Building bone to reverse osteoporosis and repair fractures

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An important, unfilled clinical need is the development of new approaches to improve fracture healing and to treat osteoporosis by increasing bone mass. Recombinant forms of bone morphogenetic protein 2 (BMP2) and BMP7 are FDA approved to promote spinal fusion and fracture healing, respectively, and the first FDA-approved anabolic drug for osteoporosis, parathyroid hormone, increases bone mass when administered intermittently but can only be given to patients in the US for two years. As we discuss here, the tremendous explosion over the last two decades in our fundamental understanding of the mechanisms of bone remodeling has led to the prospect of mechanism-based anabolic therapies for bone disorders.

There are currently a number of FDA-approved drugs for the prevention and treatment of osteoporosis that work by inhibiting bone resorption (i.e., they are anticatabolic). However, these compounds, which include bisphosphonates, calcitonin, and selective estrogen receptor modulators, only prevent further loss of bone; they do not stimulate new bone formation. The only FDA-approved compound capable of stimulating new bone formation (and thus reversing bone loss) is parathyroid hormone (PTH). However, PTH is a protein that needs to be administered subcutaneously, and patients generally prefer taking an oral medication; there are potential side effects (e.g., hypercalcemia and hypercalciuria) associated with its use; and the duration of treatment with this drug is limited to 18 months in Europe and 24 months in the US, because rodents administered high doses of PTH were shown to develop osteosarcomas (1). Thus, there is a clear clinical need to develop new bone anabolic agents (particularly small molecules that can be used orally), and understanding the molecular details of the pathways that control bone formation is critical for the development of novel approaches to reverse osteoporosis.

It might be possible to modulate these pathways not only for the treatment osteoporosis, but also to accelerate the healing of fractures and to treat the 5%–10% of fractures that fail to heal satisfactorily (2, 3). This Review focuses on current approaches, as well as those on the horizon, that have the potential to achieve these goals. Although the osteoblast, as the bone-forming cell, is the obvious target for agents that aim to mediate bone anabolism, other cells are now also being considered as therapeutic targets. For example, osteocytes, cells that have historically largely been ignored because they lie entombed in the bone matrix, and osteoclasts, the bone-resorbing cells that have long been a target for agents that are anti-catabolic, are now also potential targets for drugs that stimulate bone anabolism. The development of agents that target more than one of these cell types might be the most effective therapy, as there is some evidence to suggest that PTH utilizes all of the above cell types in achieving its anabolic effects on bone.

