## Chapter 2

# New aspects of normal bone biology

Susan M. Ott

## Introduction

Bone as a structural material must be strong enough to support the body weight and light enough for efficient movement. Some of the most important new developments in bone biology help explain the mechanisms of sensing and responding to mechanical loading. New genetic approaches combined with clinical observations have led to discoveries of signaling pathways that may eventually lead to new therapies to treat or prevent bone diseases. This chapter will focus on some recently defined factors that influence bone remodelling, with an emphasis on those that could potentially be modified to improve the bone strength.

## Basic multicellular units

Before discussing new developments, I will briefly review bone remodelling. Turnover of bone is different from dermal turnover, where the entire surface is continuously forming new skin while shedding old skin. Healthy bone is a dynamic tissue, continually resorbing bone and replacing it with new bone in discrete areas know as basic multicellular units, also called bone metabolic units (BMU). A BMU is not a permanent structure. It forms in response to a signal, performs its function, and disbands, leaving residual lining cells and osteocytes. Each BMU undergoes its functions in the same sequence: origination and organisation of the BMU, activation of osteoclasts, resorption of old bone, recruitment of osteoblasts, formation of new bone matrix, and mineralisation. On the cancellous surfaces, a BMU does not just dissolve a pit on the surface, but it spreads across the surface leaving behind an area filled with new bone. In the cortex the osteoclasts form a cutting edge and bore through the solid bone, and osteoblasts follow, filling in the tunnels and leaving behind a small vascular channel.

Some BMUs originate when the bone has been damaged; others may originate at random surfaces on the bone. The osteocytes can initiate a BMU in response to microdamage and perhaps after mechanical loading. It is not clear if systemic hormones or local cytokines initiate new BMUs, or if they work by increasing activity or lifespan of existing BMUs. The first sign of activity seen by standard microscopy is bone resorption, so early histologists thought that the first step in a remodelling cycle was performed by the osteoclasts. Now, however, it is known that osteoclasts must be activated by cells in the osteoblast lineage, a process that occurs on the molecular level. After activation of

pre-osteoclasts, which fuse and form mature osteoclasts, the second step is bone resorption. At a given spot on the bone surface, resorption is rapid for the first 10 days, and continues for about a month. The osteoclasts undergo apoptosis; the lifespan and activity of the osteoclasts determine the depth of the resorption cavity. Meanwhile, the pre-osteoblasts that were generated by the marrow stem cells have proliferated, and when the osteoclasts retire a team of osteoblasts line the cavity. The osteoblasts form a matrix and after about 14 days directs mineralisation of the matrix. The osteoblasts continue to form and to mineralise osteoid until the cavity is filled or nearly filled. The time to fill in the cavity at any given place on the surface is 124–168 days in normal individuals. After formation is concluded, that spot on the surface is quiescent for about 2–5 years, during which time the newly formed bone gradually accumulates mineral and become denser.[1] A dynamic review of bone remodelling is available online (Fig. 2.1).[2]

Recently, more attention has been paid to structures within the marrow spaces that accompany the BMU. At the initiation of a BMU, the lining cells separate from the bone matrix and merge with the marrow vasculature, forming a canopy that provides a protected space for bone resorption and formation, and provide a mechanism for the correct spacial and temporal organisation. It is likely that the stem and marrow stromal cells reside close to these vascular structures. The marrow stromal cells secrete local factors that begin to assemble the osteoclasts and the osteoblasts and the canopy restricts the action to the correct location.[3]

## The bone cells

Two major families of cells control bone remodelling: the osteoclasts and the osteoblasts.

Osteoclasts originate from cells in the hematopoetic line from precursors of macrophages. Osteoblasts are derived from stem cells in the marrow stromal that are also precursors to adipocytes and chondrocytes. The cells differentiated into pre-osteoblasts,
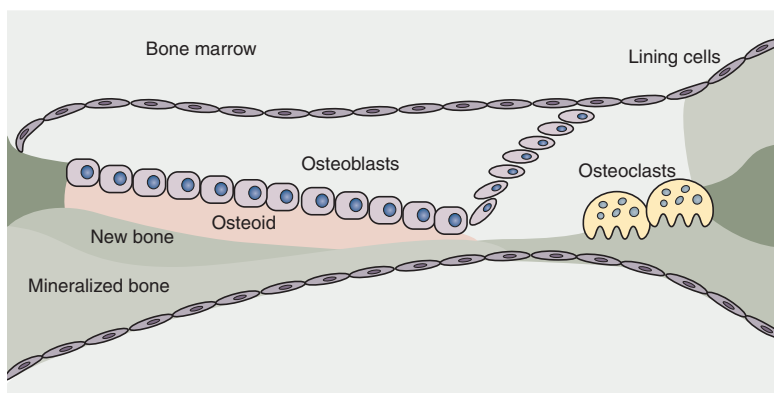


**Fig. 2.1** A BMU travelling along the surface of cancellous bone.

then mature osteoblasts that secrete collagen and form bone. The osteoblasts which do not undergo apoptosis further differentiate into lining cells or osteocytes (Fig. 2.2).

