REVIEW

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WNT signaling in bone homeostasis and disease: from human mutations to treatments

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Low bone mass and strength lead to fragility fractures, for example, in elderly individuals affected by osteoporosis or children with osteogenesis imperfecta. A decade ago, rare human mutations affecting bone negatively (osteoporosis-pseudoglioma syndrome) or positively (high–bone mass phenotype, sclerosteosis and Van Buchem disease) have been identified and found to all reside in components of the canonical WNT signaling machinery. Mouse genetics confirmed the importance of canonical Wnt signaling in the regulation of bone homeostasis, with activation of the pathway leading to increased, and inhibition leading to decreased, bone mass and strength. The importance of WNT signaling for bone has also been highlighted since then in the general population in numerous genome-wide association studies. The pathway is now the target for therapeutic intervention to restore bone strength in millions of patients at risk for fracture. This paper reviews our current understanding of the mechanisms by which WNT signalng regulates bone homeostasis.

Genetics of bone mass regulation by WNT signaling

Osteoporosis affects hundreds of millions of people worldwide, particularly postmenopausal women and older men. It is a disease in which bone density and strength decrease to a point where the skeleton cannot adequately perform its support function, predisposing it to fractures associated with substantial morbidity and mortality. Moreover, although osteoporosis is the most widespread, an array of additional high bone–fragility conditions exists, including disuse osteopenia, renal osteodystrophy and osteogenesis imperfecta. The medical community has therefore been on the lookout for many years for agents that could, unlike established antiresorptive bone drugs, not only stop bone loss but also stimulate the formation of new bone to restore bone density and strength. Whereas osteoanabolic treatment with parathyroid hormone (PTH) is already available, the best path to this therapeutic goal, which could ultimately benefit a vast number of people, may paradoxically come from a handful of patients affected by an array of genetic mutations.

Indeed, a decade ago and within the same year, mutations causing severe alterations in bone density were identified in four groups of patients, three with high bone mass and one with low bone mass, all pointing to the same pathway: the canonical branch of WNT signaling (**Fig. 1**). The WNT signaling pathway had previously emerged as a key regulator of not only developmental processes[1,2], including skeletal patterning, but also postnatal health and disease[3–5]. Interestingly, two of the four mutations causing grossly altered bone mass and density were found to occur in *LRP5*, encoding for the WNT co-receptor low density lipoprotein receptor–related protein 5, with a loss-of-function mutation in this gene being associated with low bone mass in osteoporosis-pseudoglioma syndrome (OPPG; MIM259770)[6] and a gain-of-function mutation being associated with high bone mass in otherwise healthy patients[7,8]. The other two mutations affected expression of a common gene, *SOST*, which encodes for sclerostin, a secreted antagonist to WNT signaling that binds LRP5 and the related LRP4 and LRP6 receptors and is secreted primarily from osteocytes[9,10]. Lack of sclerostin expression in bone was found to be the cause for high bone mass in sclerosteosis (MIM269500)[11] and Van Buchem disease (VBD; MIM239100)[12,13]. Notably, the high bone mass–inducing mutations in *LRP5* decrease the binding of sclerostin[14,15] and another WNT inhibitor, dickkopf 1 (DKK1) (refs. 8,14,16), thus confirming the strong link between WNT signaling and bone homeostasis. These findings, all within the last decade, sparked tremendous interest in both academic and industrial settings to explore and possibly exploit the role of WNT signaling in bone, generating a large number of mouse and human genetic studies, all of which confirmed the important role of this pathway in bone biology and disease. Here we provide an overview of what is known so far and how this evidence is being used to develop new therapeutic approaches to treat osteoporosis and other diseases with low bone mass and increased bone fragility.

WNT signaling in bone homeostasis

WNT signaling has been historically subdivided into three major branches: the WNT–β-catenin pathway, also termed the canonical WNT pathway[17], the noncanonical WNT–planar cell polarity (WNT-PCP)[18] pathway and the WNT-calcium (WNT-Ca^{2+}) pathway[19],

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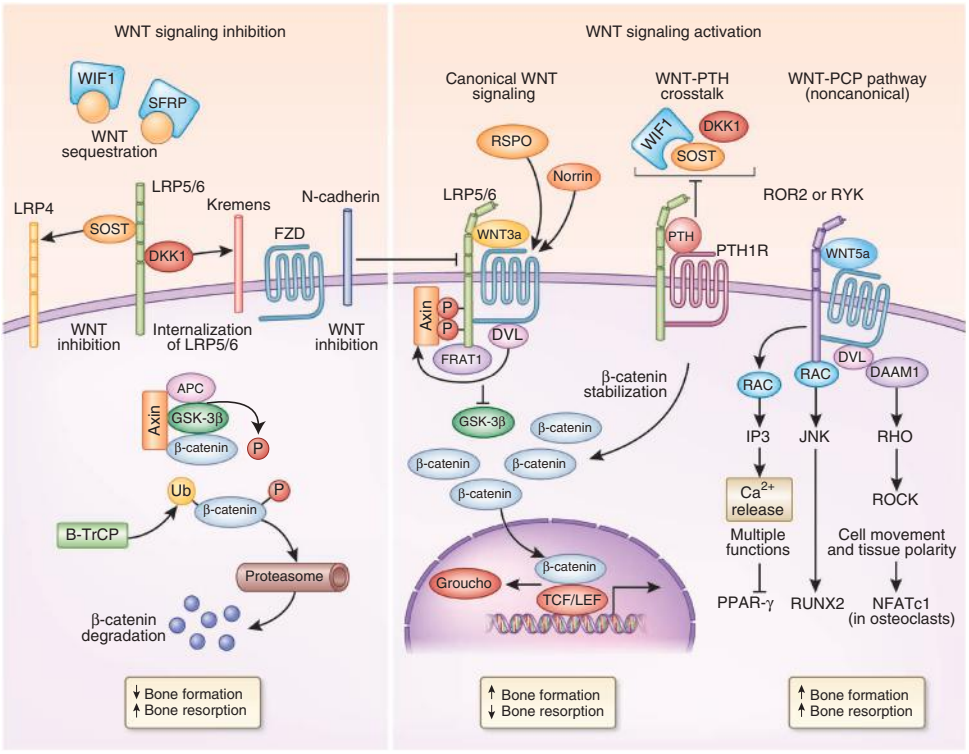


Figure 1 WNT signaling: a simplified view. In the absence of WNT, the amounts of β-catenin are low, except in adherens junctions, because of its constitutive targeting by a multiprotein destruction complex (left). The tumor suppressors axin and adenomatous polyposis (APC) bring β-catenin to GSK-3β and casein kinase 1 (CK1) (not shown), resulting in its phosphorylation (Ps in circles) at specific serine/threonine residues (left). Phosphorylated β-catenin is then targeted for polyubiquitination (Ub) (predominantly by the E3 ligase B-TrCP) and proteosomal destruction. T cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors are repressed by Groucho in the nucleus. Binding of canonical WNT ligands to a dual receptor complex comprising the WNT co-receptors LRP5 or LRP6 (LRP5/6) and one of the seven transmembrane receptors of the FZD family (right) initiates WNT–β-catenin signaling. Axin moves to the LRP5/6 tail at the membrane through its interaction with dishevelled (DVL, also called DSH), which is recruited by FZD (right). This forms a complex that also includes FRAT1 and GSK-3β, which prevents phosphorylation of β-catenin and its proteosomal degradation. β-catenin accumulates in the cytoplasm and translocates into the nucleus, where it associates with members of the TCF/LEF transcription factors while displacing Groucho to control target gene transcription. WNT signaling is modulated not only through fine tuning by a large number of WNT ligands and RSPO proteins and norrin (right) but also by extracellular antagonists such as DKK1, SOST and Wise, which bind LRP5/6 (left). Their antagonism is mediated or enhanced by receptors such as Kremen proteins and LRP4. In addition to sequestering β-catenin, N-cadherin also inhibits WNT–β-catenin signaling by interacting with LRP5 (left to right). In contrast, secreted frizzled-related proteins (SFRPs) and WNT inhibitory factor 1 (WIF1), which have ligand specificity, inhibit WNT signaling by directly sequestering WNT ligands and inhibiting both canonical and noncanonical WNT signaling (left). The PTH1 receptor can also activate the pathway in the absence of WNT ligands by forming a complex with LRP5/6 after PTH binding (right). In the WNT-PCP pathway (right), WNT binding to FZD also recruits DVL, which forms a complex with dishevelled associated activator of morphogenesis 1 (DAAM1) to trigger activation of the small G protein RHO, which in turn activates RHO-associated kinase (ROCK). Alternatively, DVL forms a complex with RAC, resulting in Jun kinase (JNK) activity. The WNT-Ca^{2+} pathway is activated by WNT5a binding to FZD and receptor-tyrosine-kinase-like orphan receptor (ROR). Intracellular calcium concentrations increase after WNT-induced coupled G protein activation of phospholipase C (PLC), resulting in dystroglycan 1 (DAG) and inositol 1,4,5-trisphosphate, type 3 (IP3) generation and cyclic GMP (cGMP)-specific phosphodiesterase (PDE) decreasing the amount of cGMP. NFATc1, nuclear factor of activated T cells, cytoplasmic, calcineurin dependent 1; PPAR-γ, peroxisome proliferator activated receptor-γ.

although as knowledge of WNT signaling increases, it becomes evident that this representation is an oversimplification. As the expanding universe of WNT signaling is reviewed elsewhere[20,21], we focus here on how the various components relate to bone metabolism. The ubiquitous nature of the WNT system and the complex regulation it is subjected to could have made it very difficult to unravel its role in bone biology and disease. However, the fact that the canonical pathway emerged as the predominant component of WNT signaling affecting bone cells has allowed the field to put together a relatively clear picture of the mechanisms by which WNT affects the skeleton.

In essence, canonical signaling (**Fig. 1**) is initiated by the binding of WNT ligands to the dual receptor complex comprised of frizzled (FZD) and either LRP5 or LRP6. This results in inactivation of the multiprotein β-catenin 'destruction complex', thus relieving the central signaling mediator β-catenin from its constitutive proteosomal degradation. β-catenin subsequently accumulates in the cytoplasm and translocates into the nucleus, where it associates with transcription factors to control target gene transcription. Importantly, all the mutations mentioned above that affect bone mass specifically target canonical WNT signaling. However, there is also increasing evidence for roles of noncanonical WNT signaling (**Fig. 1**) in bone and crosstalk between the pathways[22]. All branches of the WNT system are subjected to regulation through multiple ligands, extracellular modulators and receptors, as well as tight control by an array of extracellular inhibitors. Since the discovery of the crucial role of this pathway in human bone homeostasis, the list of pathway members that have been found to be involved in bone metabolism (**Fig. 2**) and skeletogenesis (**Box 1**) continues to increase.

WNT signaling is crucial to all bone cell types and affects hematopoiesis. The adult skeleton contains three major cell types, two of mesenchymal origin (osteoblasts and osteocytes, the latter derived from osteoblasts) and one of hematopoietic origin (osteoclasts). In general terms, osteoblasts form bone, whereas osteoclasts resorb bone, and osteocytes maintain bone and contribute to the regulation of osteoblasts and osteoclasts during bone modeling and remodeling (**Box 2**). The activity of bone cells is tightly coordinated through endocrine, paracrine, autocrine and mechanosensing signals to ensure the systemic balance of calcium-phosphate metabolism while maintaining the homeostasis and microarchitecture of bone that is

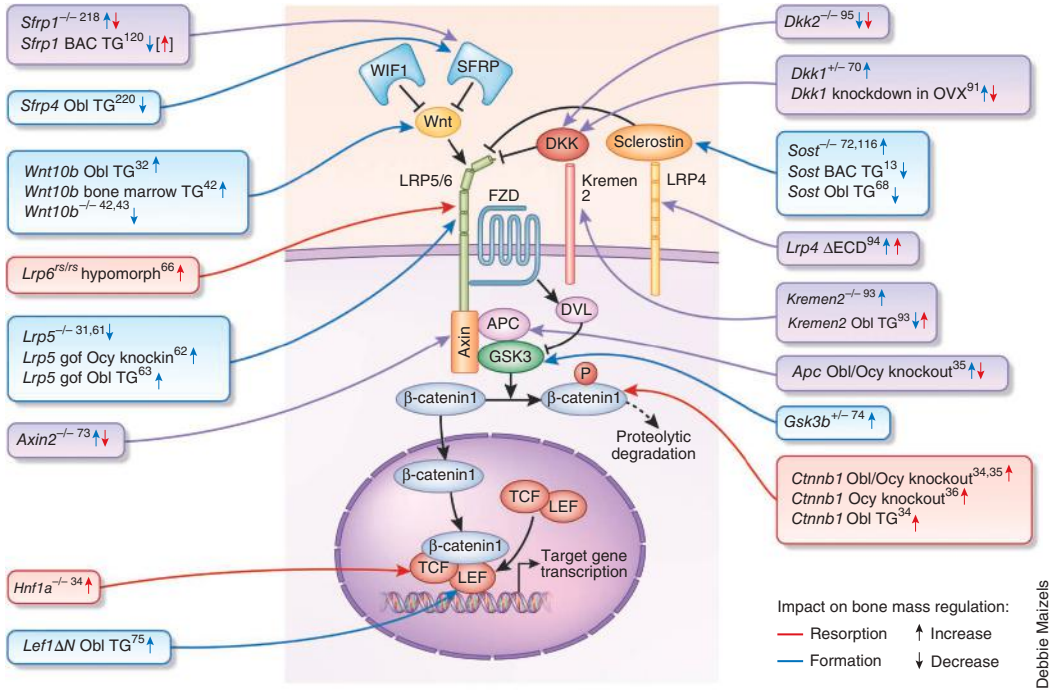


Figure 2 WNT signaling pathway members regulate bone mass: lessons from mouse genetics. Across all studies, increases in bone mass were observed as a result of pathway activation, and decreases in bone mass were observed as a result of pathway inhibition, although the relative impact on bone formation and resorption varied, reflecting the complex fine tuning of the WNT regulatory network. Alteration in the gene dosage of members of the pathway altered bone formation and resorption to varying degrees. Obl, osteoblast; Ocy, osteocyte; Obl/Ocy, osteoblast and osteocyte; TG, transgenic; gof, gain-of-function; OVX, ovariectomy; ECD, extracellular domain; Δ, deletion; BAC, bacterial artificial chromosome. Blue and red boxes indicate an effect on formation and resorption, respectively, and purple boxes indicate an effect on both. The direction of the arrows in the text boxes indicates an increase or decrease of bone resorption (red) or bone formation (blue).

adapted to biomechanical needs[23]. Moreover the interaction of bone with bone marrow is crucial to hematopoiesis and hematopoietic stem cell (HSC) function[24]. Proper maintenance of the skeleton may also be relevant for other endocrine functions of bone beyond its involvement in mineral homeostasis, such as contribution to glucose and energy metabolism[25] and fertility[26].