Bone remodeling: evolving concepts

Although macroscopically the skeleton seems to be a static organ, it is an extremely dynamic tissue at the microscopic level. Its ability to sustain the tremendous loads placed on it in everyday life depend on, among other factors, being able to remodel and repair the constant microcracks that develop both in cancellous bone — the "spongy" bone present in the vertebrae, pelvis, and ends (metaphyses) of long bones — and in cortical bone — the compact bone present in the shafts (diaphyses) of the long bones and surrounding cancellous bone in the vertebrae and pelvis. Since remodeling sites in cancellous bone in the vertebrae and pelvis are close to red marrow, which is known to contain osteoprogenitor cells (4), whereas remodeling sites in cortical bone are distant from red marrow, it had been assumed that the mechanisms of bone remodeling were likely to be different in cancellous versus cortical bone. Specifically, the assumption was that the cells needed for bone remodeling traveled directly from the red marrow to bone surfaces in cancellous bone, whereas they accessed cortical bone via the vasculature (5). However, it now seems that the fundamental mechanisms of bone remodeling might be very similar in both bone compartments, occurring in what has been termed the basic multicellular unit (BMU), which comprises the osteoclasts, osteoblasts, and osteocytes within the bone-remodeling cavity (Figure 1). Although the existence of the BMU has been established for a long time, the intimate relationship between the BMU and the vasculature, particularly in cancellous bone, was less well appreciated. This intimate relationship was initially described by Burkhardt et al. (6) more than 20 years ago and analyzed in detail in subsequent studies by Hauge and colleagues (7). These investigators demonstrated that the cells in the BMU, even in cancellous bone, were not directly contiguous to the bone marrow, but rather they were covered by a "canopy" of cells (most probably bone-lining cells) that seem to be connected to bone-lining cells on the quiescent bone surface (7). In turn, these bone-lining cells on the quiescent bone surface are in communication with osteocytes embedded within the bone matrix (7). Penetrating the canopy of bone-lining cells, and presumably serving as a conduit for the cells needed in the BMU, are capillaries; thus, the work of Hauge et al. (7) placed the BMU (consisting of osteoclasts, osteoblasts, and osteocytes), both in cancellous (Figure 1A) and in cortical (Figure 1B) bone, within the bone remodeling compartment (BRC), which comprises the BMU, the canopy of bone-lining cells, and the associated capillaries (Figure 1C and depicted schematically in Figure 1D).
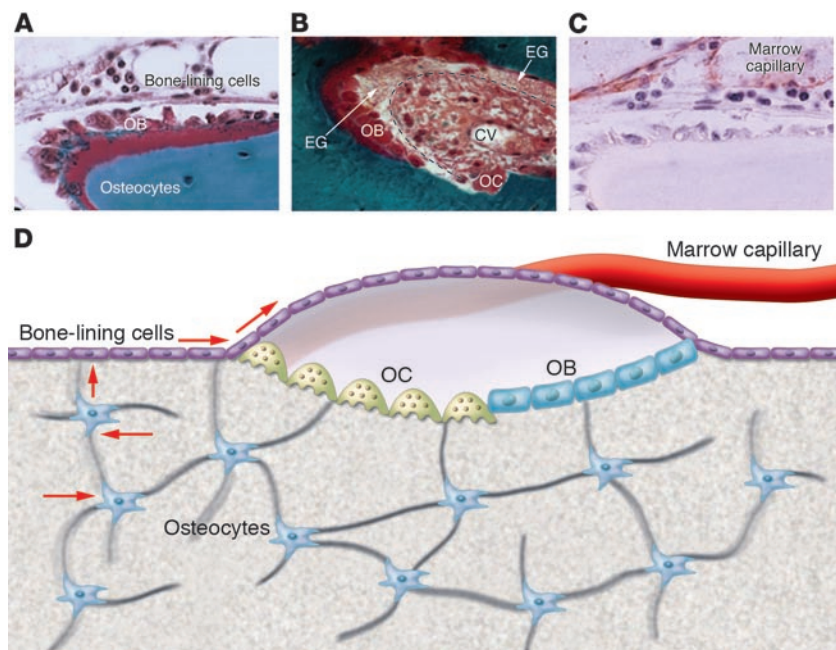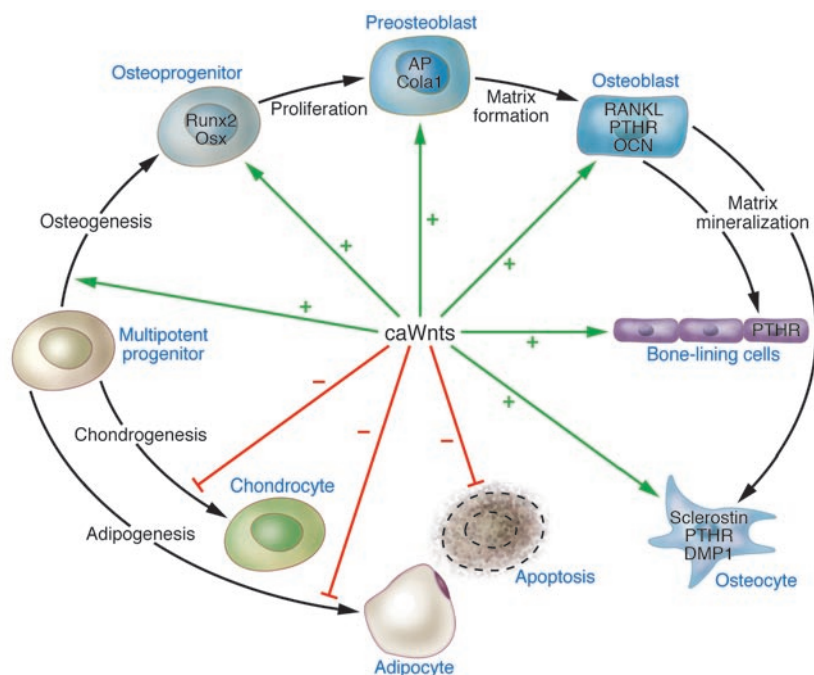
Figure 1
Histology (**A**–**C**; original magnification, ×40) and composite schematic (**D**) of the BRC, which comprises the cells constituting the BMU — specifically osteoclasts (OCs), osteoblasts (OBs), and osteocytes — as well as the canopy of bone-lining cells and the associated capillary. (**A**) BRC in cancellous bone, demonstrating the location of the OBs along the bone-forming surface. The osteocytes are shown embedded in the bone matrix and the canopy of cells consists of bone-lining cells. (**B**) BRC in cortical bone (outer demarcation indicated by the broken line) that is filled with erythrocyte ghosts (EG) and OBs; a few OCs are also seen. CV denotes the central vessel of the Haversian system, which forms the basic structural unit in cortical bone. (**C**) BRC stained with an antibody specific for CD34, which demonstrates staining of endothelial cells in the marrow capillary adjacent to the BRC. (**D**) Composite schematic of the BRC, showing connections between the osteocyte network, surface bone-lining cells, and the BRC. All cells in this network are connected with gap junctions, which might provide a pathway (arrows) by which signals generated by osteocytes deep within the bone reach the surface and elicit remodeling events by OCs and OBs. Note also the potential direct physical contact between OCs and OBs, which would allow for signaling between these cells. **A** and **C** are reproduced from Hauge et al. (7), and **B** and **D** from Eriksen et al. (10), with permission from the American Society for Bone and Mineral Research.

