Control of osteoblast function and regulation of bone mass

Shun-ichi Harada & Gideon A. Rodan

Department of Bone Biology & Osteoporosis Research, Merck Research Laboratories, West Point, Pennsylvania 19002, USA (e-mail: gideon_rodan@merck.com)

The skeleton is an efficient 'servo' (feedback-controlled/steady-state) system that continuously integrates signals and responses which sustain its functions of delivering calcium while maintaining strength. In many individuals, bone mass homeostasis starts failing in midlife, leading to bone loss, osteoporosis and debilitating fractures. Recent advances, spearheaded by genetic information, offer the opportunity to stop or reverse this downhill course.

The functions of bones include maintaining blood calcium levels, providing mechanical support to soft tissues and serving as levers for muscle action, supporting haematopoiesis, and housing the brain and spinal cord. These functions are accomplished by continuous tissue renewal, called remodelling, occurring throughout life at approximately two million microscopic sites in the adult skeleton.

Bone destruction or 'resorption' is carried out by haematopoietically derived osteoclasts. Their number and activity is determined by cell lineage allocation, proliferation and differentiation of osteoclast precursors and the resorptive efficiency of mature osteoclasts. Several effective drugs that control osteoclast generation and/or function are currently available (see review in this issue by Boyle *et al.*, page 337).

Mesenchyme-derived osteoblasts rebuild the resorbed bone by elaborating matrix that then becomes mineralized. Injectable parathyroid hormone (PTH) is the only known agent currently available for pharmacological stimulation of bone formation[1]. New insights into osteoblast regulation, reviewed here, could lead to additional bone-building treatments, for example, by controlling the activity of recently discovered genes such *LRP5*, or of newly discovered pathways such as sympathetic neurons. These potential therapies would act in conjunction with all the other factors that control bone mass in humans, some of which are still unknown.

We begin with a brief overview of skeletal homeostasis, and then address a number of questions related to this system, including hierarchies that exist among regulatory factors. This is followed by a review of recent discoveries on the regulation of osteoblast differentiation and function.

Determinants of skeletal homeostasis and bone mass

Bone mass in adults is maintained locally by the balance between osteoclastic bone resorption and osteoblastic bone formation, each of which is subject to controls aimed at fulfilling bone function. Calcium release requires bone destruction, and the principal mediators in this process are PTH and its downstream effector ($1,25(OH)_2$ vitamin D). Mechanical strength depends on bone mass and its deployment relative to mechanical forces (strain). The local structural adaptation of bones to mechanical loads is the basis for orthopaedic and orthodontic procedures. Increased mechanical loads stimulate bone formation and suppress resorption, whereas unloading has the opposite effect[2,3]. The proposed mediators for these effects include direct action on cellular stress-sensitive calcium channels and integrins, as well as paracrine mediators, such as prostaglandin E_2 (PGE_2), prostacyclin and nitric oxide (for a recent review, see ref. 4).

In addition to the important effects on the skeleton of the homeostatic demands for calcium and mechanical adaptation, a third significant input into this 'servo' system is the pronounced influence of sex steroids. Skeletal preservation by oestrogen in females[5] may be related evolutionarily to the need of calcium stores for embryonic skeletal development. In birds for example, oestrogen causes massive bone formation prior to egg laying. In mammalian adult males and females, including humans, oestrogen inhibits bone resorption by reducing osteoclast number. The mechanism most likely involves lineage allocation of monocyte/macrophage/osteoclast precursors through effects on regulatory cytokines, including interleukin (IL)-1, IL-6, tumour necrosis factor-α and PGE. Oestrogen acts via oestrogen receptor-α in human males and is required for closure of the epiphyses and for reaching or maintaining normal post-pubertal bone mass[5]. The male sex steroid testosterone, responsible for the male phenotype characterized by a larger skeleton, affects bone in several ways. It can be converted by aromatase to oestrogen to inhibit bone resorption, but in addition seems to inhibit bone resorption directly in males and stimulates bone formation in both males and females[6].

Bone resorption and formation are 'coupled' locally by mechanisms not fully understood, that is, when one goes up or down the other usually follows. But resorption is much faster than formation (it takes at least three months to rebuild bone resorbed in 2–3 weeks). Thus, increased resorption, even when accompanied by coupled increased formation, can cause bone loss owing to these kinetic differences, for example, in oestrogen deficiency or hyperparathyroidism.