## Osteoclasts

The osteoclast precursors circulate in the bloodstream. They proliferate in response to several growth factors, particularly macrocyte colony-stimulating factor that is expressed by cells in the osteoblast line. The pre-osteoclasts express surface receptor activator of nuclear factor κ-B (RANK). When RANK-ligand occupies these receptors the cells become active and merge with other pre-osteoclasts to form large, mature, multinucleated osteoclasts. The RANK-RANK-ligand signaling is the key regulator of bone resorption; many systemic hormones increase bone resorption indirectly by acting on cells in the osteoblast line, which then express the RANK-ligand. TNFα and IL-1 enhance activity of RANK-ligand. Other signaling pathways between the osteoblast and osteoclast include newly described immunoreceptor tyrosine-based activation motifs (ITAM) which are similar to systems found in immune cells and which modulate osteoclast fusion (Fig. 2.3).[4,5] After RANK activation, the intracellular pathway is mediated by calcineurin. Thus, inhibitors such as FK506 and cyclosporin A inhibit osteoclastogenesis. The activated mature osteoclast generates a complex of RANK, TRAF6, and c-Src. This leads to polarisation of the cell and cytoskeletal changes.
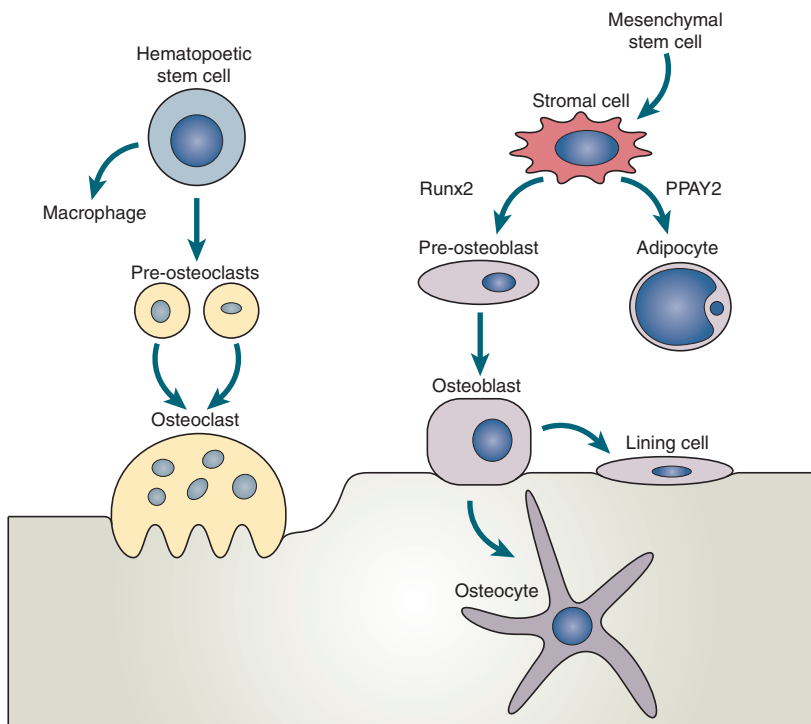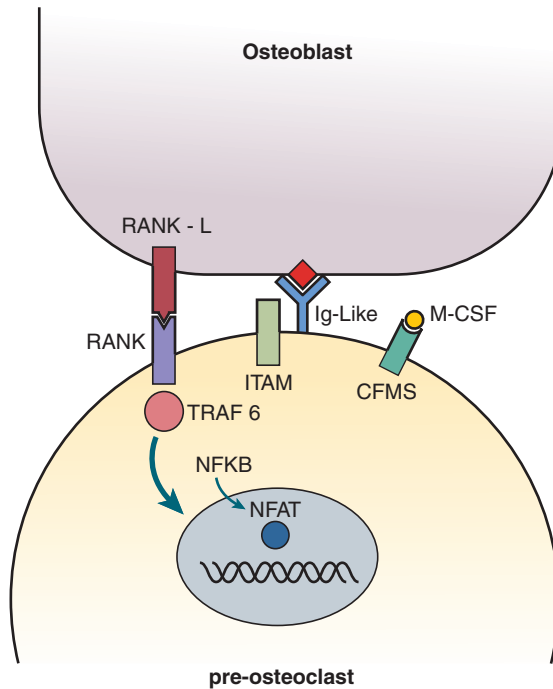


**Fig. 2.2** Bone cell lineage.

**Fig. 2.3** Osteoblast signals to pre-osteoclasts. After Takayengi & Novak.

AU: If this is to be credited please give details of accreditation.