Given the structure of the BRC, it becomes easier to understand the key role that osteocytes have in controlling bone remodeling, despite being imprisoned in the bone matrix. It is now clear that osteocytes can sense microcracks and mechanical strain and be responsive to changes in the hormonal milieu of the bone (e.g., estrogen deficiency) to essentially "trigger" bone remodeling, perhaps by communicating with bone-lining cells (8, 9). The bone-lining cells, in turn, probably initiate the process of bone resorption (8), attract osteoclasts, and, together with adjoining bone-lining cells, begin to form the roof of the BRC. By analogy with remodeling in cortical bone, which is clearly associated with growth of a blood vessel into the remodeling site (5), the presumed ingrowth of a capillary into the BRC provides the vascular supply for the cells in the BMU of cancellous bone and might also provide the necessary osteoclasts and, subsequently, the osteoblasts that are needed for bone remodeling in both cancellous and cortical bone. Within the BMU, preosteoblastic cells, which express RANKL (10), probably control the differentiation of osteoclasts from hemato-

poietic progenitors; in turn, completion of the bone resorption phase is followed by a wave of bone formation, driven, in part, by factors produced by the osteoclast that stimulate osteoblast differentiation and activity, perhaps via direct cell-cell contact between the osteoclast and osteoblast within the confines of the BRC. In the context of this orchestrated activity in the BMU, there are thus a number of cells that can be targeted by agents that increase bone mass: the osteoblast, the osteocyte, the osteoclast, and perhaps even the endothelial cell in the vasculature. Indeed, a recent study by Wang et al. (11) showed that increased VEGF production by osteoblastic cells has a marked anabolic effect on bone, apparently due to increased angiogenesis and a subsequent influx of osteoblasts onto bone surfaces. However, although the interactions of endothelial cells with osteoblasts is an active area of investigation (reviewed in ref. 12), a detailed discussion of this is beyond the scope of the present Review.

Targeting osteoblast differentiation to enhance bone formation

Overview of osteoblast differentiation. Numerous secreted factors of paracrine, autocrine, and endocrine origin influence osteoblast development and maturation. These include some bone morphogenetic proteins (BMPs), PTH, FGF, IGF, endothelin-1, and prostaglandin agonists (13–16). Only a few are used clinically in the US to promote bone formation in response to injury or aging. Recombinant human BMP2 is used clinically to mediate spinal fusions, and BMP7 (also known as OP1) is used for the treatment of nonunion of long-bone fractures that occur secondary to trauma and for which an allograft is unsuitable. Neither is approved for osteoporosis therapy, because they both have a short half-life and cannot be administered systemically. As mentioned earlier, PTH is FDA approved to increase bone formation in patients with osteoporosis; however, it can only be prescribed for two years in the US. Thus, there is a clear need for more anabolic therapies to promote osteoblast activity. Targeting the Wnt pathway is one promising avenue. Available evidence indicates that Wnts and PTH stimulate bone formation via complementary pathways (17). BMP2 also synergizes with canonical Wnts to promote bone formation, and there seems to be considerable crosstalk between the Wnt and BMP signaling pathways (18, 19).

Osteoblasts are mesenchymal cells derived from mesodermal and neural crest progenitors. As cells of the osteoblast lineage differentiate, they produce molecules essential for construction of the mineralized bone matrix and for support of hematopoiesis and angiogenesis. Sequential expression of several molecules facilitates the differentiation of the progenitor cell into a proliferating preosteoblast, then into a bone matrix–producing osteoblast, and eventually into a mechanosensory osteocyte or a bone-lining cell (Figure 2). Runt-related transcription factor 2 (Runx2) is the earliest marker of an osteoblast lineage cell and is necessary, but not

Figure 2
The central role of canonical Wnt signaling in regulating osteoblast lineage specification, expansion, and terminal differentiation. Osteoblasts are derived from multipotent mesodermal or neural crest progenitors. Activation of the canonical (ca) Wnt signaling pathway, manifest through β-catenin stabilization, prevents the formation of cartilage (chondrogenesis). Wnt10b prevents adipogenesis (40). The canonical Wnt signaling pathway promotes survival of all cells of the osteoblast lineage and induces the proliferation of preosteoblasts. +, canonical Wnt signaling promotes the process; –, canonical Wnt signaling inhibits the process; AP, alkaline phosphatase; Cola1, collagen α1; DMP1, dentin matrix protein 1; OCN, osteocalcin; Osx, osterix; PTHR, receptor for PTH.