Multiple coupling 'factors' attempt to maintain this servo system at its physiological (homeostatic) steady state (Fig. 1)[7]. Bone formation stimulatory factors released from the matrix during resorption, including insulin-like growth factor and transforming growth factor (TGF)-β, may serve this function. Several factors, such as PTH, PGE, fibroblast growth factor, TGF-β and even RANK ligand, have been

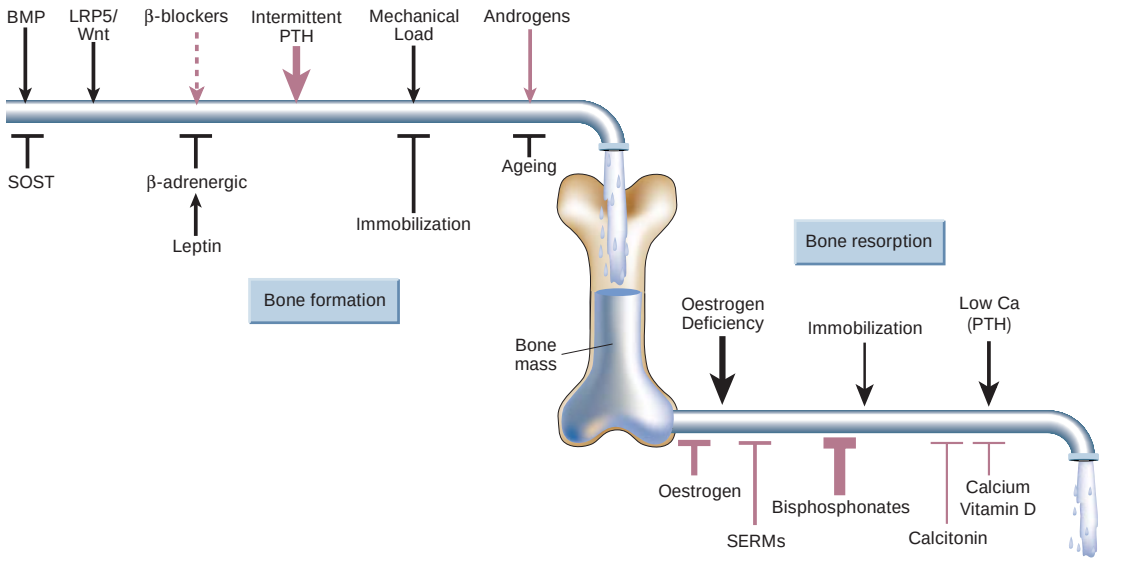

Figure 1 Determinants of skeletal homeostasis and bone mass. Schematic representation of the servo system that maintains bone mass at steady-state levels. Physiological (blue) and pharmacological (orange) stimulators and inhibitors of bone formation and resorption are listed. The relative impact, where known, is represented by the thickness of the arrows. Solid lines are current therapies and dotted lines putative ones. Abbreviations: BMP, bone morphogenetic protein(s); SOST, sclerostin; LRP5, low-density lipoprotein (LDL)-receptor-related protein 5; PTH, parathyroid hormone; SERM, selective oestrogen-receptor modulator.

shown to stimulate both resorption and formation. Mechanical effects on bone could also couple resorption to formation, as weakening of the bone as a result of resorption should engender corrective bone formation[8].

Steady state in servo systems is reached and maintained by feedback loops. Here, the best characterized is calcium control of PTH secretion, which in turn regulates circulating calcium levels via bone resorption, intestinal absorption and renal reabsorption. Sex hormone levels are regulated by feedback to the pituitary, but are controlled by the reproductive agenda, rather than skeletal needs. Mechanical feedback signals, less well defined at the molecular level, must emanate from the strain in the bone matrix, instructing osteoclasts and osteoblasts to increase, decrease or stop their activity[4].

This homeostatic system, like all others, is subject to genetic influences. This is reflected in the tight correlation of bone mineral density between identical twins compared with non-identical twins, and the lower bone density in daughters of osteoporotic mothers[9,10]. Multiple genes are undoubtedly involved, a conclusion supported by studies in inbred mice, which have identified several quantitative trait loci responsible for strain dependence of bone mass[11].

Hierarchy among factors regulating bone mass

In-depth discussion of skeletal homeostasis is beyond the scope of this review, but the following three questions will help to place recent advances in a pathophysiological and therapeutic context. First, is there a hierarchy in bone functions; second, is there a hierarchy among the missing factor(s) responsible for bone loss and osteoporosis; and third, which external (pharmacological) inputs can compensate and reverse the bone loss?

Concerning the first question, calcium mobilization overrides other functions of the skeleton. Calcium deficiency due to renal disease, malabsorption, other pathology or poor calcium diet invariably causes bone loss, mediated by elevated PTH. But owing to other servo inputs (for example, mechanical function), this loss is not random throughout the skeleton, the bone most exposed to mechanical loads being most protected[12].