The small GTPase Rab3D is necessary for formation of the ruffled border and integrins, particularly $\alpha v \beta 3$, are expressed on the surface. Integrins convey signals from the bone matrix to the osteoclast interior. The integrins are adherins that act with actin to form tight circular junctions attached to the bone mineral, creating a space between the bone and the osteoclast's ruffled border, which is separated from the rest of the marrow space (Fig. 2.4). Into this space, the osteoclast secretes cathepsin, which degrades collagen. The osteoclasts also pump hydrogen ions that dissolve the mineral. To maintain electroneutrality, a chloride channel coupled to H+ATP-ase secretes chloride into the resorption space. The hydrogen pump and carbonic anhydrase are similar to those in the nephron, and a $Cl-/HCO_3^-$ exchanger on the anti-resorbing surface maintains the cellular pH. In the acid environment, growth factors that were embedded within the bone matrix are released. These include TGFß, insulin-like growth factor (IGF), and fibroblast growth factor (FGF), which were deposited into the matrix by the previous generation of osteoblasts. Some, like TGFß, may be activated by the acid environment caused by osteoclastic proton secretion. These growth factors (delayed autocrine factors) might contribute to the coupling between resorption and formation that is seen in normal situations, but direct evidence for this theory is lacking. The osteoclast can also engulf material through endocytosis, similar to the macrophages (which are the cells most closely related to the osteoclasts). Osteoclasts thus resorb the bone, moving deeper into the bone and also spreading along the surface. Eventually, they undergo
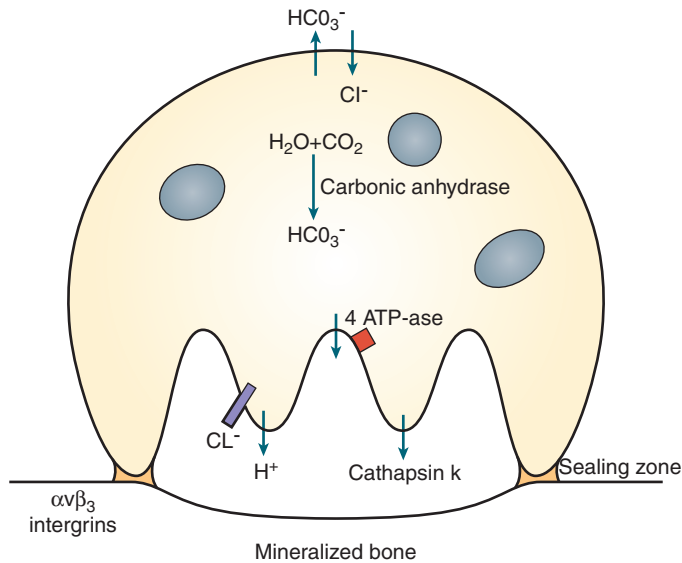
$$HC0_3^-$$

$$Cl^-$$

$$H_2O+CO_2$$

Carbonic anhydrase

$$HC0_3^-$$

4 ATP-ase

$$CL^-$$

$$H^+$$   Cathapsin k   Sealing zone

$$\alpha v \beta_3$$ intergrins

Mineralized bone

**Fig. 2.4** Mature osteoclast resorbing bone. Adapted from Novak.

AU: If this is to be credited please give details of accreditation.

apoptosis, under the influence of factors such as oestrogen (which promotes the apoptosis and thereby limits the amount of bone which is resorbed).

Although the primary function of the osteoclast is to resorb bone, there is emerging evidence that they also are necessary for the functioning of osteoblasts, which will form new bone to fill the resorbed cavities.[4] It appears that bone resorption *per se* is not necessary for bone formation.[6] Osteoclasts have been shown to excrete interleukin-6 and annexin-II, both of which could signal the osteoblasts. Patients with osteopetrosis have non-functioning osteoclasts due to mutation in the chloride channel, so bone resorption is decreased, but formation is normal. Animal studies suggest that the number of osteoclasts, and not the bone resorption, signals osteoblasts.

Excess bone resorption causes bone loss and osteoporosis. Many medications have been developed to inhibit bone resorption in order to improve the fracture risk of patients with osteoporosis. On the other hand, when osteoclasts are unable to resorb bone, patients develop osteopetrosis. The thickened bone, unfortunately, is not strong and these patients suffer from chalk-stick fractures.

## Bisphosphonate mechanism of action

Bisphosphonates are stable analogs of inorganic pyrophosphate, which is one of the body's defenses against metastatic calcification. All bisphosphonates can, therefore, inhibit mineralisation if used at high enough concentrations. The bisphosphonates bind tightly to minerals. The affinity for the hydroxyaptite crystals, the major mineral form in the bone, determines their duration of action. They enter the osteoclasts via endocytosis. The amino-bisphosphonates inhibit farnesyl pyrophosphate synthase, an enzyme within the mevalonate pathway. They bind to an aspartic acid-rich region of

the enzyme and the potency of bone resorption is related to the closeness of the fit. The downstream enzymes are necessary for prenylation of small GTP-ases, including Rho, Rab, and Rac. Therefore, the osteoclasts are not able to form a cytoskeleton.[7] Recent reports have documented very large multinucleated osteoclasts in patients who have used long-term bisphosphonate; the cells are not as close to the bone like normal osteoclasts and they do not have a ruffled border. The long-term effects of pronounced inhibition of both bone resorption and, secondarily, of bone formation remain unknown. There is one case of osteopetrosis in a boy who received excessive doses of a bisphosphonate.[8]