sufficient, for a progenitor cell to differentiate along the osteoblast lineage (20, 21). Runx2 regulates cellular proliferation, integrates numerous signaling pathways, and controls the expression of many genes (e.g., those encoding osteocalcin, VEGF, RANKL, sclerostin, and dentin matrix protein 1 [DMP1]) throughout osteoblast maturation (22, 23). Osterix is another transcription factor essential for the formation of preosteoblasts from multipotent progenitors (24). It acts downstream of Runx2, but its expression is downregulated in bone marrow stromal cells by endothelial cells (25); osterix, in turn, impairs osteoblast differentiation (24). Osterix is also a suppressor of VEGF expression following endothelin-1 exposure (26); therefore, it might be a crucial mediator of osteoblast function within the context of the BRC. During the matrix-forming and maturation phases of osteoblast differentiation, the simultaneous coexpression of alkaline phosphatase and type I collagen sets the stage for mineralization (27). Mature osteoblasts also produce osteocalcin, RANKL, and the receptor for PTH (PTHR1), which, among other actions, regulate bone formation and resorption. As osteoblasts become embedded in the mineralized matrix, they transform into osteocytes and begin expressing several molecules, including DMP1 and sclerostin, that control bone formation and phosphate metabolism (28).

Wnts and osteoblast differentiation. Wnts are secreted glycoproteins crucial for the development and homeostatic renewal of many tissues, including bone. Figure 2 depicts the central role played by the canonical Wnt signaling pathway in regulating osteoblast development. Wnts stimulate several signaling pathways by binding a receptor complex consisting of LDL receptor–related protein 5 (LRP5) or LRP6 and one of ten Frizzled (Fz) molecules (18). The canonical Wnt signaling pathway has been the most extensively studied Wnt signaling pathway in osteoblasts. It involves the stabilization of β-catenin and regulation of multiple transcription factors, namely lymphoid enhancer factor 1 (Lef1) and the T cell factors Tcf7 (often referred to as Tcf1), Tcf7L1 (often referred to as Tcf3), and Tcf7L2 (often referred to as Tcf4) (Figure 3). This pathway is active in all

cells of the osteoblast lineage, including preosteoblasts, bone-lining cells, and osteocytes (29). Notably, Wnt/β-catenin signaling is a normal physiological response to mechanical loading (30) and participates in the fracture healing process (31). Wnt signaling has three major functions in osteoblast lineage cells: dictating osteoblast specification from osteo-/chondroprogenitors; stimulating osteoblast proliferation; and enhancing osteoblast and osteocyte survival. Within the BMU, Wnts influence osteoclast maturation by regulating RANKL levels in osteoblasts (32).

The crucial role of the canonical Wnt signaling pathway in bone cells was revealed earlier this decade in seminal studies showing that loss-of-function mutations in the gene encoding LRP5 decrease bone mass, whereas gain-of-function mutations increase bone mass in both humans and mice (33–36). The mouse models also revealed that *Lrp5* is mechanically essential for regulating osteoblast number, through both increasing the proliferation of progenitors and enhancing the survival of committed osteoblasts and osteocytes (35, 36). In accordance with these findings, adding Wnts or deleting secreted Wnt inhibitors enhances osteoblast and osteocyte survival (37–39). Since the LRP5 discoveries, many other components of the Wnt signaling pathway (e.g., Wnt10b, β-catenin, Tcf1, adenomatosis polyposis coli [APC], Axin-2, secreted frizzled-related protein 1 [Sfrp1], Sfrp4, and dickkopf homolog 1 [Dkk1]) have been deleted or overexpressed in mice. The general conclusion derived from all these studies is that activation of the canonical Wnt signaling pathway facilitates osteoblast specification from mesenchymal progenitors at the expense of adipogenesis and enhances bone mass and strength, whereas suppression causes bone loss (38, 40–50). Several of these studies also revealed that the canonical Wnt signaling pathway cooperates with the essential transcription factors Runx2 and osterix to maintain and promote osteoblast maturation (41–43, 45, 50).

Although the final conclusion (that stimulation of the canonical Wnt signaling pathway increases bone mass) was similar in the above-mentioned studies (38, 40–50), the underlying mechanisms
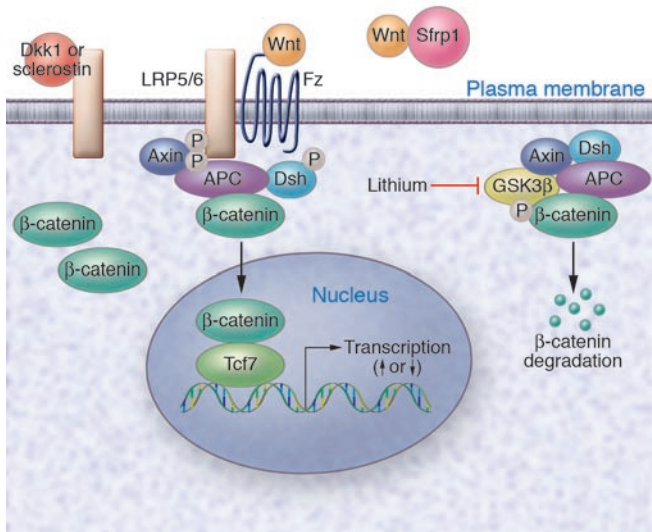
Figure 3
A partial view of the canonical Wnt signaling pathway. Wnts bind a receptor complex consisting of LRP5 or LRP6 and one of ten Fz proteins. This prevents phosphorylation of β-catenin by GSK3β and other kinases and its subsequent degradation. Of note, mutating the residues that can be phosphorylated to alanine creates stable, gain-of-function β-catenin proteins. Stabilized β-catenin accumulates and translocates to the nucleus, where it interacts with Tcf7 and related transcription factors (Lef1, Tcf7L1, Tcf7L2) to regulate gene expression. Outside the cell, molecules that sequester either LRP5 (e.g., Dkk1 and sclerostin) or the Wnt ligand (e.g., Sfrp) negatively control the canonical Wnt signaling pathway. Lithium chloride inhibits GSK3β inside the cell. APC, adenomatosis polyposis coli.