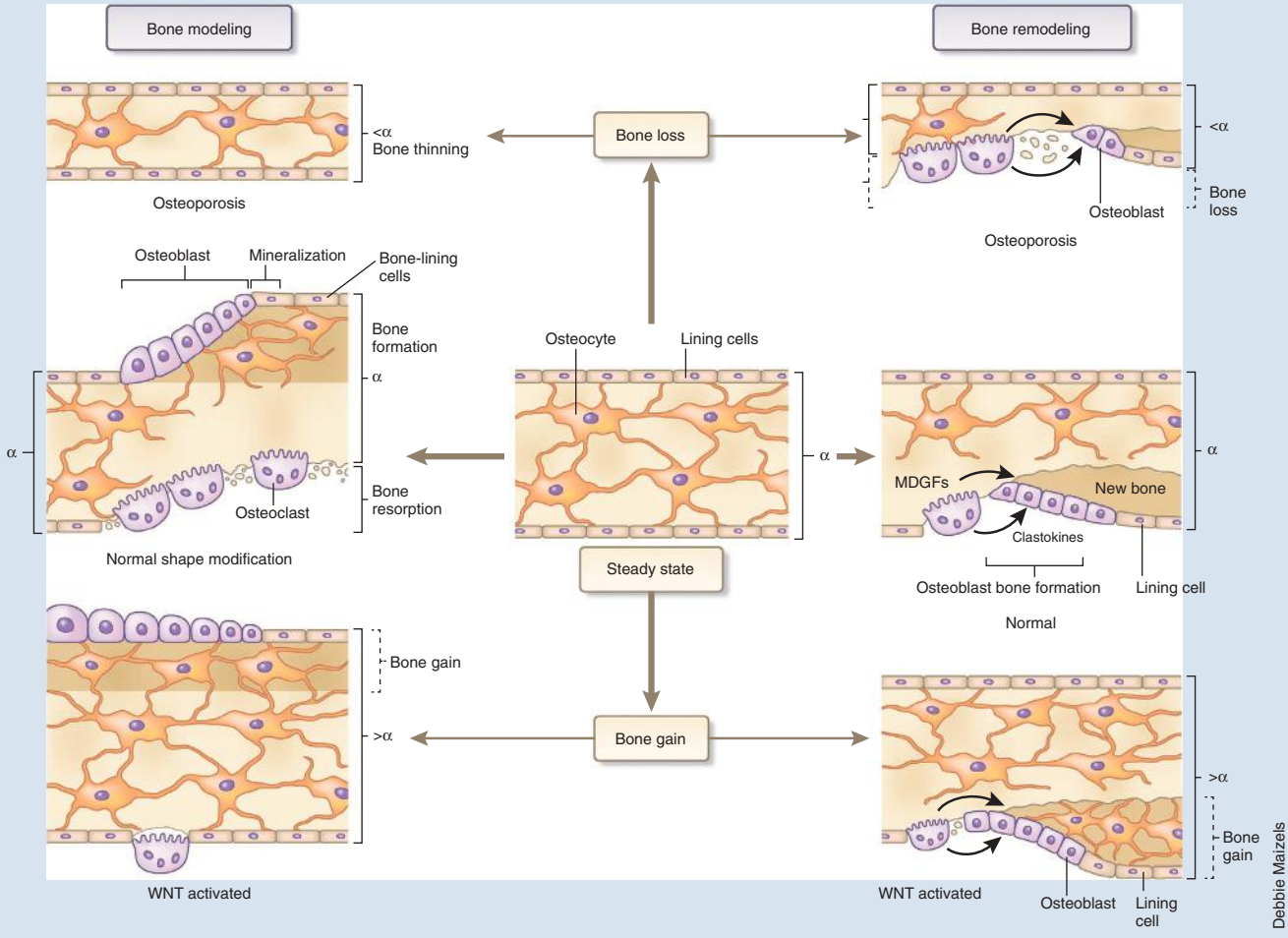
Canonical WNT signaling affects the entire osteoblastic lineage (**Fig. 3**). WNT signaling represses mesenchymal stem cell (MSC) commitment to the chondrogenic and adipogenic lineages and enhances commitment to[27–30], and differentiation along[31–33], the osteoblastic lineage. Osteoblast and osteocyte WNT–β-catenin signaling also indirectly represses osteoclast differentiation and bone resorption through the increased secretion of osteoprotegerin (OPG)[34–36]. However, osteoclasts and their precursors are also directly affected by WNT ligands, possibly as an autocrine loop[37]: β-catenin activation in early precursors favors their proliferation, whereas at later stages, WNT3a inhibits osteoclastogenesis[38,39]. In contrast, activation of the noncanonical pathway by osteoblast-expressed WNT5a stimulates differentiation of osteoclast precursors after binding to the FZD–receptor tyrosine kinase-like orphan receptor 2 (ROR2)[40] or the FZD–receptor-like tyrosine kinase (RYK)[39] receptor complex (**Box 3**). In what emerged as a feedback loop for bone remodeling, osteoclasts stimulate the local differentiation of osteoblasts (coupling process) at the end of the resorption phase by secreting WNT ligands and other chemoattractants[37]. Other cells of hematopoietic origin are also involved in WNT-dependent crosstalk with osteoblasts. Within the bone marrow, T lymphocytes express WNT10b[41], which is the best-characterized bone formation–promoting canonical WNT[32,42,43], and can trigger osteoblast WNT–β-catenin signaling in a paracrine fashion. As osteoblasts are essential in the establishment of the HSC niche, and as WNT signaling is crucial for the osteoblastic lineage, it is not surprising that WNT signaling also affects hematopoiesis. Osteoblast overexpression of the WNT antagonists *Dkk1* or

Box 1 WNT signaling in skeletal development

During skeletal development, WNT signaling is implicated in proximal-distal outgrowth and dorsoventral limb patterning and, later, in chondrogenesis, osteogenesis, myogenesis and adipogenesis, in effect playing a crucial part in MSC lineage commitment and progression[27–30]. WNT signaling consequently affects all aspects of skeletal development, including craniofacial, limb and joint formation. Mutations in several members of the canonical and noncanonical WNT signaling pathways result in skeletal malformations in mice and humans. For example, a reduction of the expressions of *Lrp5* and *Lrp6* in compound mutant mice results in limb defects[65]; many of the skeletal defects affecting bone, cartilage and joints in mouse embryos lacking both WNT co-receptors resemble those of mouse embryos lacking β-catenin[67]. In addition, mutations in the co-receptors *LRP4* and *Lrp4* result in syndactyly (webbed toes through bone fusions) in humans and mice, respectively[87,201]. Altered gene dosages of the WNT antagonists *Sost*/*SOST* give rise to limb malformations in mice[13,202] and syndactyly in humans[11]. Mutations in *WNT3*, *Wnt3a* or *WNT7A*/*Wnt7a* result in skeletal malformations in humans[203,204] and mice[205,206]. Moreover several WNT proteins, including WNT4 and WNT14, are required for joint formation[207,208], and WNT5- and Wnt5a-mediated signaling is required for normal skeletal development[209]. Another example is the secreted WNT signaling regulator RSPO2, as lack of *Rspo2* results in extensive skeletal defects in mice[210]. An implication of noncanonical WNT signaling in skeletal development is also probable, as deletion or loss-of-function mutations in the noncanonical co-receptor *Ror1* or *Ror2* lead to severe skeletal malformations in mice[211–214]. Thus, WNT signaling is involved in skeletal development, mostly through the canonical pathway but also involving noncanonical elements.

Box 2 Bone modeling and remodeling

The coordinated actions of osteoblasts, osteocytes and osteoclasts occur within two biological contexts: bone modeling and remodeling. Bone modeling is the process that shapes skeletal elements and ensures the acquisition of the appropriate bone morphology and mass during growth. This process occurs at a low rate throughout life and is required for repair and adaptation to changes in mechanical loading. In this process, bone resorption and formation occur in an uncoupled manner and on separate surfaces. In contrast, bone remodeling is the mechanism that ensures tissue turnover while maintaining bone mass and allowing for adaptation to both mechanical loading and the requirements of calcium and phosphate metabolism in the mature skeleton. Bone remodeling is based on the coupled and balanced activities of bone resorption and formation that occur in packages of cells along specific sites on the same the bone surface (basic multicellular units), mostly at the interface with the hematopoietic bone marrow. Activation of WNT–β-catenin signaling results in both situations in increased bone mass as a result of excess bone formation compared to bone resorption. Conversely, impairment of WNT–β-catenin signaling induces reduced bone mass as a result of bone resorption dominating over bone formation. MGDFs, matrix-derived growth factors; α, amount of bone at equilibrium.



Wif1 reduces WNT signaling in HSCs and disturbs HSC quiescence and self-renewal potential[44,45]. Furthermore, the WNT antagonist secreted frizzled-related protein 1 (Sfrp1) has been implicated in HSC regulation through an extrinsic mechanism, which might involve osteoblasts[46]. An additional link to hematopoiesis comes from the fact that PTH regulates the hematopoietic niche through its actions on osteoblasts[47], which also involves crosstalk with WNT signaling. Taken together, current evidence indicates that WNT directly affects both the osteoblast and the osteoclast bone cell lineages and also indirectly affects these cells through crosstalk in the bone environment, inducing an overall increase in bone formation and a decrease in bone resorption.

The impact of WNT–β-catenin signaling on adult bone homeostasis is exemplified by the bone phenotypes of a large number of mouse mutant models in which this pathway is perturbed. Although an impact on bone formation seems to be the most frequent finding, the relative contributions of bone formation compared to secondary changes in bone resorption are variable between models (**Fig. 2**). This is not entirely surprising given the extensive crosstalk between bone cells and the complex fine tuning of the WNT regulatory network in the control of bone homeostasis. Overall, and although late activation of β-catenin may exert a negative influence on osteoblasts[48], these data so far indicate that enhancement of WNT–β-catenin signaling in cells of the osteoblastic lineage increases bone mass, whereas inhibition decreases it.

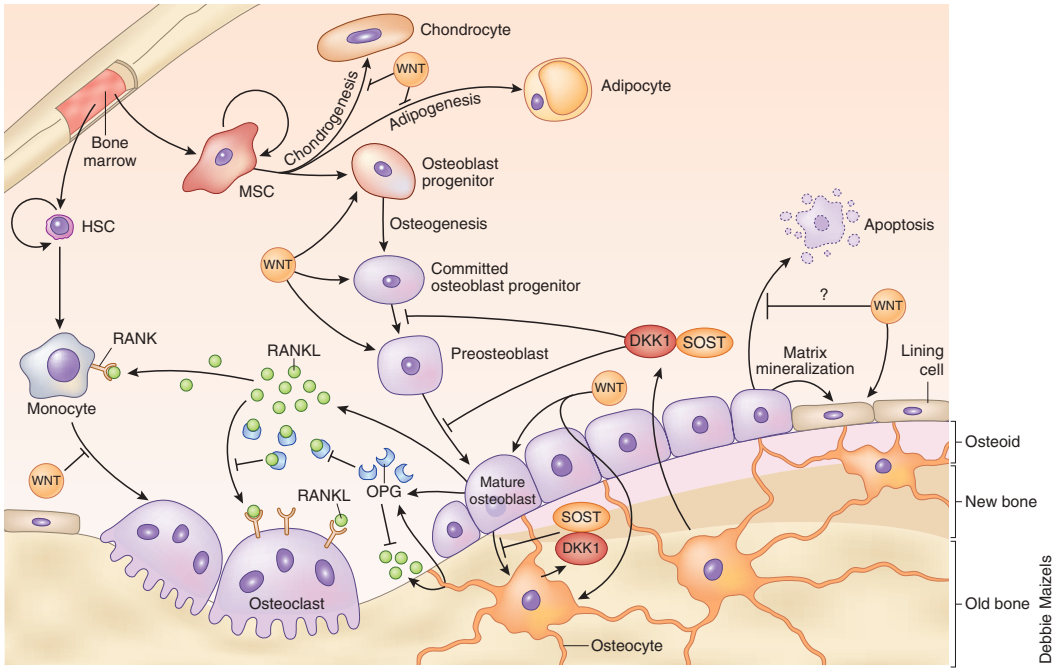


Figure 3 Impact of WNT/β-catenin signaling on bone cells. Bone-forming osteoblasts derive from pluripotent MSCs. WNT–β-catenin signaling is required for commitment of these cells to the osteoblast lineage, and it inhibits adipogenic and chondrogenic cell fate. Once commitment is ensured, canonical WNT signaling is essential for osteoblast precursor proliferation and differentiation. Wnt–β-catenin signaling has also been implicated in the downregulation of apoptosis of osteoblastic cells in some instances, though not all. Moreover, WNT–β-catenin signaling in the osteoblast lineage, including the terminally differentiated cells of this lineage and the most abundant bone cells (osteocytes), inhibits osteoclastic bone resorption. Indeed WNT–β-catenin signaling is required for osteoblast and osteocyte expression of the anti-osteoclastic factor OPG, the decoy receptor for RANKL.