### Inhibition of RANK-RANK-ligand signaling

RANK-RANK-L signaling is an obvious target for pharmaceutical intervention. Denosumab, a human monoclonal antibody to RANK-L, has been extensively studied in patients with osteoporosis, rheumatoid arthritis, and metastatic bone lesions. This antibody causes rapid and marked decrease in bone resorption, lasting for about 6 months. The bone density increases. In a recent large randomised clinical trial of postmenopausal osteoporosis the fracture rates were significantly decreased (S. R. Cummings, presented at ASBMR Annual Meeting, 2008). The RANK, RANK-L signaling pathway is not unique to the bone cells. These receptors are in the tumor necrosis factor family and are also expressed in immune cells, but to date no increase in side effects related to the immune system has been reported.

AU: Do you have the details of this reference. Can it be added to the reference list.

### Inhibition of integrins

In mice deletion of the β3 intergrin subunit causes high bone mass and one case of osteopetrosis has been reported in a patient with thrombathenia, which is caused by a mutation in the β3 intergrin. A study in women with osteoporosis given a small molecule inhibitor of αvβ3 integrin has shown evidence of decreased bone resorption, with decreases in serum and urine markers of bone resorption (collagen cross-linking molecules) and increases in the bone density.[9]

### Inhibition of cathepsin or hydrogen pump

Pycnodysostosis is a disease caused by a mutation in cathepsin. These patients have increased bone density, but the bone is fragile and, on biopsy, they have layers of unmineralised osteoid under the osteoclasts. The osteoclasts are able to form normal ruffled borders and actin rings to bind to the bone matrix and are able to secrete the hydrogen ions which dissolve the mineral from the bone, but the lack of cathepsin means the collagen matrix is not degraded.[10] Small molecules that inhibit cathepsin have been developed and tested in early clinical trials of patients with osteoporosis. The patients show improved bone density and reduced bone resorption. More studies about safety and efficacy are in progress.

The most common genetic form of osteopetrosis is carbonic anhydrase deficiency. This demonstrated the importance of the hydrogen pump for bone resorption. This has not been a target for drug therapy because inhibition could cause systemic acidosis. It is interesting to note that thiazide diuretics, which cause a mild metabolic

alkalosis, also cause a mild reduction of bone resorption, and this is possibly related to osteoclast function.

# Osteoblasts

## Stem cells

Stem cells near the vasculature within the bone marrow give rise to pre-osteoblasts,[11] which can proliferate under the influence of local and systemic growth factors. Two transcription factors control the fate of the cells. RUNX2 (formerly known as CBFA1), the earliest marker of an osteoblast lineage cell, promotes maturation into osteoblasts, but PPAR-γ causes the stem cells to become adipocytes. The lipids in the environment partly determine which of these transcription factors is prominent. Treatment of diabetic patients with roziglitazone, a PPAR-γ agonist, increased the incidence of fractures in a large randomised clinical trial. Animal studies show that this drug increases the number of marrow adipocytes, but decreases the number of osteoblasts. On the other hand, bortezomib, a proteasome inhibitor used in treatment of multiple myeloma, induces the mesenchymal stem cells to differentiate into the osteoblastic pathway, and this improves the bone mass in mice with osteoporosis.[12]

Both osteoblasts and fat cells are involved in a complex regulation of body fat and energy metabolism.[13] Fat cells convert androgens to estrogens, which are beneficial to the bones. Also they secrete the adipocyte hormones leptin and adiponectin. Epidemiological observations find a strong correlation between serum leptin levels and bone density within populations; in addition, body fat mass is closely linked to bone density. The precise role of leptin is still not clear;[14] some investigators found no local activity of leptin and hypothesised that the effects were all central, because intra-cerebroventricular injections of leptin caused bone loss that could be reversed by blockade of the sympathetic nervous system. Others have found leptin receptors on osteoblasts, and leptin directly supports osteoblastic activity, while reducing the levels of RANK-ligand.[15] Osteoblasts also increase osteocalcin production after exposure to leptin; new research suggests that the osteocalcin is associated with lower fasting glucose levels, providing a feedback loop related to energy metabolism.[16]

## Pre-osteoblasts

Growth factors such as TGF-β, fibroblast growth factor, bone morphogenic proteins, platelet-derived growth factors and colony-stimulating factors (CSF) can increase proliferation of pre-osteoblasts. More details about these factors are discussed in other chapters of this book. Bone morphogenic protein 7 is of particular interest to nephrologists because it is made in the kidney and deficiency will retard the differentiation of pre-osteoblasts into osteoblasts.[17] The spindle-shaped pre-osteoblasts accumulate near the bone surface and have been mis-named 'peri-trabecular fibrosis' for years because early histologists thought they looked like fibroblasts.