sometimes differed. For example, constitutively active β-catenin increases bone mass by inducing the expression of the gene encoding osteoprotegerin (OPG) and thereby inhibits osteoclast maturation (41, 50). In contrast, *Lrp5* gain-of-function mutations or administration of Wnt3a enhance the proliferation of preosteoblasts and prevent apoptosis of osteoblasts and osteocytes without affecting osteoclasts (35, 37, 51, 52). Thus, activation of Wnt signaling pathways through Wnt proteins binding to their receptors triggers different signaling pathways than if β-catenin is the starting point (37). β-Catenin participates in other signaling pathways, including ones induced by activated receptor tyrosine kinases and nuclear hormone receptors, and physically links E-cadherin to the actin cytoskeleton (53). Additional studies are needed to unravel the complex roles that individual Wnts have within the BMU and to determine the extent to which stabilized β-catenin can be used as a reliable surrogate for measuring Wnt activity.

Much remains to be learned about the molecular mechanisms of Wnt signaling, but it is clear that Wnts are potential targets for therapeutics designed to increase bone mass. One possible therapeutic approach would be to deliver Wnt agonists. The limitation of this approach, however, is that Wnts are historically very difficult and expensive to purify; thus, using Wnts as an anabolic agonist is impractical at this time. Efforts to therapeutically target the Wnt signaling pathway have instead focused on two alternative approaches (Figure 3): the first is to inhibit Wnt antagonists (e.g. Dkk1, sclerostin, and Sfrp1) with neutralizing antibodies (discussed below), and the second is to inhibit glycogen syn-

thase kinase 3β (GSK3β), which is a kinase that phosphorylates β-catenin and promotes its degradation. The latter approach has been accomplished in mice, whereby administration of lithium chloride and other small-molecule inhibitors of GSK3β reversed bone loss caused by aging, estrogen deficiency, and *Lrp5* mutations (54); increased the sensitivity of osteoblasts and osteocytes to mechanical loading (30); and improved fracture healing (31). Lithium chloride is a commonly prescribed mood-stabilizing drug. Two studies examining fracture risk in humans found that lithium chloride use decreased fracture incidence in a dose-dependent manner (55); however, one study found that fracture incidence did not remain lower and actually increased after lithium chloride use was discontinued (56). Thus, the long-term effects of GSK3β inhibitors and other Wnt pathway agonists on bone formation and quality require careful evaluation before they can be used clinically as bone anabolic agents.

The other therapeutic approach being actively pursued to stimulate Wnt signaling to increase bone mass is the use of humanized monoclonal antibodies to neutralize antagonists of the Wnt signaling pathway. The canonical Wnt signaling pathway can be inhibited extracellularly by sequestering either the ligand or the receptor (Figure 3). Dkk1 and sclerostin inhibit Wnt signaling by dissociating LRP5 from Fz and Wnts. Sfrps, in contrast, bind Wnts and prevent them from associating with the LRP5/Fz complex. Serum Dkk1 levels are inversely proportional to bone mass in mice (48, 49, 57) and are a prognostic biomarker of the osteolysis that is associated with multiple myeloma in humans (58). Similarly, Sfrp1 levels are inversely related to bone formation, but in mouse gene deletion studies, the results were more compelling in female mice than in male mice, indicating that Sfrp1 effects might be tied to hormonal influences (38). Sfrp1-specific neutralizing antibodies and antagonists have not yet been described. In contrast, Dkk1-specific neutralizing antibodies have been tested in several studies (59, 60). Diarra and colleagues reversed bone destruction in a mouse model of rheumatoid arthritis by administering Dkk1-specific antibodies (59). This was accompanied by increased numbers of osteoblasts, rates of bone formation, and expression of OPG and by decreased numbers of osteoclasts. Interestingly, osteophytes (bone spurs) developed in the animals treated with Dkk1-specific antibodies, which effectively converted the phenotype to an osteoarthritic model. In a different study using a mouse model of multiple myeloma, a Dkk1-specific antibody promoted bone formation in tumor-bearing and non–tumor-bearing femurs (60). The Dkk1-specific antibody reduced the number of osteoclasts expressing tartrate-resistant acid phosphatase (TRAP) and increased the number of osteoblasts producing osteocalcin. Testing of Dkk1-specific neutralizing antibodies is ongoing in preclinical animal models and these agents are likely to enter phase I clinical trials.