Canonical WNT ligands and enhancers increase bone mass. Several of the 19 known mammalian WNT proteins[21] seem to be implicated in bone homeostasis. The role of the canonical Wnt ligand Wnt10b has been best characterized so far using mouse genetic tools. Studies in which *Wnt10b* gene dosages were altered revealed that it is a positive modulator of bone formation, enhancing osteoblast differentiation and maintaining mesenchymal progenitors, osteoblast progenitors or both in adult bone[32,42,43]. Consistent with the positive role of WNT proteins on bone homeostasis, removal of wntless (encoded by *Wls*), a chaperone required for the secretion of WNT proteins, from the mature osteoblastic stage onwards results in mice with severe osteoporosis caused by impaired bone formation and increased bone resorption[49]. The greater severity of this osteoporotic phenotype compared to that of mice lacking Wnt10b[42] indicates involvement of additional WNT ligands in the control of bone homeostasis. In addition to the classical WNT ligands, mammals also express endogenous enhancers of WNT signaling, fine tuning the pathway. The best known of these are the four R-spondin (RSPO) proteins, which can bind some FZD receptors and possibly LRP6 to enhance WNT–β-catenin signaling[50] in the presence of canonical WNT proteins, perhaps by hindering receptor endocytosis[51]. Notably, deletion of *Lgr4*, which encodes the seven-transmembrane orphan receptor LGR4 that is required for RSPO enhancement of canonical WNT signaling[52,53], results in reduced bone mass caused by decreased bone formation and increased resorption[54] (**Fig. 3**). Moreover not only is RSPO2 required for skeletogenesis (**Box 1**)[55], but it also affects the maturation and mineralization of osteoblasts[56], similarly to RSPO1 (ref. 57). Whether the signaling activity of RSPO proteins is related exclusively to their function as enhancers of WNT proteins remains to be elucidated. Norrin, which has no structural resemblance to WNT or RSPO proteins but contains a cysteine-knot motif, as the WNT antagonists sclerostin and Wise do, can bind to FZD4 and activate WNT–β-catenin signaling[58]. Norrin signaling has been found to be impaired in some mutant forms of LRP5, which cause OPPG[59].

Bone-targeted deletions and mutations in Lrp5 or Lrp6 recapitulate the human phenotypes. The WNT co-receptors LRP5 and LRP6 are required for FZD proteins to activate canonical WNT signaling. These type I transmembrane receptors are characterized by an extracellular low-density lipoprotein receptor (LDLR) ligand-binding repeat. Human LRP5 and LRP6 share 71% amino acid sequence identity, and their extracellular sequences contain four of the YWTD-type β-propeller domains and the epidermal growth factor–like domains that are characteristic of this receptor family followed by three LDLR ligand-binding motifs[60]. Mice lacking Lrp5 globally[31,61] or selectively in osteocytes in bone[62] faithfully recapitulate the osteoporotic low bone–formation phenotype of humans with loss-of-function mutations in LRP5 or LRP6. Conversely, introduction of the human high–bone mass gain-of-function mutations in LRP5 β-propeller 1 induces a similar phenotype in mice when expressed ubiquitously[7,8] or selectively in the limbs or in cells of the osteoblastic lineage, including osteocytes[62,63]. An alternative model, also based on mouse genetics, has been proposed by Yadav *et al.*[64]. The authors suggested that *Lrp5* does not act as a WNT co-receptor in bone but rather controls bone formation by suppressing serotonin synthesis in the duodenum through regulation of the rate-limiting enzyme *Tph1* independently of WNT signaling. However, the majority of the currently available data and, in particular, the extensive studies of Cui *et al.*[62] involving several independent groups do not provide support for the extraskeletal and WNT-independent role of LRP5. Indeed, these studies unequivocally support the concept that the control of bone formation and bone mass by LRP5 occurs within the bone microenvironment, mostly at the level of osteocytes.

Lrp6 seems to have both distinct and overlapping functions in bone. As with *Lrp5*, haploinsufficiency for *Lrp6* results in reduced bone mass[65] but also worsens *Lrp5* deficiency–induced osteopenia in double-mutant mice, demonstrating that the functions of these two receptors are not fully redundant. Moreover, whereas bone mass reduction in *Lrp5* knockout mice[31] is related to decreased bone formation, *Lrp6* hypomorphic mice have reduced bone mass owing to

Box 3 The emerging role of noncanonical WNT signaling in bone

Although much attention has been focused on canonical WNT signaling in bone, a few studies have explored the role of noncanonical signaling. Tu *et al.*[215] showed that noncanonical WNT7b induces osteoblast differentiation through G protein–linked protein kinase C δ (PKC-δ) signaling. Moreover, WNT5a may favor osteoblastogenesis over adipogenesis through repression of PPAR-γ transcription involving chromatin inactivation[216]. Furthermore, WNT5a affects osteoclastogenesis directly[38–40].

Additional studies have explored the skeletal function of components that affect both pathways, such as FZD proteins, RSPO proteins or SFRPs. However, thus far, there are only a few mouse genetic studies that have attempted to identify which FZD proteins are implied in bone homeostasis. Loss of *Fzd9* results in decreased bone formation and, subsequently, loss of bone mass caused by impaired noncanonical WNT signaling[217], suggesting that activation of this pathway may also favor bone formation. Consistent with this hypothesis, the noncanonical receptors Ror1 and Ror2 have a role in mouse skeletogenesis, and ROR2 activation can promote *ex vivo* bone formation[40]. Notably, osteoclast precursors in mice expressed Ror2, which is activated by osteoblast-derived Wnt5a[40]. Absence of either Ror2 or Wnt5a in the respective cell lineages results in increased osteoclastogenesis and bone resorption. The WNT sequestering antagonists SFRP1, SFRP2 and SFRP4, which regulate both canonical and noncanonical signaling by quenching WNT ligands, also regulate bone homeostasis. Deletion of *Sfrp1* in mice protected against osteoblast and osteocyte apoptosis and age-related bone loss, whereas its overexpression reduced bone formation[120,218]. *Sfrp1* may also affect osteoclastogenesis, possibly independently of WNT–β-catenin signaling[219]. *Sfrp4* also negatively affects bone formation[220]; its deletion boosts bone formation and increases bone density in mice but also leads to thinning of cortices secondary to activation of noncanonical WNT signaling (Saito, H. *et al.*, unpublished data). SFRP4 was also proposed to be involved in phosphate metabolism[221], but recent evidence has failed to support this[222,223]. Although *Sfrp2*-deficient mice develop brachysyndactyly[224], changes in bone mass have not been reported; however, similar to other Sfrp proteins, SFRP2 negatively affects bone formation *in vitro* and the osteoblastic niche in the bone marrow[225]. *Wif1*, which sequesters WNT through direct binding, inhibits osteoblastic differentiation[226] and is involved in the maintenance of HSCs in the bone marrow niche[44].

From the mouse genetic data currently available, one may speculate that under physiological conditions, co-stimulation of both the canonical and noncanonical pathways occurs. Whether the net result of noncanonical activation synergizes with or antagonizes the canonical pathway and whether exclusive activation of the noncanonical pathway has positive, negative or neutral effects on bone homeostasis remains to be elucidated.

increased bone resorption[66]. That WNT–β-catenin signaling affects both bone formation and resorption to a variable degree is also reflected in many other mouse mutants (**Fig. 2**).

WNT signaling in bone is not fully mimicked by altering β-catenin. Although canonical WNT signaling is traditionally reduced to the stabilization and nuclear translocation of β-catenin, the data accumulated so far in skeletal biology suggest that if β-catenin is an important component of the machinery leading to increased bone mass, it is not sufficient to explain the whole mechanism. Deletion of β-catenin at earlier stages in limb development affects osteoblast differentiation substantially[29], confirming a key role in bone formation. Moreover, this phenotype largely overlaps with the disturbed osteoblast differentiation observed in double-mutant embryos lacking Lrp5 and Lrp6 (ref. 67). But, surprisingly, targeted constitutive activation or deletion of β-catenin in bone from the osteoblast or osteocyte stage onwards results in decreased or increased bone resorption, respectively[34–36], failing to mimic the gain-of-function or loss-of-function phenotypes of *Lrp5* mutant mice or the phenotypes of mouse models with deletion or overexpression of the WNT antagonists *Dkk1* or *Sost*[13,68–72]. Moreover, genetic alterations in components of the β-catenin destruction complex, which affect β-catenin stability, or in the β-catenin–binding transcription factors, which mediate WNT target gene expression, alter bone mass in a manner that is overall consistent with a positive role for canonical WNT signaling in bone. However, those mutant models also show variable alterations in bone formation and resorption (**Fig. 2**)[34,35,73–75]. If β-catenin were the only important signaling event downstream of canonical WNT signaling, these mutants in which β-catenin expression or activity is altered should have phenocopied the largely bone formation–driven phenotypes of the *Lrp5*, *Dkk1* or *Sost* mutants. This suggests that β-catenin stabilization may not be the only relevant event taking place downstream of the canonical WNT signaling receptor complexes. Alternatively, or in addition, the stable activation or removal of β-catenin may not be able to mimic the biological modulation of the pathway, which occurs through the interplay of activators and inhibitors *in vivo* and, in particular, through the dual co-stimulation of the canonical and noncanonical pathways by most WNT ligands. β-catenin also has a role in cadherin-based cell adhesion[76] and can associate with forkhead box O (FoxO) transcription factors[77], such that one cannot rule out the possibility that other pathways may also be affected in mice with osteoblast or osteocyte β-catenin deficiency.

Osteocyte-derived WNT antagonists are negative bone mass regulators. A high amount of fine tuning of WNT signaling in bone is provided by a number of extracellular WNT antagonists (**Figs. 1** and **2**). Sclerostin, Wise (also known as USAG-1, ectodin and SOSTDC1) and the DKK proteins are secreted glycoproteins that antagonize WNT signaling by binding to LRP5, LRP6 or both. Sclerostin and Wise are considered to be members of the DAN and Cerberus family of bone morphogenetic protein (BMP) antagonists by virtue of containing a related cysteine-knot domain. For Wise, a role in dentogenesis has emerged[78] but, in contrast to sclerostin, no role in bone homeostasis has been found. Unlike other dimeric family members, sclerostin is a monomer. Its cysteine knot contains three disulfide bonds, and an additional disulfide bond connects loops one and three of its three-looped structure[79,80]. Sclerostin is selectively secreted in bone by mature osteocytes embedded within the mineralized matrix[9,10]. Once secreted in the osteocyte lacuna, sclerostin diffuses to the surface of bone through the canalicular network and inhibits osteoblastic bone formation. In addition, autocrine effects on osteocyte WNT–β-catenin signaling seem probable[62,81]. Sclerostin was originally believed to act as a BMP antagonist in adult bone[68]. However, subsequent *in vitro* studies revealed that it antagonizes WNT signaling by binding

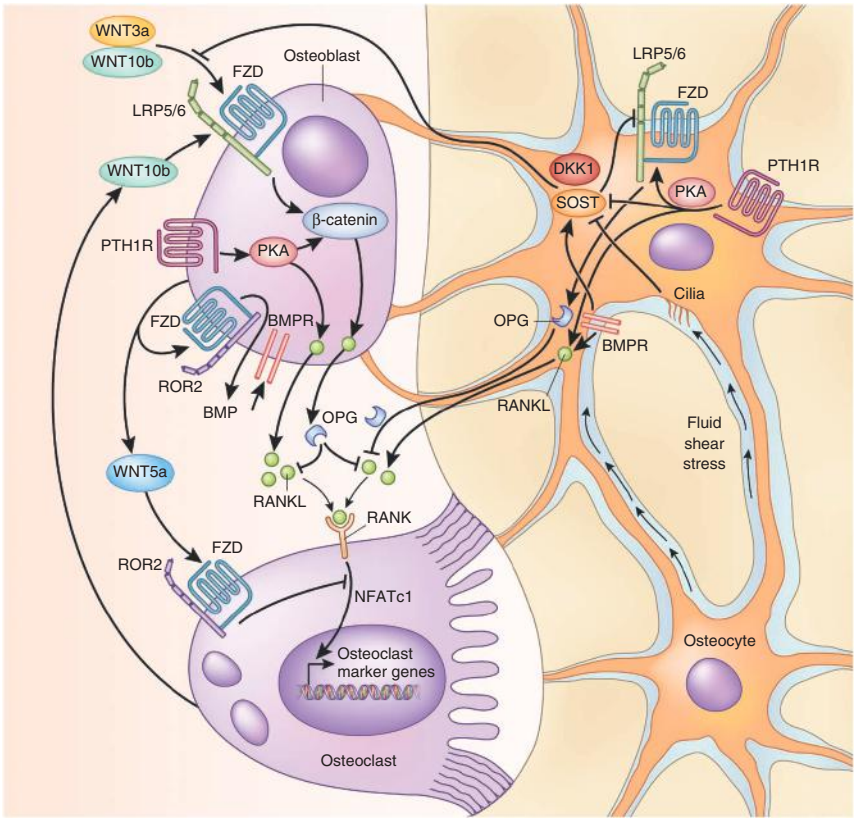


Figure 4 Crosstalk of WNT, PTH and BMP signaling between bone cells. Osteocytes control bone formation through the secretion of the WNT antagonists sclerostin (SOST) and DKK1, the expression of which is regulated by mechanosignals and signaling of PTH and BMP. PTH represses, whereas BMPR1A-mediated BMP signaling induces, expression of these antagonists. Moreover, WNT signaling in osteocytes controls the production of OPG, which is the decoy receptor for the key osteoclast differentiation factor RANKL. Osteoblast-expressed WNT5a stimulates differentiation of osteoclast precursors as a result of binding to the FZD–ROR2 receptor complex. In a feedback loop for bone remodeling, osteoclasts stimulate the local differentiation of osteoblasts at the end of the resorption phase by secreting WNT ligands. In addition, activation of PTH1R-mediated signaling in osteoblasts and osteocytes leads to β-catenin stabilization and, thus, activation of WNT signaling.

to the first β-propeller of LRP5 or LRP6 (refs. 14,15,80,82–84) and has decreased binding to high–bone mass mutant forms of LRP5 (refs. 14,15,84), possibly explaining the high–bone mass phenotype[85]. However, an additional direct role in BMP signaling has not been excluded[86]. Sclerostin also binds to LRP4, the third member of the LRP5 and LRP6 subfamily of LDLRs, probably through the third LRP4 β-propeller[87]. This interaction enhances sclerostin inhibition of WNT–β-catenin signaling in bone. The WNT antagonist DKK1 is also robustly expressed by osteocytes, though not as highly or selectively as is sclerostin. DKK proteins interact with β-propeller subregions of LRP5 and LRP6, including β-propellers 1 and 3 (refs. 84,88), thus preventing formation of the complex comprised of WNT, FZD and LRP5 or LRP6 (refs. 88,89). In addition, DKK1 also interacts with Kremen receptors and LRP4, though the functional consequences of these interactions are not understood.