The effect of oestrogen seems to trump mechanical function, as exercise is limited in its ability to maintain or restore bone mass in postmenopausal women[13] and amenorrhoeic marathon runners lose bone. Among the three modulators of bone mass — calcium availability, sex steroids and mechanical usage — the last has the least pronounced effects. Excessive reductions in bone strain produced by weightlessness (microgravity in outer space) or immobilization (paralysis, prolonged bed rest or application of casts) can cause significant bone loss, while strenuous athletic activity (for example, professional tennis) can augment certain bones[14]. However, physiological changes in physical function, such as sedentary lifestyle or moderate exercise, have only a slow and modest effect on bone mass[13].

Regarding factors responsible for osteoporosis, from an epidemiological perspective oestrogen is clearly at the top of the list. But other inputs into this servo system (such as dietary calcium, parathyroid function and genetic background) must also be important — although their contribution has not been fully quantified — as not all postmenopausal women develop osteoporosis. Additionally, bone loss can occur before menopause in women or the onset of testosterone reduction in men.

The recent discoveries discussed below could be among the inputs that may prevent or reverse bone loss, while future studies will show if and how they relate to the main skeletal modulators (calcium regulation, biomechanics and sex steroids) and what role they may have in the pathophysiology of osteoporosis. This leads to the third question, the ability of various therapies to prevent or reverse bone loss. In principle, replacement of a missing factor(s) should correct its deficiency (for example, oestrogen replacement therapy or ERT). However, in servo systems, inputs not directly related to the deficiency can move the steady-state level in the desired direction. For example, glucocorticoid-induced osteoporosis, caused at least in part by decreased bone formation, can be treated effectively with inhibitors of bone resorption (bisphosphonates). Intermittent PTH administered to stimulate bone formation does not replace PTH deficiency, but is an effective pharmacological strategy that takes advantage of one of the actions of this hormone. Any input that

can move the system in the desired direction can, in principle, have therapeutic utility. Its efficacy will depend on its role in the system in the context of the other inputs. One important feature of a servo system is that when it is brought to a new steady state by a pharmacological intervention, cessation of treatment will bring it back to the previous level (for example, bone loss following cessation of ERT or intermittent PTH).

With this background in mind we shall briefly review several recent discoveries, starting with central nervous system (CNS) control of bone mass, which came to light through observations in mice with genetic abnormalities in hypothalamic regulation of body weight and reproduction.

Central control of bone formation

The concept that the CNS may control bone formation might have been hinted at by the surge in osteogenesis following head injury, but was first proposed by Ducy and co-workers in 2000 as an explanation for the inhibitory effect of leptin on bone formation[15]. The study was prompted by the rapid recovery of bone mass following severe osteopenia (decreased bone mass) caused by inducible osteoblast ablation, which suggested precise control of bone mass homeostasis[16].

Another piece of the puzzle was provided by the surprising high bone mass (HBM) phenotype in leptin-deficient *ob*/*ob* mice that have low sex steroid and high corticosteroid concentrations[15]. Leptin, produced by fat tissue, acts in the hypothalamus to regulate body weight and fat mass through appetite suppression and increased energy expenditure. HBM, associated with increased bone formation, was also observed in leptin receptor-deficient mice as well as in leptin-deficient lean lipodystrophic mice, pointing to a role for leptin signalling per se, rather than obesity, in the bone phenotype. Moreover, no similar findings were observed in mice with mutations in the α-melanocyte-stimulating hormone (α-MSH)/agouti/melanocortin-4 (MC-4)-receptor signalling pathway or the cocaine- and amphetamine-regulated transcript (CART) pathway, all of which are involved in the anorexigenic action of leptin[17] (Fig. 2), indicating that the anti-osteogenic action of leptin can be dissociated from its anti-appetite effects. These authors found no direct effects of leptin on bone cells; leptin suppressed bone formation and reduced bone mass, but only when injected intracerebroventricularly (ICV). The failure to document humoral factor mediation, using extensive pharmacophysiological analyses (including a parabiosis study[17]), pointed to central control of bone metabolism. Other investigators reported the expression of leptin receptors in bone cells and stimulatory effects on bone[18,19], leaving open the possibility of dual action (central and peripheral) of leptin[20].

Noting the low bone mass phenotype in dopamine transporter-deficient mice, which lack dopamine uptake into presynaptic terminals[21], Takeda *et al.*[17] tested the possible involvement of the sympathetic nervous system (SNS), which is known to be activated by leptin signalling. Mice deficient in dopamine β-hydroxylase, which is necessary for production of noradrenaline and adrenaline, had HBM that did not respond to ICV infusion of leptin. In addition, bone formation and bone mass decreased after treatment with the β-adrenergic agonist isoproterenol and increased with propranolol, a β-adrenergic antagonist. These responses were resistant to leptin infusion, leading to the conclusion that SNS is the downstream mediator of leptin's central control of bone formation[17] (Fig. 2).