Although early results indicate that Dkk1-specific antibodies can promote bone formation, there are some concerns about their safety and potency. The development of osteophytes following administration of Dkk1-specific antibodies in the mouse model of rheumatoid arthritis suggests a role for Dkk1 in preventing osteoarthritis and bone spurs. However, optimization of dosage and delivery methods might control this outcome, and more studies are needed to define the appropriate doses and routes of delivery. Tumor formation is also a general concern, because the Wnt signaling pathway is activated by mutations in many cancers. However, it is well established that tumor formation requires multiple events; therefore, even though overstimulation of Wnt signaling

pathways might contribute to tumorigenesis, it would be highly unlikely to be the sole initiating event. In anecdotal support of this, neither aged *Dkk1* haploinsufficient mice nor humans using lithium chloride or with high bone mass due to gain-of-function mutations in *LRP5* have increased cancer incidence (61).

Targeting osteocyte function as a potential approach to enhancing bone anabolism

Sclerostin is another promising target of biologic therapies to increase bone mass. Sclerostin is secreted exclusively by osteocytes, apparently as a mechanism to reduce bone formation (62, 63). Accordingly, sclerostin expression levels are repressed in response to mechanical loading and intermittent PTH treatment (64–66). Sclerostin is the product of the *SOST* gene, which is mutated and downregulated in patients with sclerosteosis and van Buchem disease (67, 68), diseases characterized by high bone density. Sclerostin inhibits osteoblast activity and bone formation by sequestering LRP5 and LRP6, thereby slowing the Wnt signaling pathway (Figure 3) (69). Interestingly, an LRP5 mutant that is associated with high bone mass prevents sclerostin from binding LRP5 (70), in both mice and humans. Sclerostin also binds BMPs, but with low affinity, nondiscriminately, and without directly affecting BMP2 target genes in osteoblasts (62, 63). Sclerostin thus seems to indirectly block BMP-induced bone formation by inhibiting Wnt signaling pathways. Preliminary studies with a humanized sclerostin-specific monoclonal antibody were promising and have shown bone anabolic activities in animal models and postmenopausal women (71, 72). Thus, inhibition of sclerostin activity seems to be a bone anabolic approach that is likely to progress rapidly toward clinical testing and ultimate clinical use.

Targeting osteoblast-osteoclast cross-talk as a potential approach to enhancing bone anabolism

Under most conditions, resorbed bone is nearly precisely replaced in location and amount by new bone. Thus, it has long been recognized that bone loss through osteoclast-mediated bone resorption and bone replacement through osteoblast-mediated bone formation are tightly coupled. There is now clear evidence that osteoblasts direct osteoclast differentiation by membrane presentation of RANKL and M-CSF (73, 74). The promotion of osteoclast differentiation by RANKL can be dampened through regulation of expression of the RANKL decoy receptor, OPG (74). In many instances, suppression of OPG, usually combined with increased expression of M-CSF and RANKL, enhances bone resorption (74). However, questions remain as to how osteoblasts are recruited to the site following the resorption phase and how the amount of bone laid down is controlled. This has led to consideration of how osteoclasts and/or their activity could promote bone formation.

Stored within the bone matrix are considerable quantities of several growth factors that could promote bone formation, such as TGF-β, IGF-I, and IGF-II (75, 76). Because osteoclast-mediated bone resorption releases IGFs and TGF-β from bone, it has been posited that these play a part in coupling bone resorption to subsequent bone formation, although there is no direct evidence of this (77, 78). Examination of both mouse models and humans in whom osteoclastogenesis is perturbed has provided important insights into the role of bone resorption in promoting bone formation (79, 80). In mouse models where osteoclasts are present but not able to resorb bone (mice lacking either c-Src or chloride-7 channel), there is no defect in bone formation (81–83). Likewise, in humans

who have one of their genes encoding the chloride-7 channel inactivated, osteoclasts are present, but there is inhibition of bone resorption with no reduction in bone formation (84–86). These data are in sharp contrast to mouse models that result in defective osteoclastogenesis, such as mice lacking c-fos or M-CSF, which have no osteoclasts and also exhibit defective bone formation (87–89). Thus, it seems that the presence of osteoclasts, whether or not they are resorbing bone, is needed for normal bone formation and that the release of bone-bound factors might not be required to couple osteoclasts to the bone formation phase of bone turnover.