Indeed, some of the most compelling data supporting a role of WNT–β-catenin signaling in the regulation of bone formation come from the extensive mouse genetic studies showing that osteocyte-secreted sclerostin and DKK1 inhibit bone formation[13,68–72]. Although increased or decreased sclerostin secretion inhibits or increases bone formation, respectively, during growth and adulthood, the role of DKK1 may be more pronounced during early growth and bone repair[90]. Interestingly, and as suggested above, reduction of gene dosage or inhibition of these antagonists can also suppress bone resorption in a context-dependent manner[91,92]. Increased and decreased gene dosage of the Dkk1 co-receptor *Krm2* also results in alterations in bone formation and resorption in mice, consistent with its role as a negative bone mass regulator by binding to DKK1 and inducing internalization of the complex comprising Kremen, DKK1 and LRP5 or LRP6 (ref. 93).

Consistent with the fact that LRP4 interacts with sclerostin and DKK1, mice harboring a stop codon upstream of the transmembrane domain of *Lrp4* have increased bone formation and resorption and decreased bone mineral density[94]. However, given that the dysfunctional receptor lacking the membrane anchor could still interact with its extracellular ligands, it is unclear whether this model represents a gain or loss of function. Overall, the findings implicate mouse Lrp4 in bone mass regulation, a finding that also translates to humans[87].

It is important to emphasize here that some inhibitors of WNT signaling may have a positive role in osteoblast maturation. Dkk2 has been identified as a positive regulator of osteoblast maturation, as its absence results in disturbed bone mineralization, increased bone resorption and osteopenia[95]. This apparently surprising result actually fits well with the concept proposed by Rodda and McMahon[48] suggesting that WNT signaling needs to be toned down at later stages to allow final maturation of the osteoblasts, possibly favoring mineralization of the recently laid matrix.

WNT signaling translates mechanosignals in osteocytes. The mouse genetic studies also revealed another, and possibly crucial, function of WNT signaling in bone: the essential role of WNT signaling in osteocytes for mechanosensing and osteocyte regulation of bone mass[96]. For a long time, osteocytes were suspected to be involved in the minute-to-minute regulation of calcium and phosphate homeostasis and the skeletal responses to mechanical loading, both of which are directly related to bone mass regulation. These roles of osteocytes have been recently confirmed, and WNT signaling has been shown to be crucially involved[97,98]. Osteocytes are the main source of the WNT inhibitor sclerostin, whose expression is regulated by mechanobiology[99], and repression of sclerostin is implicated in mechanical loading–induced bone gain in mice[96]. Furthermore, sclerostin expression is markedly increased in humans during immobilization[100]. Accordingly, deletion or inhibition of sclerostin protects mice from bone loss after unloading[69,92]. In addition and as explained above, *Lrp5* mouse mutants suggest that the predominant role of *Lrp5* in the regulation of bone homeostasis is exerted in osteocytes[62], and *Lrp5* gain-of-function mutations increase mechanoresponsiveness, whereas a lack of *Lrp5* reduces it[101–103]. Furthermore, WNT–β-catenin reporter mice suggest that canonical WNT signaling is almost exclusively active in osteocytes in the adult skeleton and is regulated

by mechanical loading[81]. This general role for osteocyte WNT signaling in the control of bone homeostasis was further corroborated by the finding that a lack of β-catenin selectively in osteocytes gives rise to massive osteopenia in mice[36]. Thus, through its ability to regulate bone formation and bone resorption through the secretion of sclerostin and the production of tumor necrosis factor (ligand) superfamily, member 11 (RANKL)[104,105] and OPG[36], respectively, the osteocyte seems to be an essential coordinator of bone modeling, remodeling and homeostasis[106], linking mechanical loading and skeletal homeostasis. These regulatory loops seem to be modulated, at least in large part, by WNT signaling (**Fig. 4**).

WNT signaling crosstalk in bone homeostasis. Consistent with what is observed in other tissues, extensive crosstalk takes place between WNT signaling and other pathways in bone. Interestingly, among the numerous pathways that WNT signaling interacts with are two major bone anabolic pathways: PTH and BMP signaling.

PTH elicits both anabolic and catabolic effects on bone by stimulating both bone formation and, indirectly, bone resorption[107]. Exogenous intermittent administration of PTH, the only anabolic agent currently used for treatment of osteoporosis, enhances bone formation such that it exceeds bone resorption for an extended period of time. In contrast, continuous PTH elevation in certain conditions, including hyperparathyroidism, increases bone turnover and tends to decrease bone mass as a result of bone resorption exceeding bone formation. Parathyroid hormone exerts its action by binding the PTH-related peptide type 1 receptor (PTH1R), a G protein–coupled receptor that is expressed in several cell types in bone marrow, including stromal cells, lymphocytes and the cells of the osteoblastic lineage (for example, osteocytes). Several lines of evidence indicate that in bone, PTH signaling interacts with and regulates WNT–β-catenin signaling through multiple mechanisms. First, in animal models, PTH was found to repress expression of several secreted WNT antagonists, such as *Sost*[108,109], *Dkk1* and *Wif1* (refs. 110,111). *SOST* regulation by PTH seems to translate to the human situation, as several studies report *SOST* expression to be decreased or increased in situations of abnormally elevated or reduced endogenous amounts of PTH, respectively[112–114], and to be reduced subsequent to exogenous PTH administration[115]. Moreover, several studies indicate that PTH-induced bone gain is blunted in *Sost*-deficient[116,117] or *Sost*-overexpressing[116,118] mice, although with varying impacts on bone formation and resorption. Mouse genetic studies have also shown that altered gene dosage of the WNT-sequestering *Sfrp1* (refs. 119,120) blunts the anabolic response to PTH. In addition, mice selectively expressing constitutively active PTH1R in osteocytes show increased WNT–β-catenin signaling and bone mass, and deletion of the co-receptor *Lrp5* abolishes this bone gain[121]. Conversely, mice lacking PTH1R in osteocytes are mildly osteopenic and have increased *Sost* expression and decreased bone Wnt–β-catenin signaling[122]. Second, and in addition to this crosstalk mechanism, the PTH-PTH1R complex can associate *in vitro* with the extracellular domain of Lrp6, triggering signaling in the absence of Wnt ligands[123]. Third, activation of PTH1R by PTH leads to the phosphorylation of β-catenin at sites that stabilize it[111]. Fourth, commitment of progenitors to the osteoblast lineage is impaired in absence of the G protein αs (which stimulates cyclic AMP–dependent signaling downstream of G protein–coupled receptors) selectively in osteoblasts, and this is associated with decreased WNT signaling[124], an effect that could involve, yet may not be restricted to, PTH1R. Bone marrow–derived T lymphocytes have emerged as regulatory modulators of PTH bone anabolic action through paracrine Wnt10b signaling[41]. Thus, much of the anabolic effect of PTH on bone may indeed be mediated through WNT signaling.

BMP signaling is crucial in skeletogenesis and bone formation during development and repair, and ectopic administration of BMPs is used to enhance local bone regeneration in humans[125]. The crosstalk between BMP and WNT signaling is notoriously complex in all tissues, as it can either be synergistic or antagonistic, depending on the cellular context[126]; bone is no exception to this rule[127,128]. In line with the complexity of their crosstalk, BMP and WNT signaling have been shown to have opposing effects in osteoprogenitors, yet they seem to function, for the most part, cooperatively in osteoblasts. For example, BMP2 may regulate osteoblast function through modulation of WNT–β-catenin signaling[129]. Furthermore, BMP antagonists, such as Wise, crosstalk with WNT–β-catenin signaling by virtue of acting as WNT antagonists[130]. Moreover, *Dkk1* and *Sost* are targets of BMP receptor type 1A (BMPRIA, also known as ALK3)-mediated BMP signaling in osteoblasts and mediate the negative impact of this signaling on mature bone[128]. An analogous finding was recently shown for activin A receptor, type I (ACVR1, also known as ALK2)-dependent signaling[131]. However, mildly activating mutations in ACVR1 are associated with heterotopic ossification in the rare human genetic disorder fibrodysplasia ossificans progressiva[132]. These findings, together with the fact that exogenous BMPs can be used to induce bone regeneration and ectopic bone formation, suggest a difference in the effects of BMP signaling in bone induction, where it is an inducer, and in bone homeostasis, where BMPs may repress WNT–β-catenin signaling and subsequent bone formation in certain contexts. This may explain why inhibition of BMPRIA-dependent BMP signaling by the soluble BMPRIA receptor–Fc fusion protein was recently reported to be bone anabolic in a preclinical rodent model[133]. Similarly, deletion of *Noggin*, an inhibitor of BMPs, led to decreased bone mineral density (BMD) and bone formation in mice[134]. Thus, activation of BMP signaling in the mature skeleton decreases bone formation and bone mass, possibly through inhibition of WNT signaling.

In addition to crosstalk between PTH and BMP, β-catenin binds cadherins, which typically mediate cell adhesion and are also implicated in bone homeostasis[135], linking WNT signaling and cadherin-based cell adhesion. Moreover N-cadherin, which is strongly expressed in the osteoblastic lineage[135], not only sequesters β-catenin but also interacts with LRP5 to negatively regulate osteoblastic WNT–β-catenin signaling[136].

WNT signaling in bone disease and treatment

WNT signaling and monogenetic bone disorders. As already briefly introduced, the pivotal role of WNT–β-catenin signaling in human bone homeostasis became apparent with the discovery that loss-of-function mutations in the WNT co-receptor *LRP5* (ref. 6) cause OPPG (MIM259770), an autosomal-recessive disorder characterized by eye abnormalities and osteoporosis caused by decreased bone formation. Carriers of these mutations also have reduced bone mass and increased fracture incidence, a phenotype reproduced in *Lrp5* haploinsufficient mice[31,65]. Since this first report[6], several additional new loss-of function mutations have been found in this receptor, all giving rise to a similar phenotype[137–141]. Conversely, gain-of-function mutations result in high–bone mass syndrome (MIM601884) caused by increased bone formation[7,8]. Additional mutations identified subsequently were also associated with increased bone mass[85,142,143]. These monoallelic gain-of-function mutations are clustered in β-propeller 1 of the receptor and do not intrinsically activate the receptor but

instead decrease its affinity for the WNT antagonists sclerostin[14,15,84] and DKK1 (refs. 8,14,16,84), which are known to inhibit bone formation (**Fig. 2**). Consistent with the observations of partially overlapping functions of *Lrp5* and *Lrp6* in mouse mutant models[65] a loss-of-function missense mutation in the *LRP6* gene has been identified in a family with autosomal-dominant early coronary artery disease characterized not only by metabolic syndrome (high concentrations of low-density lipoprotein and triglycerides, hypertension and diabetes) but also by osteoporosis[144]. Moreover mutations in *LRP4*, which interacts *in vitro* with several WNT antagonists including sclerostin[87,94], result in the loss of LRP4 function as a sclerostin facilitator, causing a sclerosteosis-like phenotype[87]. The relative importance of different LRP proteins for the action of sclerostin remains to be elucidated.

Among the most notable high–bone mass phenotypes related to mutations in modulators of the WNT–β-catenin signaling pathway are those related to the alteration of sclerostin expression (**Fig. 2**). Humans homozygous for the autosomal-recessive diseases sclerosteosis (MIM269500)[11] and VBD (MIM239100)[12] and dominant-heritable craniodiaphyseal dysplasia (CDD; MIM122860)[145] have massive generalized bone overgrowth caused by a lack of sclerostin expression within bone. In individuals with sclerosteosis, this is a result of mutations in *SOST*[11], whereas people with VBD lack a downstream noncoding region containing an enhancer element implicated in adult *SOST* bone expression[13]. CDD is associated with a missense mutation in the signal peptide of *SOST*[145]. Because the phenotype of patients with CDD is more severe than that of patients lacking sclerostin, this suggests that the mutant protein may acquire new functions. Further investigations are required to address this point. Most importantly, studies of BMD and biochemical markers in patients and carriers show that carriers, in whom the concentrations of circulating sclerostin are decreased, have increased bone mass but normal markers of bone turnover once they have reached skeletal maturity and have none of the side effects observed in homozygous patients[146].

Whereas no monogenetic bone mass disorders have been thus far attributed to *DKK1* mutations, there is compelling evidence that increased DKK1 production in cancer cells is associated with the development of osteolytic lesions in human metastatic bone disease[147] and that *DKK1* has a crucial role in multiple myeloma[148]. Deregulated WNT–β-catenin signaling is also implicated in the development of osteosarcoma, although the high number of WNT–β catenin pathway components makes it challenging to dissect the putatively multiple relevant WNT signaling events. Epigenetic silencing of the WNT antagonist encoding WIF1 is associated with osteosarcoma progression[149], and *LRP5* has been identified as a candidate marker for disease progression. Mutations in the *WTX* gene, which encodes for the WNT inhibitor and tumor suppressor WTX (also known as Amer1), have been shown to give rise to cranial sclerosis in X-linked dominant osteopathia striata[150], which is in part related to the misregulation of mesenchymal progenitor commitment[151].