Central control was also supported by increased bone formation and bone mass following inactivation of the Y2 neuropeptide Y (NPY) receptor in brain[22]. Although no bone phenotype has been reported in *NPY*-null mice, this observation is consistent with the decrease in bone formation caused by ICV injection of NPY, suggesting the central control of bone formation by Y2 and its ligands[15]. Because leptin suppresses NPY, this pathway does not mediate the effects of leptin on bone. Nevertheless, it raises the

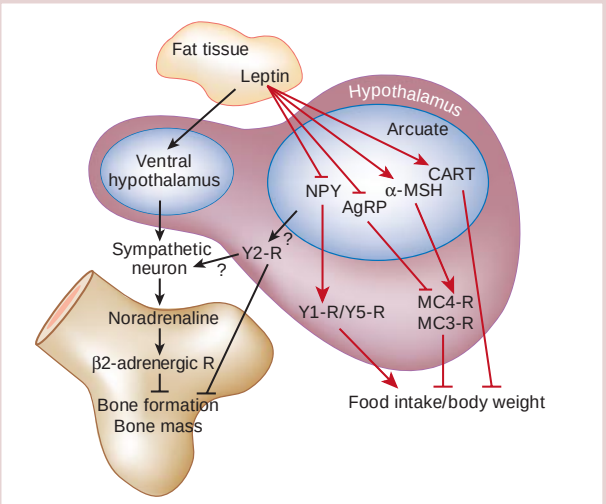

Figure 2 Leptin signalling pathways. In the regulation of body weight, leptin acts on the arcuate nucleus to induce the anorexigenic peptides α-melanocyte-stimulating hormone (α-MSH) and cocaine- and amphetamine-related transcript (CART), and to repress the orexigenic peptides neuropeptide Y (NPY) and agouti-related protein (AgRP), an antagonist for the melanocortin-3 (MC-3) and MC-4 receptors. These combined effects of leptin lead to increased body weight mainly via MC-3 and MC-4 receptors and Y1 and Y5 NPY receptors. Leptin also increases energy expenditure by activating sympathetic neurons. In contrast to leptin's anorexigenic action, during bone formation the anti-osteogenic action of leptin is mediated by the sympathetic nervous system (SNS) via the ventral hypothalamus. Noradrenaline released through activation of the SNS acts on β2-adrenergic receptors expressed in osteoblasts to inhibit bone formation. Activation of Y2 NPY receptors in the hypothalamus by its ligands also represses bone formation.

question of whether leptin and Y2 act on bone through a common pathway. While Y1 and Y5 receptors mediate orexigenic effects of NPY[23], pharmacological data suggest that the Y2 receptor could be involved in NPY regulation of sympathetic neuronal activity[24,25]. But NPY action on the autonomic nervous system is complex, involving both peripheral and central pathways, and additional studies are needed to evaluate whether the effects of NPY on bone and the SNS are linked.

These exciting discoveries raise a number of questions that future studies should address. The mediator of the central anti-osteogenic action of leptin in bone is the β2-adrenergic receptor. This receptor is known to activate the cAMP signalling pathway, which has been implicated in the bone formation stimulatory effect of PTH and prostaglandins. These pro- and antianabolic effects could be mediated by different target cells or cAMP may have different effects based on the pattern of its changes.

Another question pertains to the role of CNS and SNS in the hierarchy of physiological and pathological regulators of bone mass. Does this central control represent the master regulator of bone formation that, for example, is important in restoring bone mass after osteoblast ablation?

Last, do the CNS and SNS, and for that matter leptin, have similar roles in the regulation of bone formation in humans as they do in mice. For example, bone loss observed in anorexia nervosa patients or amenorrhoeic female athletes, both with low levels of leptin and oestrogens, suggests that, unlike in mice, leptin deficiency cannot override bone loss induced by oestrogen deficiency in humans[26]. Stimulatory effects of propranolol on fracture repair have been reported in rats[27], but limited information has been available on the role for SNS in the human skeleton. Supportive evidence is offered by the association of osteoporosis with 'reflex sympathetic dystrophy'. This condition is caused by hyperadrenergic activity and is treated