What are possible osteoclast-osteoblast coupling factors? One intriguing recent discovery is the bidirectional signaling mediated by the transmembrane proteins ephrinB2 and EPH receptor B4 (EphB4), which are expressed on osteoclasts and osteoblasts, respectively (90). The engagement of these molecules stimulates osteoblast differentiation and represses osteoclast differentiation. A potential scenario is that osteoclast ephrinB2 stimulates osteoblast differentiation by binding EphB4 on osteoprogenitor cells, promoting bone formation. Conversely, EphB4 on osteoblastic cells binding ephrinB2 on osteoclast precursors represses further osteoclast differentiation. These interactions require close physical contact of osteoblast lineage cells with preosteoclasts and mature osteoclasts and are likely to occur in the BRC, as depicted in Figure 1D. Indeed, the close proximity of osteoblast lineage cells to preosteoclasts and mature osteoclasts has already been demonstrated by the finding that membrane-bound RANKL and M-CSF on osteoblast lineage cells promote osteoclast differentiation (91, 92). There is evidence that osteoclasts might also recruit osteoblast lineage cells. Ryu et al. (93) have shown that sphingosine 1-phosphate (S1P), which is produced by osteoclasts, stimulates osteoblast migration, as well as promoting osteoblast survival. Thus, osteoclasts might recruit osteoprogenitors to sites of bone resorption by secretion of S1P and stimulate osteoblast differentiation by stimulating EphB4 signaling. In turn, osteoblast-stimulated reverse signaling through EphB4 binding to ephrinB2 on osteoclast precursors would repress osteoclast differentiation. This sequence of events would shut down bone resorption and set the stage for the next phase of bone remodeling, when osteoblasts are recruited to the site to replace the resorbed bone. Interest is growing in whether this stage of the bone remodeling cycle could provide novel anabolic targets.

PTH uses multiple pathways to promote bone formation

Although an excess of PTH, as in severe hyperparathyroidism, is often catabolic for bone, Fuller Albright observed almost 80 years ago that PTH can also have anabolic effects on bone (94). This led ultimately to a clinical trial clearly demonstrating that intermittent exposure of bone to PTH can increase bone formation and bone mass in humans (95). This is in marked contrast to continuous PTH exposure, which often leads to bone loss (96). Even though PTH is now approved by the FDA as a bone anabolic agent for patients with osteoporosis, determining the precise mechanism(s) by which it exerts its anabolic effects on bone still remains an area of intensive investigation. Emerging evidence indicates that part of the difficulty in unequivocally defining the mechanism(s) for the anabolic effects of PTH is that these effects probably involve multiple cell types and pathways. Thus, as summarized in Figure 4 and discussed below, PTH probably impacts osteoblasts, bone-lining cells, osteocytes, and osteoclasts and activates multiple pathways that collectively result in stimulation of bone formation. This
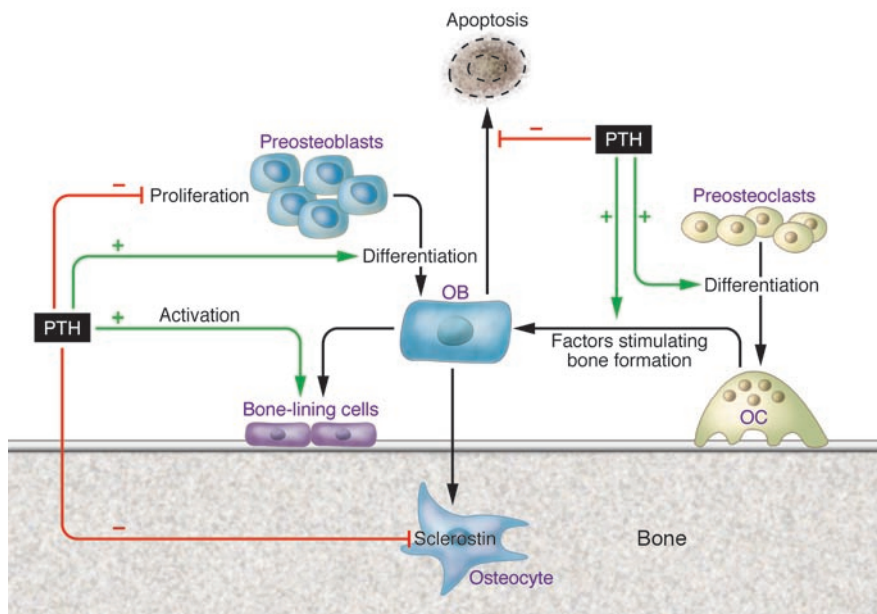
Figure 4
Potential cellular targets for the anabolic effects of PTH. PTH targets multiple cell types to mediate its bone anabolic effects. Specifically, PTH decreases apoptosis and proliferation of osteoblasts (OBs), as well as increasing their differentiation. It can activate bone-lining cells into functioning osteoblasts and decrease sclerostin production by osteocytes, which would be expected to increase Wnt signaling to osteoblasts. Finally, PTH is postulated to stimulate factors produced by osteoclasts (OCs) that stimulate osteoblasts.

complexity has recently been demonstrated in microarray analyses of bones harvested from rats treated with PTH, where a large number of genes (~1,000) were modulated by PTH (97). These included genes involved in Wnt signaling (Wnt4 and Sfrp4), transcription factors (cAMP responsive element modulator [CREM]), growth factors (amphiregulin [AREG]), and chemokines (CCL2, also known as MCP-1]), and understanding how theses genes and gene regulatory networks ultimately result in an anabolic effect on bone is clearly an ongoing challenge.