WNT signaling and genetic association to BMD. The role of *LRP5*, *LRP4* and *SOST* is further corroborated by genetic association studies illustrating that natural variants within these genes have an influence on BMD, which is currently the most widely used predictor of fracture risk in the general population, whereas the findings involving *LRP6* are still ambiguous[152–154]. A wide array of studies have identified an association between common *LRP5* single nucleotide polymorphisms and BMD[155], results that were reconfirmed in a genome-wide association study (GWAS)[156] and a meta-analysis of five GWASs[157]. Furthermore, a recent GWAS in individuals of European descent has shown that nonsynonymous single nucleotide polymorphisms in *LRP4* are also associated with BMD[157,158] and confirmed previous findings of the association of *SOST* with BMD[152,159,160]. Other WNT pathway members, such as *WNT10B* and *WNT16* (encoding for canonical and noncanonical WNT ligands, respectively), the WNT modulator–encoding *RSPO*, WNT antagonists–encoding *SFRP1* and *SFRP4* and the β-catenin–encoding *CTNNB1* have been identified as further osteoporosis susceptibility candidate genes[152,161]. Moreover *WLS*, encoding the chaperone protein wntless, which is required for WNT secretion, was also found to be associated with BMD[157]. *MEF2C* (myocyte enhancer factor 2 C), recently found to be associated with BMD by a meta-analysis of five GWASs[157], is also indirectly linked to WNT–β-catenin signaling given its role in the control of the enhancer element that is required for adult *SOST* bone expression[162]. The largest meta-analysis on lumbar spine and femoral neck BMD so far, including 17 GWASs and over 30,000 individuals of European and Asian ancestry, recently confirmed the association of *LRP4*, *LRP5*, *SOST* and *MEF2C* with BMD and also identified an array of other pathway members, such as the WNT ligand–encoding *WNT4*, *WNT16* and *WNT5b* and the WNT antagonist–encoding *DKK1* and *SFRP4* (ref. 154). This convergence of multiple analyses of very large cohorts of human populations of different genetic backgrounds is both unusual and very noteworthy, strongly suggesting that WNT signaling may indeed be the most dominant regulator of bone mass in humans, making it a therapeutic target of choice.

Thus there is ample evidence that activation of WNT signaling in mice and humans, particularly of the canonical pathway, leads to substantial increases in bone formation and bone mass, opening new avenues for therapeutic intervention in diseases where bone mass needs to be increased. However, activation of WNT signaling might also be associated with uncontrolled growth of bone cells and cancers, calling for particularly careful scrutiny of current preclinical and clinical studies.

The WNT–β-catenin pathway as an osteoanabolic drug target. The extensive genetic validation of the importance of WNT–β-catenin signaling in the regulation of adult bone homeostasis has prompted industry to pursue various therapeutic approaches to target this pathway to increase bone formation and bone mass. If successful, such therapeutics would be of the most benefit to individuals with osteoporosis, but they would also have applications in other diseases that are characterized by low bone mass and high bone fragility, such as immobilization and osteogenesis imperfecta, and perhaps in fracture repair. Several therapies that reduce osteoclast-mediated bone resorption are available for the prevention, treatment or both of osteoporosis, including bisphosphonates and an antibody to RANKL[163,164]. However, although these therapies reduce the risk of new vertebral fractures up to 70%, nonvertebral fracture risk is only reduced by 20–25%, and, most importantly, the risk for deleterious hip fractures is only reduced by a maximum of ~40%. Consequently, there is a strong medical need for bone-building therapies that actively increase bone mass by stimulating new bone formation instead of, or together with, antiresorptive treatments. Recombinant PTH has been developed as such an anabolic treatment for osteoporosis[165]; however, this treatment involves daily subcutaneous injection of PTH_{1-84} or PTH_{1-34} fragments, which is cumbersome. Furthermore, over time, this treatment increases not only bone formation but also osteoblast-mediated secretion of RANKL and bone resorption, resulting in high bone turnover and limiting its beneficial effects on bone mass[166,167]. Consequently, a bone-building therapy that does not require daily

injections, increases bone formation without increasing bone resorption, is independent of bone turnover and has improved efficacy with respect to reduction of hip fracture risk is highly desirable.

Human and mouse genetic data point to positive modulation of the WNT–β-catenin pathway as the potential solution. Blocking the function of the WNT antagonist sclerostin has emerged as the most promising approach so far for three reasons. First, sclerostin is an extracellular target that is amenable to the use of biologics. Second, sclerostin is the only pathway modulator that is highly selective for bone, with sclerostin secretion being restricted to osteocytes for the most part. Third, the absence of sclerostin is known to result in marked increases in bone mass and strength (resulting from high bone formation without a concomitant increase, and perhaps with a decrease, in bone resorption)[72,116]. In line with a mostly bone-restricted role of sclerostin, the complications related to the absence of this WNT antagonist in humans (sclerosteosis and VBD) seem to all be related to massive bone overgrowth, including unwanted foramen closures and nerve compressions, and no other gross abnormalities have been observed[11,12]. Moreover, haploinsufficiency of sclerostin in people results in a more moderate increase in bone mass[146] with none of the undesirable effects of full deletion, possibly mimicking the effects of a properly dosed therapeutic intervention. The link of sclerostin production to mechanosensing suggests the possibility that new bone formation could be directed to the appropriate locations within the skeleton. Consequently, the most advanced therapeutic effort to target WNT signaling in bone consists of sclerostin inhibition by an antibody. In line with the genetic data, this treatment induces generalized bone gain in animal models and humans[168,169], and this bone mass increase is caused by an increase in bone formation. However, and in contrast to intermittent PTH, patients treated with antibodies to sclerostin not only showed no increase but instead showed a decrease in bone resorption markers[167,169], consistent with mouse studies showing that canonical WNT signaling represses bone resorption[34–36].

Hence, whereas some aspects of bone anabolism induced by PTH or sclerostin inhibition partially overlap, consistent with *SOST* being negatively regulated by PTH, they also diverge[170]. Preclinical animal models indicate that all bone envelopes respond to either principle with bone formation. However, those envelopes adjacent to bone marrow, namely the endocortical and cancellous bone envelope (endosteum), are most responsive to PTH treatment, whereas the outer cortical (subperiosteal) envelope is less reactive[171]. This may relate to the requirement of resorptive activity for a full PTH anabolic effect mediated largely through bone remodeling (**Box 2**)[172]. In contrast, inhibition of sclerostin results in robust bone-forming responses in both endosteal and subperiosteal bone surfaces[173], indicating a dominant bone modeling effect (**Box 2**) that has been documented in nonhuman primate studies[174]. The overall effects on cortical bone are particularly encouraging for the clinic, as observational studies suggest that hip fractures are predominantly associated with a poor cortical bone template and increased intracortical porosity[175–177].

However, the currently preferred approach of targeting sclerostin is not the only approach considered for the treatment of bone diseases. Consistent with the genetic mouse data, inhibition of DKK1, which is highly but not selectively expressed in bone, also induces bone gain in preclinical animal models[90,178]. Although the effects seem to be more variable and less prominent than those obtained with antibodies to sclerostin, such biologics have potential for the inhibition of osteolytic lesions in multiple myeloma[179,180] on the basis of the clear implication of DKK1 in the pathophysiology of this disease[147,148]. The role of DKK1 in joint remodeling requires further exploration[181]; preclinical animal model data suggest that inhibition of Dkk1 may attenuate erosive bone destruction in rheumatoid arthritis[182] and could possibly serve as an adjunct therapy in this disease. In line with this finding, RSPO1, which antagonizes DKK1, also protected structural joint integrity in a mouse model of arthritis[183]. In other instances, preclinical data indicate that downregulation of WNT–β-catenin signaling by enhancement or restoration of DKK1 function may help treating ankylosing spondylitis by inhibiting new bone formation to prevent joint fusion[184]. This may also apply for sclerostin, as impaired expression of this protein has been demonstrated in people with this disease[185].

Preclinical data so far indicate that enhancement of WNT–β-catenin signaling by sclerostin inhibition promotes fracture healing and implant osseointegration[186,187]. Consistent with a positive role of canonical WNT signaling in fracture repair, *Sfrp1*- and *Lrp5*-deficient mice show improved and decreased fracture healing, respectively[188,189]. Likewise, antibody-mediated inhibition of the WNT antagonist DKK1 enhances fracture healing and implant osseointegration in some preclinical settings[90,189,190]. Blocking the inhibitors of WNT signaling may therefore also have therapeutic applications in these indications. Recent preclinical evidence revealed that impaired osteocyte WNT–β-catenin signaling may be implicated in the progression of renal osteodystrophy, which is a hallmark of chronic kidney disease and is characterized by disturbed bone turnover, high bone fragility and frequent fractures[98]. Further investigations are required to establish whether enhancement of WNT signaling would also improve bone health in this condition. Interestingly, preliminary data derived from mouse mutant models of osteogenesis imperfecta suggest that enhancement of WNT signaling by sclerostin inhibition might also have therapeutic potential in this disease[191]. Osteogenesis imperfecta summarizes an array of mutations characterized by bone matrix abnormalities caused by an impaired collagen phase, resulting in decreased quantity and quality of bone and leading to disturbed bone growth and multiple bone fractures. Whether enhancement of Wnt signaling improves bone strength in these preclinical models exclusively by correcting the decreased quantity of bone remains to be explored.

Whereas targeting WNT signaling with biologics seems very promising, the development of small molecules has thus far been challenging. Small-molecule antagonists to SFRP1 have so far only been shown to stimulate bone growth in organ culture[192–194]. Inhibition of glycogen synthase kinase 3 β (GSK-3β) by lithium or a dual inhibitor of GSK-3α and GSK-3β was shown to be bone protective in preclinical bone loss models[61,195,196]. Epidemiologic studies indicate that lithium, which is used as psychotropic medication, might also be protective against osteoporosis fractures in humans[197,198]. However, although it provides further validation, this approach is less attractive because of a lack of bone selectivity. Whether therapeutic approaches aiming at direct application of RSPO proteins, modulation of LRP5 function or general pathway activation[199,200] will be successful remains to be determined. The recent elucidation of the structural basis of the interactions of LRP5 and LRP6 with sclerostin and DKK1 (ref. 84) may help determine the rationale of the design of low–molecular weight modulators, but this remains a challenging task.

Conclusion and perspectives

Since the discovery that mutations in the WNT pathway cause severe alterations in bone density in humans, a large amount of data has accumulated in the last decade identifying the pathway as key to practically all aspects of bone homeostasis, including mechanosensation. Moreover, several components of the WNT signaling machinery have emerged as being strongly linked to bone mass, bone fractures or both

in multiple GWASs. Although a crucial role for canonical WNT signaling has been strongly established, much remains to be discovered in respect to its fine tuning and crosstalk with other pathways in bone. Likewise, the relative role of noncanonical WNT signaling in bone homeostasis remains to be further elucidated. Taken together, the evidence suggests that positive modulation of the WNT–β-catenin signaling pathway by targeting relatively bone-specific extracellular modulators, such as sclerostin, has great promise for rebuilding bone in conditions of bone fragility, including, but not restricted to, osteoporosis and fracture repair. Advanced clinical trials are underway, and although there is little doubt as to the efficacy of these approaches, the kinetics of bone formation changes over time remains to be explored.

In addition, potential concerns related to the mode of action, such as foramen closures caused by excessive bone formation or putative oncogenicity caused by to derepression of the pathway, will need to be addressed as development of these drugs progresses. However, studies in mice and human carriers of mutations enhancing WNT signaling have been reassuring so far, making targeting of WNT signaling in bone a promising approach for the treatment of bone fragility and enhancing bone repair.

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R.B. and M.K. wrote the paper and drew the figures.

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The authors declare competing financial interests: details are available in the online version of the paper.