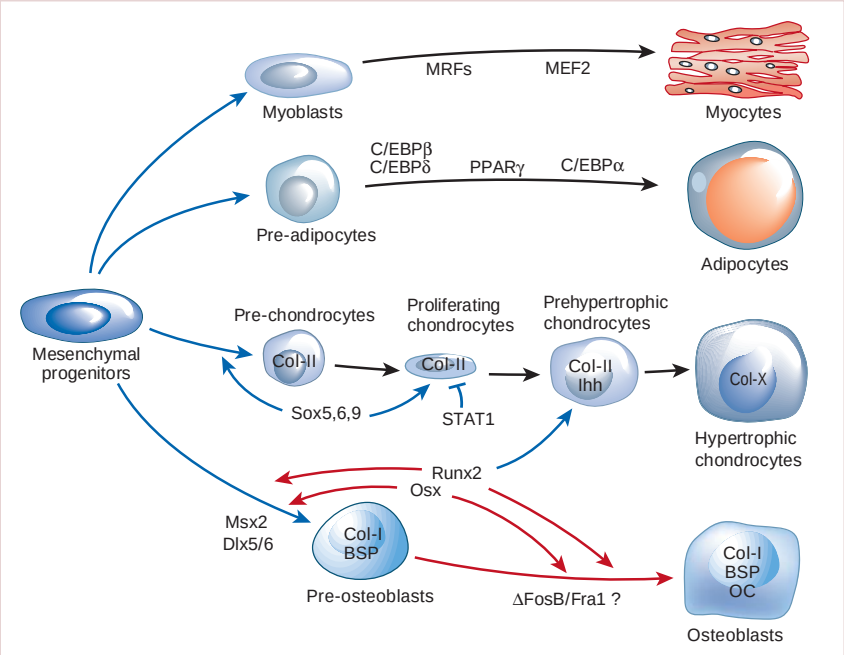

Figure 3 Transcriptional control of osteoblastic, chondrocytic, adipocytic and myocytic differentiation. Osteoblasts differentiate from mesenchymal progenitor cells that also give rise to myocytes, under the control of MRFs and MEF2[31], to adipocytes under the control of C/EBPα, β and δ and PPARγ[30], and to chondrocytes under the control of Sox5, -6 and -9[33] and STAT1 (see review in this issue by Kronenberg, page 332). Runx2 is essential for osteoblast differentiation and is also involved in chondrocyte maturation. Osterix (Osx) acts downstream of Runx2 to induce mature osteoblasts that express osteoblast markers, including osteocalcin. Abbreviations: MRFs, myogenic regulatory factors (including MyoD, myogenin, myogenic factor 5 and myogenic regulatory factor 4); MEF2, myocyte-enhancer factor 2; C/EBP, CCAAT-enhancer-binding protein; PPARγ, peroxisome proliferator-activated receptor γ; STAT1, signal transducers and activators of transcription-1; Runx2, runt-related transcription factor 2; Col-I/II/X, type I/II/X collagen; Ihh, Indian hedgehog; BSP, bone sialoprotein; OC, osteocalcin.

with β-blockers[28]. As β-blockers have been widely used in the clinic, insights into their effect on the skeleton could be obtained retrospectively.

Transcriptional regulation of bone formation

Genetic studies in mice have also provided new insights into the transcriptional regulation of osteoblast differentiation. Osteoblasts are derived from mesenchymal precursor cells that also give rise to chondrocytes, myoblasts, adipocytes and tendon cells[29]. Transcriptional control of myogenesis and adipogenesis consists of a cascade of transcriptional events driven by a series of transcription factors that control each other's phenotype-specific gene expression[30,31] (Fig. 2). Understanding the transcriptional control of osteoblast/chondrocyte differentiation has been more limited, largely owing to the lack of cell-culture systems that fully recapitulate osteoblast/chondrocyte differentiation in a synchronized fashion. Based on skeletal phenotype associated with genetic mutations in animals and humans and the analysis of transcriptional complexes formed with osteoblast/chondrocyte-specific enhancers, a number of transcription factors that control osteogenesis and chondrogenesis have been identified[32]. These include runt-related transcription factor 2 (Runx2) and sex determining region Y-box 9 (Sox9), 'master' regulators of osteogenesis and chondrogenesis[33–36] (see review in this issue by Kronenberg, page 332).

The essential role of *Runx2*, also known as *Cbfa1*, *Osf2* and *AML3*, in osteoblast differentiation was documented unambiguously in null-mutation mice that had a cartilaginous skeleton with complete absence of osteoblasts[34–36]. Heterozygous mice had a defect in intramembranous ossification that resembles cleidocranial dysplasia in humans, caused by mutation of *RUNX2* (ref. 36; and see review in this issue by Zelzer and Olsen, page 343). Although cartilage develops in *Runx2*-null mice, careful histological analysis showed delayed chondrocyte maturation[37], consistent with *Runx2* expression in hypertrophic chondrocytes. Moreover, transgenic expression of *Runx2* in chondrocytes, via the chondrocyte-specific type II collagen promoter, results in ectopic chondrocyte hypertrophy and endochondral ossification[38,39], indicating that *Runx2* controls differentiation of hypertrophic chondrocytes and osteoblasts.