The pre-osteoblasts have receptors for many of the hormones and cytokines that were classically felt to increase bone resorption (such as PTH, interleukins, and calcitriol). Pre-osteoblasts express RANK-ligand on their surface, which stimulates

the pre-osteoclasts. The gene profiles of the cells in the osteoblast lineage change in a complicated way during differentiation. The cells have decreasing proliferative capacity as they become more differentiated, under the influence of transcription factors such as RUNX2 and OSTERIX.[18] There are different developmental routes to the same endpoint and mature osteoblasts have heterogeneous gene profiles.

## Mature osteoblasts

The mature osteoblasts secrete collagen and bone matrix proteins, as well as several growth factors. Osteocalcin and osteopontin are expressed in these mature cells, as well as osteoprotegerin (OPG), a decoy receptor for RANK-ligand that will block osteoclast formation. When a team of osteoblasts first starts forming matrix, the cells are cuboidal and plump, and have many mitochondria. As they fill in the resorption cavity, they become flatter and less metabolically active. Some of them stop making matrix, and are left behind the other cells and surrounded by matrix. These cells differentiate into the osteocytes. Some of the osteoblasts undergo apoptosis. When the cells have finished making osteoid they differentiate into lining cells. These cells are flat, pancake-like cells that form tight junctions with each other and essentially separate the bone from the rest of the body. The osteocytes remain in contact with the lining cells. The lining cells make alkaline phosphatase, which prevents pyrophosphate from entering the bone and dissolving the mineral. Patients with hypophosphatasia have mutations in the alkaline phosphatase and develop severe fatal osteomalacia.

The osteoblasts and the vascular sinusoids in the marrow function as a niche for the hematopoetic stem cells. Recent studies using real-time imaging have shown that these cells home to the endosteum surface in irradiated mice, near preosteoblasts that are positive for N-cadherin. This special zone normally maintains the stem cells, but promotes their expansion in response to bone marrow damage.[19]

## The Wnt-signalling pathway

In 1997 clinical investigators from Creighton University reported a kindred with high bone mass.[20] The proband was an 18-year-old girl who had back pain after an automobile accident. Radiographs showed dense, but otherwise normal bones and the bone mineral density was 5.6 SD above average for age. Her mother had similarly dense bones. This led to an extended survey of this family whose members carried the 'high bone mass' gene. Using linkage analysis, they demonstrated that the family members with high bone mass had a single point mutation in the *LRP-5* gene (low-density-lipoprotein-receptor-related protein 5). This was not on any list of candidate genes involved in bone metabolism. Shortly afterwards, an unrelated kindred was reported that carried the identical mutation. In both studies, the inheritance was autosomal dominant. Meanwhile, another group of investigators discovered that a different mutation in the *LRP-5* gene caused the osteoporosis-pseudoglioma syndrome, a devastating disease manifested by multiple fractures and blindness which was inherited as an autosomal recessive disorder. *LRP-5* is a member of the Wnt-signalling pathway (Fig. 2.5). The Wnt genes are homologous to segment-polarity genes that are critical in embryonic development.
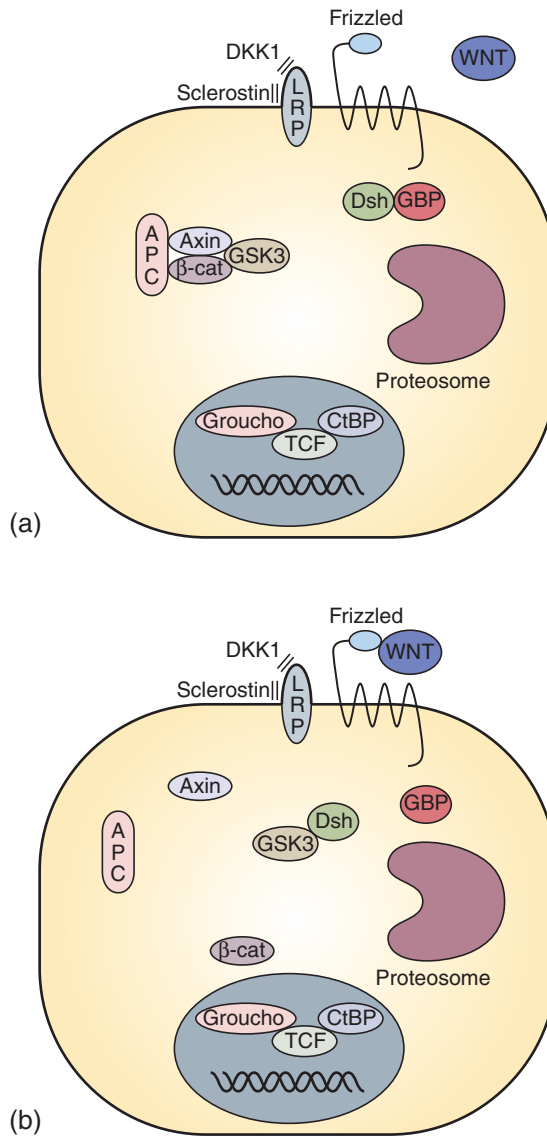
**Fig. 2.5** The Wnt signaling pathway. (a) No signal. (b) Ligand occupies receptor and pathway is activated.

