafico A, Liu G, Yaniv A, Gazit A, Aaronson SA** 2001 Novel mechanism of Wnt signalling inhibition mediated by Dickkopf-1 interaction with LRP6/Arrow. Nat Cell Biol 3:683–686
67. **Brott BK, Sokol SY** 2002 Regulation of Wnt/LRP signaling by distinct domains of Dickkopf proteins. Mol Cell Biol 22:6100–6110
68. **Mao B, Wu W, Li Y, Hoppe D, Stannek P, Glinka A, Niehrs C** 2001 LDL-receptor-related protein 6 is a receptor for Dickkopf proteins. Nature 411:321–325
69. **Semenov MV, Tamai K, Brott BK, Kuhl M, Sokol S, He X** 2001 Head inducer Dickkopf-1 is a ligand for Wnt coreceptor LRP6. Curr Biol 11:951–961
70. **Li L, Mao J, Sun L, Liu W, Wu D** 2002 Second cysteine-rich domain of Dickkopf-2 activates canonical Wnt signaling pathway via LRP-6 independently of dishevelled. J Biol Chem 277:5977–5981
71. **Gregory CA, Perry AS, Reyes E, Conley A, Gunn WG, Prockop DJ** 2005 Dkk-1-derived synthetic peptides and lithium chloride for the control and recovery of adult stem cells from bone marrow. J Biol Chem 280:2309–2323
72. **Davidson G, Mao B, del Barco Barrantes I, Niehrs C** 2002 Kremen proteins interact with Dickkopf1 to regulate anteroposterior CNS patterning. Development 129:5587–5596
73. **Mao B, Wu W, Davidson G, Marhold J, Li M, Mechler BM, Delius H, Hoppe D, Stannek P, Walter C, Glinka A, Niehrs C** 2002 Kremen proteins are Dickkopf receptors that regulate Wnt/β-catenin signalling. Nature 417:664–667
74. **Mao B, Niehrs C** 2003 Kremen2 modulates Dickkopf2 activity during Wnt/LRP6 signaling. Gene 302:179–183
75. **Krupnik VE, Sharp JD, Jiang C, Robison K, Chickering TW, Amaravadi L, Brown DE, Guyot D, Mays G, Leiby K, Chang B, Duong T, Goodearl AD, Gearing DP, Sokol SY, McCarthy SA** 1999 Functional and structural diversity of the human Dickkopf gene family. Gene 238:301–313
76. **Barrantes Idel B, Montero-Pedrazuela A, Guadano-Ferraz A, Obregon MJ, Martinez de Mena R, Gailus-Durner V, Fuchs H, Franz TJ, Kalaydjiev S, Klempt M, Holter S, Rathkolb B, Reinhard C, Morreale de Escobar G, Bernal J, Busch DH, Wurst W, Wolf E, Schulz H, Shtrom S, Greiner E, Hrabe de Angelis M, Westphal H, Niehrs C** 2006 Generation and characterization of dickkopf3 mutant mice. Mol Cell Biol 26:2317–2326
77. **Mukhopadhyay M, Shtrom S, Rodriguez-Esteban C, Chen L, Tsukui T, Gomer L, Dorward DW, Glinka A, Grinberg A, Huang SP, Niehrs C, Belmonte JC, Westphal H** 2001 Dickkopf1 is required for embryonic head induction and limb morphogenesis in the mouse. Dev Cell 1:423–434
78. **Roodman GD** 2006 New potential targets for treating myeloma bone disease. Clin Cancer Res 12:6270s–6273s
79. **Wang FS, Ko JY, Lin CL, Wu HL, Ke HJ, Tai PJ** 2006 Knocking down dickkopf-1 alleviates estrogen deficiency induction of bone loss. A histomorphological study in ovariectomized rats. Bone 40:485–492
80. **Guise TA, Mohammad KS, Clines G, Stebbins EG, Wong DH, Higgins LS, Vessella R, Corey E, Padalecki S, Suva L, Chirgwin JM** 2006 Basic mechanisms responsible for osteolytic and osteoblastic bone metastases. Clin Cancer Res 12:6213s–6216s