This dual role for Runx2 suggested the presence of additional factors that act in osteoblasts to control osteogenesis. One such factor is *Osterix* (*Osx*)[40]. Osx is a zinc finger-containing protein induced in C2C12 myoblasts in response to bone morphogenetic protein (BMP), a potent stimulator of bone formation when injected into muscle or dermis. *Osx*-null mice develop a perfectly patterned skeleton composed entirely of cartilage, lacking osteoblasts and mineralized bone matrix. Unlike *Runx2*-null mice, the cartilage of *Osx*-null mice is normal, containing fully mineralized, terminally differentiated hypertrophic chondrocytes, pointing to a specific role for *Osx* in osteoblast differentiation. *Osx* is not expressed in *Runx2*-null mice, while the expression of *Runx2* is normal in *Osx*-null mice. Interestingly, *Osx*-null osteoblast precursors in the periosteum of membranous bones express chondrocyte markers, such as *Sox9* and *Col2a1*, suggesting that *Osx* acts downstream of *Runx2* to induce osteoblastic differentiation in bipotential chondro-osteo progenitor cells.

A number of transcription factors/cofactors have been shown to interact with Runx2. AJ18, a zinc finger-containing factor, inhibits Runx activity by competing for its DNA-binding sequence[41]. Core-binding factor-β (Cbfβ) is a heterodimerizing partner of Runx1 and Runx3 that is essential for haematopoiesis. Transgenic rescue of embryonic lethal *Cbfb*-null mice and 'knock-in' of *Cbfb* fused in-frame to a cDNA encoding green fluorescent protein[42,43] resulted in mice that exhibited delayed ossification, indicating a role for Cbfβ in bone. However, unlike *Runx2*-null mice that completely lack bone and osteoblasts, ossification is initiated in these mice, suggesting that Runx2 can act in the absence of Cbfβ.

Distal-less homeobox 5 (*Dlx5*) and *msh homeobox homologue 2* (*Msx2*) are homeobox-containing transcription factors expressed in early stages of osteoblast differentiation. Functional analysis *in vitro* points to reciprocal roles of *Dlx5* and *Msx2* as activator and repressor of transcription. Murine and human mutations show that they are essential for normal intramembranous ossification[44]. *Dlx5*/*Dlx6*-null mice also indicate a role for *Dlx5*/*Dlx6* in the axial and appendicular skeleton[45].

Changes in Fos-family transcription factors affect both osteoblasts and osteoclasts[46]. Transgenic expression of *cfos* in mice causes osteosarcoma, whereas *cfos*-null mutation causes osteopetrosis. Transgenic expression of *fra-1* or *ΔFosB* (an alternatively spliced form of FosB), both widely expressed in many tissues in addition to bone, stimulates bone formation and increases bone mass in