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1. Nüsslein-Volhard, C. & Wieschaus, E. Mutations affecting segment number and polarity in *Drosophila*. *Nature* **287**, 795–801 (1980).
2. Cabrera, C.V., Alonso, M.C., Johnston, P., Phillips, R.G. & Lawrence, P.A. Phenocopies induced with antisense RNA identify the wingless gene. *Cell* **50**, 659–663 (1987).
3. Nusse, R. & Varmus, H.E. Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. *Cell* **31**, 99–109 (1982).
4. Kinzler, K.W. *et al.* Identification of a gene located at chromosome 5q21 that is mutated in colorectal cancers. *Science* **251**, 1366–1370 (1991).
5. Nishisho, I. *et al.* Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. *Science* **253**, 665–669 (1991).
6. Gong, Y. *et al.* Osteoporosis-Pseudoglioma Syndrome Collaborative Group. LDL receptor–related protein 5 (LRP5) affects bone accrual and eye development. *Cell* **107**, 513–523 (2001).
7. Little, R.D. *et al.* 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 (2002).
8. Boyden, L.M. *et al.* High bone density due to a mutation in LDL-receptor–related protein 5. *N. Engl. J. Med.* **346**, 1513–1521 (2002).
9. Poole, K.E. *et al.* Sclerostin is a delayed secreted product of osteocytes that inhibits bone formation. *FASEB J.* **19**, 1842–1844 (2005).
10. van Bezooijen, R.L. *et al.* Sclerostin is an osteocyte-expressed negative regulator of bone formation, but not a classical BMP antagonist. *J. Exp. Med.* **199**, 805–814 (2004).
11. Brunkow, M.E. *et al.* Bone dysplasia sclerosteosis results from loss of the *SOST* gene product, a novel cystine knot-containing protein. *Am. J. Hum. Genet.* **68**, 577–589 (2001).
12. Balemans, W. *et al.* Identification of a 52 kb deletion downstream of the *SOST* gene in patients with van Buchem disease. *J. Med. Genet.* **39**, 91–97 (2002).
13. Loots, G.G. *et al.* Genomic deletion of a long-range bone enhancer misregulates sclerostin in Van Buchem disease. *Genome Res.* **15**, 928–935 (2005).
14. Balemans, W. *et al.* The binding between sclerostin and LRP5 is altered by DKK1 and by high–bone mass LRP5 mutations. *Calcif. Tissue Int.* **82**, 445–453 (2008).
15. Ellies, D.L. *et al.* Bone density ligand, Sclerostin, directly interacts with LRP5 but not LRP5G171V to modulate Wnt activity. *J. Bone Miner. Res.* **21**, 1738–1749 (2006).
16. Ai, M., Holmen, S.L., Van Hul, W., Williams, B.O. & Warman, M.L. Reduced affinity to and inhibition by DKK1 form a common mechanism by which high bone mass–associated missense mutations in LRP5 affect canonical Wnt signaling. *Mol. Cell. Biol.* **25**, 4946–4955 (2005).
17. Cadigan, K.M. & Peifer, M. Wnt signaling from development to disease: insights from model systems. *Cold Spring Harb. Perspect. Biol.* **1**, a002881 (2009).
18. Jenny, A. Planar cell polarity signaling in the *Drosophila* eye. *Curr. Top. Dev. Biol.* **93**, 189–227 (2010).
19. Kohn, A.D. & Moon, R.T. Wnt and calcium signaling: β-catenin–independent pathways. *Cell Calcium* **38**, 439–446 (2005).
20. van Amerongen, R. & Nusse, R. Towards an integrated view of Wnt signaling in development. *Development* **136**, 3205–3214 (2009).
21. Kikuchi, A., Yamamoto, H. & Sato, A. Selective activation mechanisms of Wnt signaling pathways. *Trends Cell Biol.* **19**, 119–129 (2009).
22. Liu, Y. *et al.* The orphan receptor tyrosine kinase Ror2 promotes osteoblast differentiation and enhances *ex vivo* bone formation. *Mol. Endocrinol.* **21**, 376–387 (2007).
23. Long, F. Building strong bones: molecular regulation of the osteoblast lineage. *Nat. Rev. Mol. Cell Biol.* **13**, 27–38 (2012).
24. Bianco, P. Minireview: the stem cell next door: skeletal and hematopoietic stem cell "niches" in bone. *Endocrinology* **152**, 2957–2962 (2011).
25. Confavreux, C.B., Levine, R.L. & Karsenty, G. A paradigm of integrative physiology, the crosstalk between bone and energy metabolisms. *Mol. Cell. Endocrinol.* **310**, 21–29 (2009).
26. Oury, F. *et al.* Endocrine regulation of male fertility by the skeleton. *Cell* **144**, 796–809 (2011).
27. Kennell, J.A. & MacDougald, O.A. Wnt signaling inhibits adipogenesis through β-catenin–dependent and –independent mechansims. *J. Biol. Chem.* **280**, 24004–24010 (2005).
28. Day, T.F., Guo, X., Garrett-Beal, L. & Yang, Y. Wnt/β-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. *Dev. Cell* **8**, 739–750 (2005).
29. Hill, T.P., Später, D., Taketo, M.M., Birchmeier, W. & Hartmann, C. Canonical Wnt/β-catenin signaling prevents osteoblasts from differentiating into chondrocytes. *Dev. Cell* **8**, 727–738 (2005).
30. Hu, H. *et al.* Sequential roles of Hedgehog and Wnt signaling in osteoblast development. *Development* **132**, 49–60 (2005).
31. Kato, M. *et al.* 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 (2002).
32. Bennett, C.N. *et al.* Wnt10b increases postnatal bone formation by enhancing osteoblast differentiation. *J. Bone Miner. Res.* **22**, 1924–1932 (2007).
33. Miclea, R.L. *et al.* Adenomatous polyposis coli-mediated control of β-catenin is essential for both chondrogenic and osteogenic differentiation of skeletal precursors. *BMC Dev. Biol.* **9**, 26 (2009).
34. Glass, D.A. II *et al.* Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. *Dev. Cell* **8**, 751–764 (2005).
35. Holmen, S.L. *et al.* Essential role of β-catenin in postnatal bone acquisition. *J. Biol. Chem.* **280**, 21162–21168 (2005).
36. Kramer, I. *et al.* Osteocyte Wnt/β-catenin signaling is required for normal bone homeostasis. *Mol. Cell. Biol.* **30**, 3071–3085 (2010).
37. Pederson, L., Ruan, M., Westendorf, J.J., Khosla, S. & Oursler, M.J. Regulation of bone formation by osteoclasts involves Wnt/BMP signaling and the chemokine sphingosine-1-phosphate. *Proc. Natl. Acad. Sci. USA* **105**, 20764–20769 (2008).
38. Wei, W. *et al.* Biphasic and dosage-dependent regulation of osteoclastogenesis by β-catenin. *Mol. Cell. Biol.* **31**, 4706–4719 (2011).
39. Santiago, F., Oguma, J., Brown, A.M. & Laurence, J. Noncanonical Wnt signaling promotes osteoclast differentiation and is facilitated by the human immunodeficiency virus protease inhibitor ritonavir. *Biochem. Biophys. Res. Commun.* **417**, 223–230 (2012).
40. Maeda, K. *et al.* Wnt5a-Ror2 signaling between osteoblast-lineage cells and osteoclast precursors enhances osteoclastogenesis. *Nat. Med.* **18**, 405–412 (2012).
41. Terauchi, M. *et al.* T lymphocytes amplify the anabolic activity of parathyroid hormone through Wnt10b signaling. *Cell Metab.* **10**, 229–240 (2009).
42. Bennett, C.N. *et al.* Regulation of osteoblastogenesis and bone mass by Wnt10b. *Proc. Natl. Acad. Sci. USA* **102**, 3324–3329 (2005).
43. Stevens, J.R. *et al.* Wnt10b deficiency results in age-dependent loss of bone mass and progressive reduction of mesenchymal progenitor cells. *J. Bone Miner. Res.* **25**, 2138–2147 (2010).
44. Fleming, H.E. *et al.* Wnt signaling in the niche enforces hematopoietic stem cell quiescence and is necessary to preserve self-renewal *in vivo*. *Cell Stem Cell* **2**, 274–283 (2008).
45. Schaniel, C. *et al.* Wnt-inhibitory factor 1 dysregulation of the bone marrow niche exhausts hematopoietic stem cells. *Blood* **118**, 2420–2429 (2011).
46. Renström, J. *et al.* Secreted frizzled-related protein 1 extrinsically regulates cycling activity and maintenance of hematopoietic stem cells. *Cell Stem Cell* **5**, 157–167 (2009).
47. Calvi, L.M. *et al.* Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature* **425**, 841–846 (2003).

48. Rodda, S.J. & McMahon, A.P. Distinct roles for Hedgehog and canonical Wnt signaling in specification, differentiation and maintenance of osteoblast progenitors. *Development* **133**, 3231–3244 (2006).
49. Zhong, Z. *et al.* Wntless functions in mature osteoblasts to regulate bone mass. *Proc. Natl. Acad. Sci. USA* **109**, E2197–E2204 (2012).
50. Nam, J.S., Turcotte, T.J., Smith, P.F., Choi, S. & Yoon, J.K. Mouse cristin/R-spondin family proteins are novel ligands for the Frizzled 8 and LRP6 receptors and activate β-catenin–dependent gene expression. *J. Biol. Chem.* **281**, 13247–13257 (2006).
51. Binnerts, M.E. *et al.* R Spondin1 regulates Wnt signaling by inhibiting internalization of LRP6. *Proc. Natl. Acad. Sci. USA* **104**, 14700–14705 (2007).
52. Carmon, K.S., Gong, X., Lin, Q., Thomas, A. & Liu, Q. R-spondins function as ligands of the orphan receptors LGR4 and LGR5 to regulate Wnt/β-catenin signaling. *Proc. Natl. Acad. Sci. USA* **108**, 11452–11457 (2011).
53. de Lau, W. *et al.* Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling. *Nature* **476**, 293–297 (2011).
54. Luo, J. *et al.* Regulation of bone formation and remodeling by G-protein–coupled receptor 48. *Development* **136**, 2747–2756 (2009).
55. Jin, Y.R., Turcotte, T.J., Crocker, A.L., Han, X.H. & Yoon, J.K. The canonical Wnt signaling activator, R-spondin2, regulates craniofacial patterning and morphogenesis within the branchial arch through ectodermal-mesenchymal interaction. *Dev. Biol.* **352**, 1–13 (2011).
56. Friedman, M.S., Oyserman, S.M. & Hankenson, K.D. Wnt11 promotes osteoblast maturation and mineralization through R-spondin 2. *J. Biol. Chem.* **284**, 14117–14125 (2009).
57. Lu, W. *et al.* R-spondin1 synergizes with Wnt3A in inducing osteoblast differentiation and osteoprotegerin expression. *FEBS Lett.* **582**, 643–650 (2008).
58. Smallwood, P.M., Williams, J., Xu, Q., Leahy, D.J. & Nathans, J. Mutational analysis of Norrin-Frizzled4 recognition. *J. Biol. Chem.* **282**, 4057–4068 (2007).
59. Ai, M., Heeger, S., Bartels, C.F. & Schelling, D.K. Osteoporosis-Pseudoglioma Collaborative Group. Clinical and molecular findings in osteoporosis-pseudoglioma syndrome. *Am. J. Hum. Genet.* **77**, 741–753 (2005).
60. He, X., Semenov, M., Tamai, K. & Zeng, X. LDL receptor-related proteins 5 and 6 in Wnt/β-catenin signaling: arrows point the way. *Development* **131**, 1663–1677 (2004).
61. Clément-Lacroix, P. *et al.* 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 (2005).
62. Cui, Y. *et al.* Lrp5 functions in bone to regulate bone mass. *Nat. Med.* **17**, 684–691 (2011).
63. Babij, P. *et al.* High bone mass in mice expressing a mutant *LRP5* gene. *J. Bone Miner. Res.* **18**, 960–974 (2003).
64. Yadav, V.K. *et al.* Lrp5 controls bone formation by inhibiting serotonin synthesis in the duodenum. *Cell* **135**, 825–837 (2008).
65. Holmen, S.L. *et al.* Decreased BMD and limb deformities in mice carrying mutations in both Lrp5 and Lrp6. *J. Bone Miner. Res.* **19**, 2033–2040 (2004).
66. Kubota, T. *et al.* Lrp6 hypomorphic mutation affects bone mass through bone resorption in mice and impairs interaction with Mesd. *J. Bone Miner. Res.* **23**, 1661–1671 (2008).
67. Joeng, K.S., Schumacher, C.A., Zylstra-Diegel, C.R., Long, F. & Williams, B.O. Lrp5 and Lrp6 redundantly control skeletal development in the mouse embryo. *Dev. Biol.* **359**, 222–229 (2011).
68. Winkler, D.G. *et al.* Osteocyte control of bone formation via sclerostin, a novel BMP antagonist. *EMBO J.* **22**, 6267–6276 (2003).
69. Lin, C. *et al.* Sclerostin mediates bone response to mechanical unloading through antagonizing Wnt/β-catenin signaling. *J. Bone Miner. Res.* **24**, 1651–1661 (2009).
70. Morvan, F. *et al.* 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 (2006).
71. Li, J. *et al.* Dkk1-mediated inhibition of Wnt signaling in bone results in osteopenia. *Bone* **39**, 754–766 (2006).
72. Li, X. *et al.* Targeted deletion of the sclerostin gene in mice results in increased bone formation and bone strength. *J. Bone Miner. Res.* **23**, 860–869 (2008).
73. Yan, Y. *et al.* Axin2 controls bone remodeling through the β-catenin–BMP signaling pathway in adult mice. *J. Cell Sci.* **122**, 3566–3578 (2009).
74. Kugimiya, F. *et al.* GSK-3β controls osteogenesis through regulating Runx2 activity. *PLoS ONE* **2**, e837 (2007).
75. Hoeppner, L.H., Secreto, F.J., Razidlo, D.F., Whitney, T.J. & Westendorf, J.J. Lef1ΔN binds β-catenin and increases osteoblast activity and trabecular bone mass. *J. Biol. Chem.* **286**, 10950–10959 (2011).
76. Di Benedetto, A. *et al.* N-cadherin and cadherin 11 modulate postnatal bone growth and osteoblast differentiation by distinct mechanisms. *J. Cell Sci.* **123**, 2640–2648 (2010).
77. Almeida, M., Han, L., Martin-Millan, M., O'Brien, C.A. & Manolagas, S.C. Oxidative stress antagonizes Wnt signaling in osteoblast precursors by diverting β-catenin from T cell factor– to forkhead box O–mediated transcription. *J. Biol. Chem.* **282**, 27298–27305 (2007).
78. Ahn, Y., Sanderson, B.W., Klein, O.D. & Krumlauf, R. Inhibition of Wnt signaling by Wise (Sostdc1) and negative feedback from Shh controls tooth number and patterning. *Development* **137**, 3221–3231 (2010).
79. Veverka, V. *et al.* Characterization of the structural features and interactions of sclerostin: molecular insight into a key regulator of Wnt-mediated bone formation. *J. Biol. Chem.* **284**, 10890–10900 (2009).
80. Weidauer, S.E. *et al.* NMR structure of the Wnt modulator protein Sclerostin. *Biochem. Biophys. Res. Commun.* **380**, 160–165 (2009).
81. Hens, J.R. *et al.* TOPGAL mice show that the canonical Wnt signaling pathway is active during bone development and growth and is activated by mechanical loading *in vitro*. *J. Bone Miner. Res.* **20**, 1103–1113 (2005).
82. Li, X. *et al.* Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling. *J. Biol. Chem.* **280**, 19883–19887 (2005).
83. Semënov, M., Tamai, K. & He, X. SOST is a ligand for LRP5/LRP6 and a Wnt signaling inhibitor. *J. Biol. Chem.* **280**, 26770–26775 (2005).
84. Bourhis, E. *et al.* Wnt antagonists bind through a short peptide to the first β-propeller domain of LRP5/6. *Structure* **19**, 1433–1442 (2011).
85. Pangrazio, A. *et al.* Identification of the first deletion in the *LRP5* gene in a patient with autosomal dominant osteopetrosis type I. *Bone* **49**, 568–571 (2011).
86. Krause, C. *et al.* Distinct modes of inhibition by sclerostin on bone morphogenetic protein and Wnt signaling pathways. *J. Biol. Chem.* **285**, 41614–41626 (2010).
87. Leupin, O. *et al.* Bone overgrowth-associated mutations in the *LRP4* gene impair Sclerostin facilitator function. *J. Biol. Chem.* **286**, 19489–19500 (2011).
88. Bourhis, E. *et al.* Reconstitution of a frizzled8.Wnt3a.LRP6 signaling complex reveals multiple Wnt and Dkk1 binding sites on LRP6. *J. Biol. Chem.* **285**, 9172–9179 (2010).
89. Semënov, M.V., Zhang, X. & He, X. DKK1 antagonizes Wnt signaling without promotion of LRP6 internalization and degradation. *J. Biol. Chem.* **283**, 21427–21432 (2008).
90. Li, X. *et al.* Dickkopf-1 regulates bone formation in young growing rodents and upon traumatic injury. *J. Bone Miner. Res.* **26**, 2610–2621 (2011).
91. Wang, F.S. *et al.* Knocking down dickkopf-1 alleviates estrogen deficiency induction of bone loss. A histomorphological study in ovariectomized rats. *Bone* **40**, 485–492 (2007).
92. Tian, X., Jee, W.S., Li, X., Paszty, C. & Ke, H.Z. Sclerostin antibody increases bone mass by stimulating bone formation and inhibiting bone resorption in a hindlimb-immobilization rat model. *Bone* **48**, 197–201 (2011).
93. Schulze, J. *et al.* Negative regulation of bone formation by the transmembrane Wnt antagonist Kremen-2. *PLoS ONE* **5**, e10309 (2010).
94. Choi, H.Y., Dieckmann, M., Herz, J. & Niemeier, A. Lrp4, a novel receptor for Dickkopf 1 and sclerostin, is expressed by osteoblasts and regulates bone growth and turnover *in vivo*. *PLoS ONE* **4**, e7930 (2009).
95. Li, X. *et al.* Dkk2 has a role in terminal osteoblast differentiation and mineralized matrix formation. *Nat. Genet.* **37**, 945–952 (2005).
96. Robling, A.G. *et al.* Mechanical stimulation of bone *in vivo* reduces osteocyte expression of Sost/sclerostin. *J. Biol. Chem.* **283**, 5866–5875 (2008).
97. Bonewald, L.F. & Johnson, M.L. Osteocytes, mechanosensing and Wnt signaling. *Bone* **42**, 606–615 (2008).
98. Sabbagh, Y. *et al.* Repression of osteocyte Wnt/β-catenin signaling is an early event in the progression of renal osteodystrophy. *J. Bone Miner. Res.* **27**, 1757–1772 (2012).
99. Turner, C.H. *et al.* Mechanobiology of the skeleton. *Sci. Signal.* **2**, pt3 (2009).
100. Gaudio, A. *et al.* Increased sclerostin serum levels associated with bone formation and resorption markers in patients with immobilization-induced bone loss. *J. Clin. Endocrinol. Metab.* **95**, 2248–2253 (2010).
101. Robinson, J.A. *et al.* Wnt/β-catenin signaling is a normal physiological response to mechanical loading in bone. *J. Biol. Chem.* **281**, 31720–31728 (2006).
102. Sawakami, K. *et al.* The Wnt co-receptor LRP5 is essential for skeletal mechanotransduction but not for the anabolic bone response to parathyroid hormone treatment. *J. Biol. Chem.* **281**, 23698–23711 (2006).
103. Saxon, L.K., Jackson, B.F., Sugiyama, T., Lanyon, L.E. & Price, J.S. Analysis of multiple bone responses to graded strains above functional levels, and to disuse, in mice *in vivo* show that the human Lrp5 G171V high bone mass mutation increases the osteogenic response to loading but that lack of Lrp5 activity reduces it. *Bone* **49**, 184–193 (2011).
104. Nakashima, T. *et al.* Evidence for osteocyte regulation of bone homeostasis through RANKL expression. *Nat. Med.* **17**, 1231–1234 (2011).
105. Xiong, J. *et al.* Matrix-embedded cells control osteoclast formation. *Nat. Med.* **17**, 1235–1241 (2011).
106. Bonewald, L.F. The amazing osteocyte. *J. Bone Miner. Res.* **26**, 229–238 (2011).
107. Qin, L., Raggatt, L.J. & Partridge, N.C. Parathyroid hormone: a double-edged sword for bone metabolism. *Trends Endocrinol. Metab.* **15**, 60–65 (2004).
108. Keller, H. & Kneissel, M. *SOST* is a target gene for PTH in bone. *Bone* **37**, 148–158 (2005).
109. Bellido, T. *et al.* 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 (2005).
110. Li, X. *et al.* Determination of dual effects of parathyroid hormone on skeletal gene expression *in vivo* by microarray and network analysis. *J. Biol. Chem.* **282**, 33086–33097 (2007).
111. Guo, J. *et al.* Suppression of Wnt signaling by Dkk1 attenuates PTH-mediated stromal cell response and new bone formation. *Cell Metab.* **11**, 161–171 (2010).