As recently summarized by Jilka (98), the osteoblast is clearly a target for PTH action. Studies in mice have demonstrated that intermittent PTH treatment inhibits osteoblast apoptosis, at least in cancellous bone, leading to the suggestion that this might account, in large part, for the increase in osteoblast numbers and subsequent increase in bone formation seen following PTH therapy (99, 100). However, inhibition of osteoblast apoptosis by PTH is yet to be confirmed in humans (101); this might, in part, be due to the much lower basal prevalence of osteoblast apoptosis in mice versus humans (98). In terms of the specific mechanisms by which apoptosis is inhibited, studies in cultured osteoblastic cells have shown that PTH rapidly activates antiapoptotic signaling pathways that involve cAMP-mediated activation of PKA and its subsequent phosphorylation and inactivation of the proapoptotic protein BCL2-antagonist of cell death (Bad), as well as increased expression of survival genes such as B cell leukemia/lymphoma 2 (Bcl-2) (100). PTH also seems to decrease osteoblast proliferation, leading to a reciprocal increase in osteoblast differentiation (102); these effects might be due to decreased expression of cyclin D1, which is required for cell cycle progression, as well as increased expression of cyclin-dependent kinase inhibitors (102, 103). Concomitantly, PTH transiently increases the level and activity of Runx2 (100), and there are data supporting a role for LRP5-mediated Wnt signaling in mediating at least part of the anabolic effect of PTH (104). Some of the effects of PTH on osteoblastic cells might also be due to increased production and subsequent autocrine/paracrine effects of IGF-I and FGF-2 (105, 106). In addition, there is also evidence that PTH can "activate" previously quiescent

bone-lining cells on bone surfaces (107), which might explain the very early increase in bone formation seen following PTH therapy. In contrast to these effects on differentiated osteoblastic cells, PTH does not seem to influence the proliferation or differentiation of uncommitted osteoprogenitor cells (99), in part because the PTH receptor is not expressed until these cells have progressed down the osteoblast lineage (108).

As noted earlier, until recently, the osteocyte was regarded as a relatively inert, quiescent cell entrapped in the bone matrix, but there is increasing evidence of a key role for the osteocyte in the regulation of bone remodeling (9). Consistent with this role, PTH has been shown to cause a transient reduction in the level of mRNA encoding sclerostin in osteocytes (65). This would, in turn, lead to increased Wnt signaling in the local microenvironment, and this might be an important mechanism by which PTH regulates osteoblast development via actions on the osteocyte.

Prior to the approval of PTH as a therapy for osteoporosis, there were a number of FDA-approved drugs, such as alendronate, that inhibited bone resorption. Since PTH stimulates bone formation, a logical hypothesis was that the simultaneous administration of PTH with an anticatabolic drug would lead to even larger increases in bone mass than administration of either agent alone. Surprisingly, however, this has proven not to be the case: studies in both women (109) and men (110) show that alendronate in fact blunts the anabolic action of PTH, at least in cancellous bone. This clinical observation — combined with animal work showing that absence of c-fos, which leads to a block in osteoclast differentiation, also results in loss of the anabolic response to PTH (111) — has led to the plausible hypothesis that at least part of the anabolic actions of PTH on bone are mediated by its activation of the osteoclast (79). Indeed, as discussed earlier, there is intense interest in identifying the factor(s) produced by osteoclasts that stimulate bone formation by osteoblasts.

The future of bone anabolic therapies

It is clear that within the closed compartment constituting the BRC, there is an intimate cross-talk among osteoblasts, osteo-

clasts, and bone-lining cells forming the canopy of the BRC. Moreover, the connections between the bone-lining canopy cells and bone-lining cells on the bone surface, which communicate with osteocytes, also allow the osteocytes embedded deep within the bone matrix to influence the numbers and activity of the osteoblasts. Understanding better these complex cellular interactions and the molecules that mediate them, such as those discussed in this Review, will ultimately lead to the development of new compounds, such as the sclerostin-specific antibody discussed above, with potent bone anabolic effects. This would, in turn, fulfill an important clinical need, since, although there are numerous compounds available that inhibit bone resorption, treatment options for reversing bone loss with bone formation–stimulating agents are currently limited to PTH. Interestingly,

it is somewhat ironic that despite the tremendous advances in basic bone biology that might lead to targeted anabolic therapies, PTH, which was discovered empirically to have anabolic effects on bone, continues to remain somewhat of a mystery in terms of its specific mechanisms of action.

Acknowledgments

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