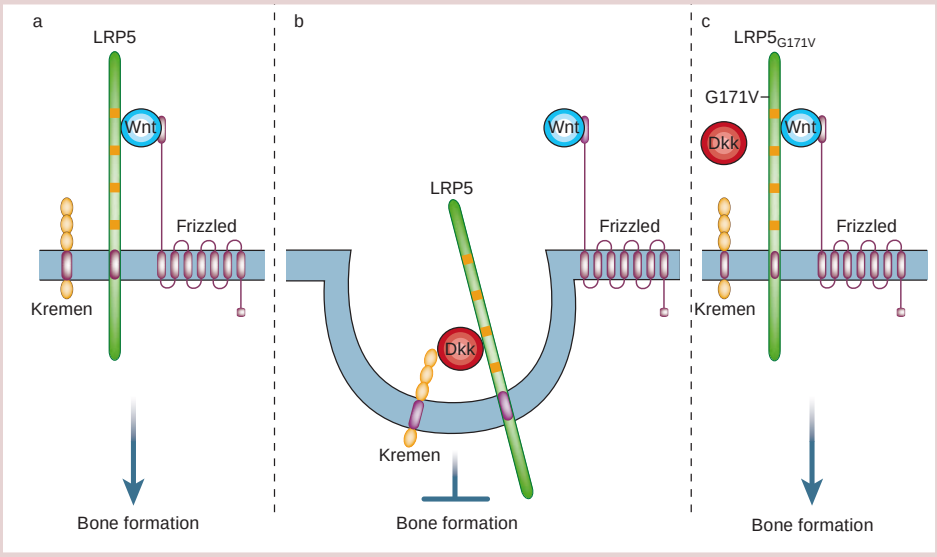

Figure 4 A model for LRP5 signalling pathway in bone. **a**, Wnt induces the complex of LRP5 (a co-receptor for Wnt) and Frizzled (the receptor for Wnt), which activates the canonical Wnt signalling pathway, leading to bone formation. **b**, LRP5 can also form a ternary complex with Dickkopf-1 (Dkk; a Wnt inhibitor) and Kremen (a receptor of Dkk), which triggers rapid internalization and depletion of cell-surface LRP5, leading to inhibition of the canonical Wnt signalling pathway[67]. **c**, $LRP5_{G171V}$ — the mutant LRP5 that causes high bone mass — probably forms complexes preferentially with Wnt and Frizzled in bone tissue, leading to constitutive activation of Wnt signalling and bone formation (hypothetical model). Figure modified from ref. 67.

mice[47,48]. It is not known how these Fos-family proteins, which lack transactivation domains, increase bone formation.

Recent insights into the transcriptional regulation of osteoblast differentiation during development are largely due to advances in human and mouse genetics (Fig. 3). But information on the transcriptional regulation of bone formation and bone mass in the adult, which could be most useful for therapeutic applications, remains limited. Transgenic expression of a dominant negative form of *Runx2* in mature osteoblasts, under the mouse osteoblast-specific osteocalcin promoter, decreases bone formation and reduces bone mass, supporting a role for Runx2 in osteoblast activity in adult mice[49]. Transgenic expression of *Runx2* under the type I collagen promoter, an early marker for osteoblast commitment, results in a surprising osteopenic phenotype caused by impaired osteoblast maturation and increased bone resorption[50,51]. These results suggest a potential role for Runx2 in bone resorption, consistent with the absence of osteoclasts in *Runx2*-null mice, and support the notion that temporal control of Runx2 activity is important for normal bone development. Conditional inactivation of genes, in a spatial and temporal fashion, should clarify the fine aspects of transcriptional regulation of bone cell differentiation and activity.

New genes responsible for high bone mass in humans

New genes responsible for hereditary skeletal disorders could provide unexpected therapeutic opportunities. These include mutations associated with increased bone formation manifested as HBM. The affected individuals are generally healthy, suggesting that these genes have tissue-specific roles. Two such genes are *LRP5* and *SOST*.

LRP5 encodes the low-density lipoprotein (LDL)-receptor-related protein 5, responsible for HBM in one kindred with strikingly dense bone and no other abnormalities. This trait was mapped to chromosome 11q13 (the long arm of chromosome 11 at band 13) where a single mutation, G171V, was found in all affected individuals[52]. The identical mutation was identified in another HBM kindred[53]. *LRP5* was also mapped as the locus for osteoporosis-pseudoglioma syndrome (OPPG), an autosomal recessive disease characterized by severe osteoporosis due to decreased bone formation and pseudoglioma resulting from failed regression of primary vitreal vasculature[54]. Thus, while gain of function leads to HBM, an autosomal dominant trait, loss of function leads to osteoporosis.

The role of *Lrp5* in bone formation and bone mass was further confirmed genetically in mice. Null mutation of *Lrp5* results in post-natal bone loss, due to decreased bone formation and osteoblast proliferation, in a Runx2-independent manner[55]. Conversely, increased bone formation and higher bone mass was reported in transgenic mice that express in osteoblasts *LRP5* with the HBM mutation G171V (reported by F. Bex and co-workers at the 2002 meeting of the American Society for Bone and Mineral Research), supporting a cell-autonomous role for *Lrp5* in osteoblasts. No developmental abnormalities were observed in the skeleton of these mice, consistent with a unique role of *Lrp5* in bone homeostasis.

The identification of *LRP5* as a key molecule in bone regulation was surprising for several reasons. *LRP5* is expressed at low levels in almost all tissues and shows little temporal changes. Second, the LRP5 signal transduction pathways were not known to control bone formation. *LRP5* was identified originally as a member of the LDL-receptor superfamily with low affinity to apolipoprotein E[56]. However, recent *in vitro* findings support a role for *LRP5* in Wingless/Wnt signalling, largely because of its close homology to *Lrp6*, a mammalian homologue of Arrow, which is a co-receptor for Wingless signalling in *Drosophila*. Null mutation of *Lrp6* in mice results in loss of Wnt signalling, which is important for many developmental processes[57]. Consistent with its structural homology to LRP6, LRP5 interacts *in vitro* in cell culture with a subfamily of Wnts, including Wnt1 and Wnt3a, to form a complex with Frizzled, a well characterized receptor for Wnts, leading to activation of the canonical Wnt signalling pathway[55,58] (Fig 4a). Although Wnt/Wingless signalling has been linked to limb development and chondrogenesis, there was little information on its role in bone and osteoblasts. Activation of Wnt signalling in osteoblast precursor cells by Wnt3a, a constitutively active form of β-catenin or LiCl (an activator of Wnt signalling), was shown recently to promote osteoblastic differentiation[54,59].