Several of the Wnt-related proteins have been shown to be important in the regulation of bone metabolism. The signal is Wnt, a protein secreted by many cell types (the name is a combination of the *Drosophila* gene *wingless* and the mouse gene *int*), which occupies Frizzled, a 7-transmembrane domain receptor, and *LRP-5* is a co-factor necessary for signal transduction. Intracellular proteins include Dishevelled, Axin, GSK3,

and ß-catenin. When there is no signal, the intracellular GSK phosphorylates ß-catenin, so it is ubiquitinated and broken down by the proteosome. When Wnt binds the Frizzled receptor, it phosphorylates and activates Dishevelled, which represses GSK3, which in turn releases Axin from the ß-catenin. The Axin is then restrained by the intracellular part of LRP-5, and so the ß-catenin accumulates, and enters the nucleus and binds TCF transcription factors. This pathway, that involves Wnt and ß-catenin, is called the canonical Wnt-signalling pathway. Several extracellular proteins can inhibit this process. Dickkopf (Dkk) ties up LRP-5 by binding to it and another membrane protein called Kremen. Sclerostin, homologue of Wise, also binds to the LRP-5 protein. These each prevent the LRP-5 from restraining Axin. Secreted Frizzled-related protein (sFRP) acts as a decoy receptor and binds to Wnt. In patients with the G171V mutation and high bone mass, the mutated LRP-5 resists binding to Dkk. Therefore, the Wnt-signalling pathway is more active. In the patients with osteoporosis pseudoglioma syndrome, the protein is non-functional and so the Wnt-signalling pathway is inhibited.[21]

Gene array analysis on bone marrow cells from patients with myeloma compared those who had bone lesions on MRI scans with those without lesions found that those with bone lesions expressed Dkk1, which is one of the extracellular proteins that inhibits Wnt signaling. Animal models of myeloma show response to pharmacological inhibition of Dkk1 and transgenic mice without the *Dkk1* gene have high bone density.

The Wnt-signalling pathway is involved in pleomorphic processes. Gene array technology shows that expression of 879 of 39,000 genes are statistically different after treatment with Wnt3a. These genes included ones that control cell differentiation (promotion of osteoblastic as opposed to adipocytic phenotypes), inhibition of apoptosis (longer lifespan), and other proteins in the Wnt-signalling pathway (feedback). Of particular interest is the finding that Wnt 3a up-regulates osteoprotegerin (OPG), a secreted protein that inhibits bone resorption. Thus, the Wnt-signalling pathway not only increases osteoblastic cell differentiation and bone formation, but also inhibits bone resorption by blocking the RANK-L/RANK interaction.[22]

During the last several years there has been an epidemic of transgenic mice with knock-outs or over-expression of proteins within the Wnt-signalling pathway, and they all have either high bone mass or osteopenia as would be predicted by whether the pathway was activated or inhibited, respectively. Drugs that enhance this pathway, however, could have side effects because many other systems also use the same pathway. Malignancies could occur with over-stimulation of the pathway; recently it was demonstrated that osteosarcomas silence one of the inhibitors of Wnt.

## Treatment with intermittent parathyroid hormone

There is nothing new about the fact that PTH increases the bone formation rate and that the osteoblasts have PTH receptors. When PTH is consistently elevated, the bone resorption rate exceeds the bone formation rate and thus bone is lost, especially on the endocortical surfaces. However, when given intermittently (once a day) a different set of genes is expressed by the bone cells, and the net effect is a net increase in bone volume because bone formation is greater than bone resorption. There are more

osteoblasts, they are more active, and the mineral apposition rate is faster. Teriparatide (1–34 human PTH) is now used to treat serious osteoporosis. The mechanisms for the differential effects of continuous versus intermittent PTH are still not known.

## Osteocytes

The osteocytes, once thought to be 'trapped' in the bone mineral, are actually quite active within their lacunae and canaliculae.[6] They have multiple long cell processes that extend within the bone and form junctions with other osteocytes. The resulting network resembles a neuronal network and the osteocytes could be considered the brains of the bones. A special cell process like a cilia can be found on these extensions, which is firmly attached to the mineral of the canalicular wall. When mechanical loads are applied to the bone, the cilia can detect the fluid movement, and thus the osteocytes can sense mechanical strains. The osteocytes can secrete several stimulatory factors into the marrow, including prostaglandins, nitric oxide, and IGF. When there is more serious micro-damage, the osteocytes undergo apoptosis. This signals the surface cells to originate a new BMU, most likely because the osteocytes tonically secrete sclerostin, which inhibits bone formation.