112. Costa, A.G. *et al.* Circulating Sclerostin in disorders of parathyroid gland function. *J. Clin. Endocrinol. Metab.* **96**, 3804–3810 (2011).
113. Kaji, H., Imanishi, Y., Sugimoto, T. & Seino, S. Comparisons of serum sclerostin levels among patients with postmenopausal osteoporosis, primary hyperparathyroidism and osteomalacia. *Exp. Clin. Endocrinol. Diabetes* **119**, 440–444 (2011).
114. van Lierop, A.H., Witteveen, J.E., Hamdy, N.A. & Papapoulos, S.E. Patients with primary hyperparathyroidism have lower circulating sclerostin levels than euparathyroid controls. *Eur. J. Endocrinol.* **163**, 833–837 (2010).
115. Drake, M.T. *et al.* Effects of parathyroid hormone treatment on circulating sclerostin levels in postmenopausal women. *J. Clin. Endocrinol. Metab.* **95**, 5056–5062 (2010).
116. Kramer, I., Loots, G.G., Studer, A., Keller, H. & Kneissel, M. Parathyroid hormone (PTH)-induced bone gain is blunted in SOST overexpressing and deficient mice. *J. Bone Miner. Res.* **25**, 178–189 (2010).
117. Robling, A.G. *et al.* Anabolic and catabolic regimens of human parathyroid hormone 1–34 elicit bone- and envelope-specific attenuation of skeletal effects in Sost-deficient mice. *Endocrinology* **152**, 2963–2975 (2011).
118. Rhee, Y. *et al.* PTH receptor signaling in osteocytes governs periosteal bone formation and intracortical remodeling. *J. Bone Miner. Res.* **26**, 1035–1046 (2011).
119. Bodine, P.V., Seestaller-Wehr, L., Kharode, Y.P., Bex, F.J. & Komm, B.S. Bone anabolic effects of parathyroid hormone are blunted by deletion of the Wnt antagonist secreted frizzled-related protein-1. *J. Cell. Physiol.* **210**, 352–357 (2007).
120. Yao, W. *et al.* Overexpression of secreted frizzled-related protein 1 inhibits bone formation and attenuates parathyroid hormone bone anabolic effects. *J. Bone Miner. Res.* **25**, 190–199 (2010).
121. O'Brien, C.A. *et al.* Control of bone mass and remodeling by PTH receptor signaling in osteocytes. *PLoS ONE* **3**, e2942 (2008).
122. Powell, W.F. Jr. *et al.* Targeted ablation of the PTH/PTHrP receptor in osteocytes impairs bone structure and homeostatic calcemic responses. *J. Endocrinol.* **209**, 21–32 (2011).
123. Wan, M. *et al.* Parathyroid hormone signaling through low-density lipoprotein–related protein 6. *Genes Dev.* **22**, 2968–2979 (2008).
124. Wu, J.Y. *et al.* Gsα enhances commitment of mesenchymal progenitors to the osteoblast lineage but restrains osteoblast differentiation in mice. *J. Clin. Invest.* **121**, 3492–3504 (2011).
125. Sieber, C., Kopf, J., Hiepen, C. & Knaus, P. Recent advances in BMP receptor signaling. *Cytokine Growth Factor Rev.* **20**, 343–355 (2009).
126. Guo, X. & Wang, X.F. Signaling cross-talk between TGF-β/BMP and other pathways. *Cell Res.* **19**, 71–88 (2009).
127. Lin, G.L. & Hankenson, K.D. Integration of BMP, Wnt, and Notch signaling pathways in osteoblast differentiation. *J. Cell. Biochem.* **112**, 3491–3501 (2011).
128. Kamiya, N. The role of BMPs in bone anabolism and their potential targets SOST and DKK1. *Curr. Mol. Pharmacol.* **5**, 153–163 (2012).
129. Bain, G., Müller, T., Wang, X. & Papkoff, J. Activated β-catenin induces osteoblast differentiation of C3H10T1/2 cells and participates in BMP2 mediated signal transduction. *Biochem. Biophys. Res. Commun.* **301**, 84–91 (2003).
130. Lintern, K.B., Guidato, S., Rowe, A., Saldanha, J.W. & Itasaki, N. Characterization of wise protein and its molecular mechanism to interact with both Wnt and BMP signals. *J. Biol. Chem.* **284**, 23159–23168 (2009).
131. Kamiya, N., Kaartinen, V.M. & Mishina, Y. Loss-of-function of ACVR1 in osteoblasts increases bone mass and activates canonical Wnt signaling through suppression of Wnt inhibitors SOST and DKK1. *Biochem. Biophys. Res. Commun.* **414**, 326–330 (2011).
132. Kaplan, F.S. *et al.* Investigations of activated ACVR1/ALK2, a bone morphogenetic protein type I receptor, that causes fibrodysplasia ossificans progressiva. *Methods Enzymol.* **484**, 357–373 (2010).
133. Baud'huin, M. *et al.* A soluble bone morphogenetic protein type IA receptor increases bone mass and bone strength. *Proc. Natl. Acad. Sci. USA* **109**, 12207–12212 (2012).
134. Canalis, E., Brunet, L.J., Parker, K. & Zanotti, S. Conditional inactivation of noggin in the postnatal skeleton causes osteopenia. *Endocrinology* **153**, 1616–1626 (2012).
135. Mbalaviele, G., Shin, C.S. & Civitelli, R. Cell-cell adhesion and signaling through cadherins: connecting bone cells in their microenvironment. *J. Bone Miner. Res.* **21**, 1821–1827 (2006).
136. Haÿ, E. *et al.* N-cadherin interacts with axin and LRP5 to negatively regulate Wnt/β-catenin signaling, osteoblast function, and bone formation. *Mol. Cell. Biol.* **29**, 953–964 (2009).
137. Barros, E.R., Dias da Silva, M.R., Kunii, I.S. & Lazaretti-Castro, M. Three years follow-up of pamidronate therapy in two brothers with osteoporosis-pseudoglioma syndrome (OPPG) carrying an *LRP5* mutation. *J. Pediatr. Endocrinol. Metab.* **21**, 811–818 (2008).
138. Cheung, W.M. *et al.* A family with osteoporosis pseudoglioma syndrome due to compound heterozygosity of two novel mutations in the *LRP5* gene. *Bone* **39**, 470–476 (2006).
139. Laine, C.M. *et al.* Novel mutations affecting LRP5 splicing in patients with osteoporosis-pseudoglioma syndrome (OPPG). *Eur. J. Hum. Genet.* **19**, 875–881 (2011).
140. Streeten, E.A. *et al.* Osteoporosis-pseudoglioma syndrome: description of 9 new cases and beneficial response to bisphosphonates. *Bone* **43**, 584–590 (2008).
141. Korvala, J. *et al.* Mutations in LRP5 cause primary osteoporosis without features of OI by reducing Wnt signaling activity. *BMC Med. Genet.* **13**, 26 (2012).
142. Rickels, M.R., Zhang, X., Mumm, S. & Whyte, M.P. Oropharyngeal skeletal disease accompanying high bone mass and novel LRP5 mutation. *J. Bone Miner. Res.* **20**, 878–885 (2005).
143. Van Wesenbeeck, L. *et al.* 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 (2003).
144. Mani, A. *et al.* LRP6 mutation in a family with early coronary disease and metabolic risk factors. *Science* **315**, 1278–1282 (2007).
145. Kim, S.J. *et al.* Identification of signal peptide domain SOST mutations in autosomal dominant craniodiaphyseal dysplasia. *Hum. Genet.* **129**, 497–502 (2011).
146. van Lierop, A.H. *et al.* Patients with sclerosteosis and disease carriers: human models of the effect of sclerostin on bone turnover. *J. Bone Miner. Res.* **26**, 2804–2811 (2011).
147. Hall, C.L., Kang, S., MacDougald, O.A. & Keller, E.T. Role of Wnts in prostate cancer bone metastases. *J. Cell. Biochem.* **97**, 661–672 (2006).
148. Pinzone, J.J. *et al.* The role of Dickkopf-1 in bone development, homeostasis, and disease. *Blood* **113**, 517–525 (2009).
149. Kansara, M. *et al.* Wnt inhibitory factor 1 is epigenetically silenced in human osteosarcoma, and targeted disruption accelerates osteosarcomagenesis in mice. *J. Clin. Invest.* **119**, 837–851 (2009).
150. Perdu, B. *et al.* Osteopathia striata with cranial sclerosis owing to WTX gene defect. *J. Bone Miner. Res.* **25**, 82–90 (2010).
151. Moisan, A. *et al.* The WTX tumor suppressor regulates mesenchymal progenitor cell fate specification. *Dev. Cell* **20**, 583–596 (2011).
152. Sims, A.M. *et al.* Genetic analyses in a sample of individuals with high or low BMD shows association with multiple Wnt pathway genes. *J. Bone Miner. Res.* **23**, 499–506 (2008).
153. van Meurs, J.B. *et al.* GENOMOS Study. Large-scale analysis of association between LRP5 and LRP6 variants and osteoporosis. *J. Am. Med. Assoc.* **299**, 1277–1290 (2008).
154. Estrada, K. *et al.* Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture. *Nat. Genet.* **44**, 491–501 (2012).
155. Li, W.F. *et al.* Genetics of osteoporosis: accelerating pace in gene identification and validation. *Hum. Genet.* **127**, 249–285 (2010).
156. Richards, J.B. *et al.* Bone mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study. *Lancet* **371**, 1505–1512 (2008).
157. Rivadeneira, F. *et al.* Twenty bone-mineral-density loci identified by large-scale meta-analysis of genome-wide association studies. *Nat. Genet.* **41**, 1199–1206 (2009).
158. Styrkarsdottir, U. *et al.* Multiple genetic loci for bone mineral density and fractures. *N. Engl. J. Med.* **358**, 2355–2365 (2008).
159. Styrkarsdottir, U. *et al.* New sequence variants associated with bone mineral density. *Nat. Genet.* **41**, 15–17 (2009).
160. Huang, Q.Y., Li, G.H. & Kung, A.W. The –9247 T/C polymorphism in the *SOST* upstream regulatory region that potentially affects C/EBPα and FOXA1 binding is associated with osteoporosis. *Bone* **45**, 289–294 (2009).
161. Yerges, L.M. *et al.* High-density association study of 383 candidate genes for volumetric BMD at the femoral neck and lumbar spine among older men. *J. Bone Miner. Res.* **24**, 2039–2049 (2009).
162. Leupin, O. *et al.* Control of the SOST bone enhancer by PTH using MEF2 transcription factors. *J. Bone Miner. Res.* **22**, 1957–1967 (2007).
163. Miller, P.D. & Derman, R.J. What is the best balance of benefits and risks among anti-resorptive therapies for postmenopausal osteoporosis? *Osteoporos. Int.* **21**, 1793–1802 (2010).
164. Baron, R., Ferrari, S. & Russell, R.G. Denosumab and bisphosphonates: different mechanisms of action and effects. *Bone* **48**, 677–692 (2011).
165. Rubin, M.R. & Bilezikian, J.P. Parathyroid hormone as an anabolic skeletal therapy. *Drugs* **65**, 2481–2498 (2005).
166. Hodsman, A.B. *et al.* Parathyroid hormone and teriparatide for the treatment of osteoporosis: a review of the evidence and suggested guidelines for its use. *Endocr. Rev.* **26**, 688–703 (2005).
167. McClung, M.R. *et al.* Opposite bone remodeling effects of teriparatide and alendronate in increasing bone mass. *Arch. Intern. Med.* **165**, 1762–1768 (2005).
168. Paszty, C., Turner, C.H. & Robinson, M.K. Sclerostin: a gem from the genome leads to bone-building antibodies. *J. Bone Miner. Res.* **25**, 1897–1904 (2010).
169. Padhi, D., Jang, G., Stouch, B., Fang, L. & Posvar, E. Single-dose, placebo-controlled, randomized study of AMG 785, a sclerostin monoclonal antibody. *J. Bone Miner. Res.* **26**, 19–26 (2011).
170. Kramer, I., Keller, H., Leupin, O. & Kneissel, M. Does osteocytic SOST suppression mediate PTH bone anabolism? *Trends Endocrinol. Metab.* **21**, 237–244 (2010).
171. Fox, J. *et al.* Effects of daily treatment with parathyroid hormone 1–84 for 16 months on density, architecture and biomechanical properties of cortical bone in adult ovariectomized rhesus monkeys. *Bone* **41**, 321–330 (2007).
172. Lindsay, R. *et al.* Effects of a one-month treatment with PTH(1–34) on bone formation on cancellous, endocortical, and periosteal surfaces of the human ilium. *J. Bone Miner. Res.* **22**, 495–502 (2007).