Involvement of the Wnt pathway in the action of LRP5 on bone is supported by the observation that LRP5 with the HBM mutation prevents inhibition of Wnt signalling by Dickkopf-1[53,60] (Fig. 4b, c). And recent subtractive hybridization and microarray analysis identified a series of genes involved in Wnt signalling as signature genes induced during fracture repair in rodents[61]. Taken together, these genetic and limited biological observations support a previously unrecognized role for *LRP5* and Wnt signalling in bone formation (Fig. 4). It remains to be shown how LRP5 plays such a selective role in bone. Six additional mutations of the *LRP5* gene in the amino-terminal domain near G171 were identified recently in individuals with increased bone density, notably in cortical bone[62]. This exciting discovery points to the power of genetics in the identification of new mechanisms that could not have been hypothesized based on previous knowledge.

The recent discovery of the gene *Sclerostin* (*SOST*) in chromosome 17q12–q21, provided the first evidence for a role of BMP in bone mass regulation. *SOST* is the locus for sclerosteosis, an autosomal recessive HBM trait initially found in South African Dutch descendants with elevated bone formation. Five distinct mutations that lead to SOST inactivation have been identified in families with HBM in South Africa, Senegal, Brazil and the United States[63,64]. *SOST* encodes a protein homologous to the secreted factors that regulate BMPs, such as Noggin, Chordin and Gremlin[65]. Unlike other BMP-binding proteins that are expressed in many tissues, *SOST* expression is restricted to bone and cartilage, except for low expression in liver and kidney[64]. Preliminary data suggest that SOST decreases bone formation by suppressing BMP activity in bone. This is the first evidence for a potential role of BMP in adult bone since its discovery 30 years ago as a potent inducer of ectopic osteogenesis.

From the bench to the clinic

The spectacular advances in genomics and genetics promised to usher in an unprecedented era of understanding and treating disease. Three years after deciphering the human genome it is clear that the progress is slow, for several reasons. Many common diseases, such as osteoporosis, are multigenic and result from the interplay between genetic and environmental factors. Even when disease rate-limiting genes are identified, they have to be amenable to drug discovery — that is, available pharmaceutical tools should be able to mimic or compensate for their absence (lack of function) or excessive activity (gain of function). Another requirement is sufficient tissue selectivity, to avoid mechanism-based 'side effects'. There are, however, examples in bone diseases where these requirements could be met[66].

In bone resorption, genetic approaches have identified osteoprotegerin, cathepsin K and the chloride channel 7 (CICN7) as rate limiting for osteoclast generation and activity, and all three are now drug discovery targets (see review in this issue by Boyle and co-workers, page 337). Some of the genetic discoveries described above could lead to treatments that increase bone formation. Therapeutic modulation of transcription factors, except for nuclear receptors controlled by cognate ligands, has been historically difficult, as it occurs beyond the cellular and nuclear membranes and involves multiple large interactive proteins. Thus, exploiting for therapeutic purposes the information provided by *RunX2*, *Osx*, *Dxl*/*Msx* and *Fos*, for example, faces significant challenges, which could possibly be met on a case by case basis.

There may be better prospects in using the SOST discovery for therapeutic purposes, as SOST interaction with BMP most likely occurs extracellularly. One could conceive that a blocking anti-SOST antibody could interact with the binding domain and mimic its deletion.

The LRP5 mutation was suggested to increase skeletal responsiveness to mechanical stimulation. At the biochemical level it apparently increases Wnt signalling in bone. As mentioned above, one needs to understand the tissue specificity of this effect and try to mimic it pharmacologically. The challenges in this case are the ubiquitous expression of LRP5 and involvement of Wnt in multiple processes, including cancer.

Central regulation of bone mass via the SNS offers a new dimension for potential bone therapies. There is currently too little information to speculate if central or peripheral approaches to activate this pathway, if applicable to humans, are more promising. As with other potential therapies, achieving tissue selectivity will be the main challenge here. However, each of these three potential therapies involving SOST, LRP5 and SNS deal with modulation of receptor–ligand interactions that have in many cases been amenable to successful therapeutic approaches. □

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