### Sclerostin

Within the group of bone diseases with high bone mass are Van Buchem's disease and sclerosteosis. Patients with Van Buchem's disease have abnormally thick skulls, square jaws, and may have abnormalities in fingers. The bones can be painful. Some of the patients were found to have mutations in the *LRP-5* gene, but others did not. Sclerosteosis is a serious, autosomal recessive disease. Patients have very thick bones, especially in the skull, with entrapment of cranial nerves leading to deafness and facial nerve palsy, increased intracranial pressure and greater risk of stroke. Bones in the rest of the skeleton are also thick and dense, and frequently syndactyly is present. Most of the patients are Afrikaans from South Africa, and bone fractures are not seen. Patients with sclerosteosis were found to have homozygous mutations in the SOST gene and those with Van Buchem's disease had mutations in an area on the same chromosome as *SOST*, which is upstream and is involved in regulation of the gene transcription.

Relatives of patients with sclerosteosis were tested to see if they were heterozygous for the mutation in the *SOST* gene. The bone densities of these heterozygous persons were high and they did not have fractures. The carriers were healthy and had no symptoms of skeletal dysfunction. Sclerosteosis is, therefore, a human model of a gene knock-out showing a beneficial effect in heterozygotic carriers, but serious disease in homozygote knock-outs.

Sclerostin, the *SOST* gene product, was initially thought to function as a BMP (bone morphogenetic protein) antagonist. This antagonistic function, however, is weak and does not really explain the disease. Now we know that sclerostin is a circulating inhibitor of the Wnt-signalling pathway, which acts to inhibit LRP-5 function. Sclerostin is expressed almost exclusively in the osteocytes. In fact, the sclerostin is not present in osteocytes near the bone surface, but only in the more mature osteocytes that are deeper in the bone. The osteocytes tonically suppress the lining cells via sclerostin, and

then stop secreting it when the need arises to form new bone. While the exact mechanism is not known, it now seems likely that sclerostin plays an important role in skeletal adaptation to mechanical forces.

An elegant study by Robling and colleagues[23] established the importance of sclerostin as a mechanisms to link biomechanical forces to bone formation. They studied mice and rats whose forearms were loaded by mild bending. The opposite limbs were used as controls. In the control limb, the osteocytes diffusely expressed sclerostin. This was seen by immunohistochemistry and also documented by *in situ* hydridisation. In the loaded forearm, the osteocytes appeared normal on routine staining, proving that they were still alive, but on the third day, the sclerostin secretion was inhibited. Furthermore, the suppression of sclerostin secretion was very specifically located to the area of the bone that was most stressed. The mRNA for sclerostin was decreased in locations corresponding to the decreased immunohistochemistry, so the mechanical loading response of the *SOST* gene was partly at the transcriptional level. The animals were labeled with fluorochrome, and the bone formation rates 10 days later were increased in proportion to the decrease in the sclerostin.

Antibodies against sclerostin have been given to normal mice and rats, and their bone density increases. One small study of 45 post-menopausal women showed that an antibody against human sclerostin increased the biochemical markers of bone formation. Sclerostin inhibition has potential for becoming a useful anabolic agent because it is targeted to the bone, and because there is a genetic model of partial suppression in which bones are strong, but otherwise the people are healthy.

## Mechanical loading

One of the most novel new aspects of bone biology is the ability of bone to show an anabolic response to mechanical loading at precise loading stress and frequency. This response is seen at a variety of vibration frequencies and mechanical loads, but always with a particular ratio between the frequency and the load. Thus, low frequency-high load and high-frequency-low loads can both give anabolic responses. This led to the development of low-level, high-frequency platforms (30Hz, 0.3*g*), which have been shown in pilot studies to increase the bone density of the legs and spine. These loads are similar to those caused by muscle contractibility during postural control. Further studies are in progress, and to date there are not enough subjects to tell if this approach will reduce the risk of fractures.[24]

## Systemic hormones

It seems that every systemic hormone influences bone biology, but the response depends on the context. Factors such as age, stage of development, level of the hormone, whether the level is changing or not, levels of other hormones and minerals, and degree of mechanical loading all can play a role. Bone cells have receptors for a long list of hormones, including the traditional mineral-regulating hormones (PTH, PTHrp, calcitonin, and its gene-related product, and calcitriol), the gonadal hormones, IGF1, energy-related hormones (thyroid, insulin, leptin, ghrelin), glucocorticoids, and serotonin. They even have receptors for calcium, and probably for

phosphate, although that has not yet been definitely identified. Details about all of these are beyond the scope of this chapter, but a few new findings deserve mention.

## Estrogen

New studies have not tarnished the important role of oestrogen in maintaining bone health. Even in males, estrogens are important regulators of bone resorption, and testosterone is converted into oestrogen by aromatases. Several mechanisms are involved:

- oestrogen increases osteoclast apoptosis;
- by suppressing interleukins and pro-inflammatory cytokine expression in bone marrow cells, oestrogen decreases the number of osteoclasts;
- by inhibiting the production of RANK-ligand, oestrogen reduces the number and activity of osteoclasts;
- by increasing stromal cell/osteoblast cell expression of TGFβ, oestrogen inhibits osteoclast activity.