81. **Hall CL, Bafico A, Dai J, Aaronson SA, Keller ET** 2005 Prostate cancer cells promote osteoblastic bone metastases through Wnts. Cancer Res 65:7554–7560
82. **Yaccoby S, Ling W, Zhan F, Walker R, Barlogie B, Shaughnessy Jr JD** 2006 Antibody-based inhibition of DKK1 suppresses tumor-induced bone resorption and multiple myeloma growth in-vivo. Blood 109:2906–2911
83. **Pacifici M, Koyama E, Shibukawa Y, Wu C, Tamamura Y, Enomoto-Iwamoto M, Iwamoto M** 2006 Cellular and molecular mechanisms of synovial joint and articular cartilage formation. Ann NY Acad Sci 1068:74–86
84. **Ellies DL, Viviano B, McCarthy J, Rey JP, Itasaki N, Saunders S, Krumlauf R** 2006 Bone density ligand, Sclerostin, directly interacts with LRP5 but not LRP5G171V to modulate Wnt activity. J Bone Miner Res 21:1738–1749
85. **Semenov M, Tamai K, He X** 2005 SOST is a ligand for LRP5/LRP6 and a Wnt signaling inhibitor. J Biol Chem 280:26770–26775
86. **Semenov MV, He X** 2006 LRP5 mutations linked to high bone mass diseases cause reduced LRP5 binding and inhibition by SOST. J Biol Chem 281:38276–38284
87. **Brunkow ME, Gardner JC, Van Ness J, Paeper BW, Kovacevich BR, Proll S, Skonier JE, Zhao L, Sabo PJ, Fu Y, Alisch RS, Gillett L, Colbert T, Tacconi P, Galas D, Hamersma H, Beighton P, Mulligan J** 2001 Bone dysplasia sclerosteosis results from loss of the SOST gene product, a novel cystine knot-containing protein. Am J Hum Genet 68:577–589
88. **Bellido T, Ali AA, Gubrij I, Plotkin LI, Fu Q, O'Brien CA, Manolagas SC, Jilka RL** 2005 Chronic elevation of parathyroid hormone in mice reduces expression of sclerostin by osteocytes: a novel mechanism for hormonal control of osteoblastogenesis. Endocrinology 146:4577–4583
89. **Keller H, Kneissel M** 2005 SOST is a target gene for PTH in bone. Bone 37:148–158
90. **Robling AG, Bellido T, Turner CH** 2006 Mechanical stimulation in vivo reduces osteocyte expression of sclerostin. J Musculoskelet Neuronal Interact 6:354
91. **Winkler DG, Sutherland MK, Geoghegan JC, Yu C, Hayes T, Skonier JE, Shpektor D, Jonas M, Kovacevich BR, Staehling-Hampton K, Appleby M, Brunkow ME, Latham JA** 2003 Osteocyte control of bone formation via sclerostin, a novel BMP antagonist. EMBO J 22:6267–6276
92. **Ominsky M, Warmington KS, Asuncion FJ, Tan HL, Grisanti MS, Geng Z, Stephens P, Henry A, Lawson A, Lightwood D, Perkins V, Kirby P, Moore A, Popplewell A, Robinson M, Li X, Kostenuik PJ, Simonet WS, Lacey DL, Paszty C** 2006 Sclerostin monoclonal antibody treatment increases bone strength in aged osteopenic ovariectomized rats. J Bone Miner Res 21:S44
93. **Ominsky M, Stouch B, Doellgast G, Gong J, Gao Y, Haldankar R, Winters A, Chen Q, Graham K, Zhou L, Hale M, Henry A, Lightwood D, Moore A, Popplewell A, Robinson M, Vlasseros F, Jolette J, Smith SY, Kostenuik PJ, Simonet WS, Lacey DL, Paszty C** 2006 Administration of sclerostin monoclonal antibodies to female Cynomologous monkeys results in increased bone formation, bone mineral density, and bone strength. J Bone Miner Res 21:S44

Endocrinology **is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**