173. Li, X. *et al.* Sclerostin antibody treatment increases bone formation, bone mass, and bone strength in a rat model of postmenopausal osteoporosis. *J. Bone Miner. Res.* **24**, 578–588 (2009).
174. Ominsky, M.S. *et al.* Two doses of sclerostin antibody in cynomolgus monkeys increases bone formation, bone mineral density, and bone strength. *J. Bone Miner. Res.* **25**, 948–959 (2010).
175. Crabtree, N. *et al.* Intracapsular hip fracture and the region-specific loss of cortical bone: analysis by peripheral quantitative computed tomography. *J. Bone Miner. Res.* **16**, 1318–1328 (2001).
176. Epstein, S. Is cortical bone hip? What determines cortical bone properties? *Bone* **41** (suppl. 1), S3–S8 (2007).
177. Zebaze, R.M. *et al.* Intracortical remodelling and porosity in the distal radius and post-mortem femurs of women: a cross-sectional study. *Lancet* **375**, 1729–1736 (2010).
178. Glantschnig, H. *et al.* A rate-limiting role for dickkopf-1 in bone formation and the remediation of bone loss in mouse and primate models of postmenopausal osteoporosis by an experimental therapeutic antibody. *J. Pharmacol. Exp. Ther.* **338**, 568–578 (2011).
179. Fulciniti, M. *et al.* Anti-DKK1 mAb (BHQ880) as a potential therapeutic agent for multiple myeloma. *Blood* **114**, 371–379 (2009).
180. Heath, D.J. *et al.* Inhibiting Dickkopf-1 (Dkk1) removes suppression of bone formation and prevents the development of osteolytic bone disease in multiple myeloma. *J. Bone Miner. Res.* **24**, 425–436 (2009).
181. Sieper, J. Spondyloarthropathies in 2010: new insights into therapy—TNF blockade and beyond. *Nat. Rev. Rheumatol.* **7**, 78–80 (2011).
182. Diarra, D. *et al.* Dickkopf-1 is a master regulator of joint remodeling. *Nat. Med.* **13**, 156–163 (2007).
183. Krönke, G. *et al.* R-spondin 1 protects against inflammatory bone damage during murine arthritis by modulating the Wnt pathway. *Arthritis Rheum.* **62**, 2303–2312 (2010).
184. Uderhardt, S. *et al.* Blockade of Dickkopf (DKK)-1 induces fusion of sacroiliac joints. *Ann. Rheum. Dis.* **69**, 592–597 (2010).
185. Appel, H. *et al.* Altered skeletal expression of sclerostin and its link to radiographic progression in ankylosing spondylitis. *Arthritis Rheum.* **60**, 3257–3262 (2009).
186. Agholme, F., Li, X., Isaksson, H., Ke, H.Z. & Aspenberg, P. Sclerostin antibody treatment enhances metaphyseal bone healing in rats. *J. Bone Miner. Res.* **25**, 2412–2418 (2010).
187. Ominsky, M.S. *et al.* Inhibition of sclerostin by monoclonal antibody enhances bone healing and improves bone density and strength of nonfractured bones. *J. Bone Miner. Res.* **26**, 1012–1021 (2011).
188. Gaur, T. *et al.* Secreted frizzled related protein 1 is a target to improve fracture healing. *J. Cell. Physiol.* **220**, 174–181 (2009).
189. Komatsu, D.E. *et al.* Modulation of Wnt signaling influences fracture repair. *J. Orthop. Res.* **28**, 928–936 (2010).
190. Agholme, F., Isaksson, H., Kuhstoss, S. & Aspenberg, P. The effects of Dickkopf-1 antibody on metaphyseal bone and implant fixation under different loading conditions. *Bone* **48**, 988–996 (2011).
191. Sinder, B.P. *et al.* Sclerostin antibody improves skeletal parameters in a Brtl/+ mouse model of osteogenesis imperfecta. *J. Bone Miner. Res.* **28**, 73–80 (2013).
192. Bodine, P.V. *et al.* A small molecule inhibitor of the Wnt antagonist secreted frizzled-related protein-1 stimulates bone formation. *Bone* **44**, 1063–1068 (2009).
193. Moore, W.J. *et al.* Modulation of Wnt signaling through inhibition of secreted frizzled-related protein I (sFRP-1) with N-substituted piperidinyl diphenylsulfonyl sulfonamides: part II. *Bioorg. Med. Chem.* **18**, 190–201 (2010).
194. Shi, M. *et al.* Identification of iminooxothiazolidines as secreted frizzled related protein-1 inhibitors. *Bioorg. Med. Chem. Lett.* **19**, 6337–6339 (2009).
195. Warden, S.J. *et al.* Psychotropic drugs have contrasting skeletal effects that are independent of their effects on physical activity levels. *Bone* **46**, 985–992 (2010).
196. Kulkarni, N.H. *et al.* 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 (2006).
197. Bolton, J.M. *et al.* Fracture risk from psychotropic medications: a population-based analysis. *J. Clin. Psychopharmacol.* **28**, 384–391 (2008).
198. Vestergaard, P., Rejnmark, L. & Mosekilde, L. Reduced relative risk of fractures among users of lithium. *Calcif. Tissue Int.* **77**, 1–8 (2005).
199. Borchert, K.M. *et al.* High-content screening assay for activators of the Wnt/Fzd pathway in primary human cells. *Assay Drug Dev. Technol.* **3**, 133–141 (2005).
200. Verkaar, F., van der Stelt, M., Blankesteijn, W.M., van der Doelen, A.A. & Zaman, G.J. Discovery of novel small molecule activators of β-catenin signaling. *PLoS ONE* **6**, e19185 (2011).
201. Johnson, E.B., Hammer, R.E. & Herz, J. Abnormal development of the apical ectodermal ridge and polysyndactyly in Megf7-deficient mice. *Hum. Mol. Genet.* **14**, 3523–3538 (2005).
202. Collette, N.M., Genetos, D.C., Murugesh, D., Harland, R.M. & Loots, G.G. Genetic evidence that SOST inhibits WNT signaling in the limb. *Dev. Biol.* **342**, 169–179 (2010).
203. Niemann, S. *et al.* Homozygous WNT3 mutation causes tetra-amelia in a large consanguineous family. *Am. J. Hum. Genet.* **74**, 558–563 (2004).
204. Woods, C.G. *et al.* Mutations in WNT7A cause a range of limb malformations, including Fuhrmann syndrome and Al-Awadi/Raas-Rothschild/Schinzel phocomelia syndrome. *Am. J. Hum. Genet.* **79**, 402–408 (2006).
205. Galceran, J., Farinas, I., Depew, M.J., Clevers, H. & Grosschedl, R. Wnt3a$^{-/-}$-like phenotype and limb deficiency in Lef1$^{-/-}$Tcf1$^{-/-}$ mice. *Genes Dev.* **13**, 709–717 (1999).
206. Parr, B.A. & McMahon, A.P. Dorsalizing signal Wnt-7a required for normal polarity of D-V and A-P axes of mouse limb. *Nature* **374**, 350–353 (1995).
207. Guo, X. *et al.* Wnt/β-catenin signaling is sufficient and necessary for synovial joint formation. *Genes Dev.* **18**, 2404–2417 (2004).
208. Hartmann, C. & Tabin, C.J. Wnt-14 plays a pivotal role in inducing synovial joint formation in the developing appendicular skeleton. *Cell* **104**, 341–351 (2001).
209. Wang, B., Sinha, T., Jiao, K., Serra, R. & Wang, J. Disruption of PCP signaling causes limb morphogenesis and skeletal defects and may underlie Robinow syndrome and brachydactyly type B. *Hum. Mol. Genet.* **20**, 271–285 (2011).
210. Jin, Y.R., Turcotte, T.J., Crocker, A.L., Han, X.H. & Yoon, J.K. The canonical Wnt signaling activator, R-spondin2, regulates craniofacial patterning and morphogenesis within the branchial arch through ectodermal-mesenchymal interaction. *Dev. Biol.* **352**, 1–13 (2011).
211. DeChiara, T.M. *et al.* Ror2, encoding a receptor-like tyrosine kinase, is required for cartilage and growth plate development. *Nat. Genet.* **24**, 271–274 (2000).
212. Lyashenko, N. *et al.* Mice lacking the orphan receptor ror1 have distinct skeletal abnormalities and are growth retarded. *Dev. Dyn.* **239**, 2266–2277 (2010).
213. Mikels, A., Minami, Y. & Nusse, R. Ror2 receptor requires tyrosine kinase activity to mediate Wnt5A signaling. *J. Biol. Chem.* **284**, 30167–30176 (2009).
214. Witte, F., Chan, D., Economides, A.N., Mundlos, S. & Stricker, S. Receptor tyrosine kinase-like orphan receptor 2 (ROR2) and Indian hedgehog regulate digit outgrowth mediated by the phalanx-forming region. *Proc. Natl. Acad. Sci. USA* **107**, 14211–14216 (2010).
215. Tu, X. *et al.* Noncanonical Wnt signaling through G protein–linked PKCδ activation promotes bone formation. *Dev. Cell* **12**, 113–127 (2007).
216. Takada, I. *et al.* A histone lysine methyltransferase activated by non-canonical Wnt signalling suppresses PPAR-γ transactivation. *Nat. Cell Biol.* **9**, 1273–1285 (2007).
217. Albers, J. *et al.* Control of bone formation by the serptentine receptor Frizzled-9. *J. Cell Biol.* **192**, 1057–1072 (2011).
218. Bodine, P.V. *et al.* The Wnt antagonist secreted frizzled-related protein-1 is a negative regulator of trabecular bone formation in adult mice. *Mol. Endocrinol.* **18**, 1222–1237 (2004).
219. Häusler, K.D. *et al.* Secreted frizzled-related protein-1 inhibits RANKL-dependent osteoclast formation. *J. Bone Miner. Res.* **19**, 1873–1881 (2004).
220. Nakanishi, R. *et al.* Osteoblast-targeted expression of Sfrp4 in mice results in low bone mass. *J. Bone Miner. Res.* **23**, 271–277 (2008).
221. Berndt, T. *et al.* Secreted frizzled-related protein 4 is a potent tumor-derived phosphaturic agent. *J. Clin. Invest.* **112**, 785–794 (2003).
222. Cho, H.Y. *et al.* Transgenic mice overexpressing secreted frizzled-related proteins (sFRP)4 under the control of serum amyloid P promoter exhibit low bone mass but did not result in disturbed phosphate homeostasis. *Bone* **47**, 263–271 (2010).
223. Christov, M., Koren, S., Yuan, Q., Baron, R. & Lanske, B. Genetic ablation of sfrp4 in mice does not affect serum phosphate homeostasis. *Endocrinology* **152**, 2031–2036 (2011).
224. Morello, R. *et al.* Brachy-syndactyly caused by loss of Sfrp2 function. *J. Cell. Physiol.* **217**, 127–137 (2008).
225. Nakajima, H. *et al.* Wnt modulators, SFRP-1, and SFRP-2 are expressed in osteoblasts and differentially regulate hematopoietic stem cells. *Biochem. Biophys. Res. Commun.* **390**, 65–70 (2009).
226. Cho, S.W. *et al.* Wnt inhibitory factor (WIF)-1 inhibits osteoblastic differentiation in mouse embryonic mesenchymal cells. *Bone* **44**, 1069–1077 (2009).