Oestrogen also has some positive effects on bone formation by acting as a mitogen to cells early in the osteoblast line, reducing apoptosis of osteoblasts, and increasing expression of TGFβ, bone morphogenetic proteins, and IGF-I.[25]

## Glucocorticoids

These hormones cause devastating loss of bone mineral and increase in fracture rates, and in the past it was taught that they increased bone resorption rates. Now it has been shown that the major physiological action of these steroids is to inhibit bone formation—this effect is so strong that decreased markers of bone formation can be detected in healthy people after 1 week of treatment with prednisone. Contrary to popular opinion, the direct effect of glucocorticoids on osteoclasts is actually an inhibition of resorption.[4]

## Serotonin

A recent series of genetic studies in mice has suggested a role of gut-derived serotonin in the control of bone formation.[26] Mice with knock-out of the *LRP-5* gene developed serious osteoporosis. The protein profile of these mice, compared with normal littermates, showed high levels of tryptophan hydroxylase, the enzyme that converts tryptophan to serotonin. The serum levels of serotonin were markedly elevated in the LRP-5 knock-out animals. Of interest, the serotonin levels were also elevated in humans with the osteoporosis pseudoglioma syndrome, who have an inactivating mutation in *LRP-5* gene. Furthermore, in the knock-out animals, either a tryptophan-reduced diet or an inhibitor of serotonin reduced serotonin serum levels normalised the bone density. Serotonin receptors were identified in the osteoblasts, and when activated they inhibited osteoblast activity. All of these findings suggest that serum serotonin plays a role in osteoblast function. In the LRP-5 knock-out mice, the origin of the excess serotonin was the gut. Selected knock-out in the gut reproduced the osteopenia, but selected knock-out in osteoblast cells had no effect. The authors felt

this meant that the Wnt-signalling pathway in the osteoblasts was not responsible for the disease in the LRP-knockout, and that the gut serotonin was inhibiting the bone.[26]

Other laboratories have found that mice with selective knock-out of various steps within the Wnt-signalling pathway do have osteoporosis. Results from genetically modified animal models must be interpreted with some caution, because deleting one gene could activate alternate pathways.

Also, the osteoblast family is heterogeneous, and it is not yet clear which of the cells are most involved with either Wnt or serotonin signaling. Although it is plausible that a gut-derived hormone could modulate bone formation, it makes heuristic sense that the major regulators would be factors derived from the local osteocytes. Physiological bone formation should be targeted to the part of the skeleton which is bearing the most mechanical load. Further research will help to clarify the exact role of the LRP-5, and serotonin in both normal and pathological conditions.

Serotonin also is an important neuro-transmitter. This small molecule does not pass through the blood–brain barrier, so medications that increase brain serotonin levels may not necessarily increase the systematic serum serotonin levels. There is, however, an increasing body of evidence that patients treated with selective serotonin reuptake inhibitors have increased fracture rates and/or decreased bone density. The mechanisms are still unclear.[27]

## Summary

This chapter has briefly reviewed the normal sequence of bone remodelling, and discussed new findings about the differentiation, function, and interactions of the bone cells. The importance of mechanical loading was stressed. Some mechanisms of medications used for osteoporosis, as well as potential new targets for influencing the bone strength are particularly interesting to clinicians and to the millions of patients with metabolic bone diseases.

## References

1. Ott S. Histomorphometric analysis of bone remodelling. In: J Bilezikian, L Raisz, Ga R (eds) *Principles of Bone Biology*. San Diego: Academic Press; 2002:303–20.

[AU: Please give date accessed]

2. Ott SM. *Osteoporosis and Bone Physiology*. Available at: http://courses.washington.edu/bonephys (accessed 2009).

3. Eriksen EF, Eghbali-Fatourechi GZ, Khosla S. Remodelling and vascular spaces in bone. *J Bone Miner Res* 2007;**22:**1–6.

4. Novack DV, Teitelbaum SL. The osteoclast: friend or foe? *Ann Rev Pathol* 2008;**3:**457–84.

5. Takayanagi H. Osteoimmunology: shared mechanisms and crosstalk between the immune and bone systems. *Nat Rev Immunol* 2007;**7**(4):292–304.

[AU: Please update reference]

6. Henriksen K, Neutzsky-Wulff AV, Bonewald LF, Karsdal MA. Local communication on and within bone controls bone remodelling. *Bone 2009.*

7. Russell RG, Xia Z, Dunford JE, et al. Bisphosphonates: an update on mechanisms of action and how these relate to clinical efficacy. *Ann N Y Acad Sci* 2007;**1117:**209–57.

8. Whyte MP, McAlister WH, Novack DV, Clements KL, Schoenecker PL, Wenkert D. Bisphosphonate-induced osteopetrosis: novel bone modeling defects, metaphyseal osteopenia, and osteosclerosis fractures after drug exposure ceases. *J Bone Miner Res* 2008;**23:**1698–707.

9. Murphy MG, Cerchio K, Stoch SA, Gottesdiener K, Wu M, Recker R. Effect of L-000845704, an alphaVbeta3 integrin antagonist, on markers of bone turnover and bone mineral density in postmenopausal osteoporotic women. *J Clin Endocrinol Metab* 2005;**90:**2022–8.

10. Fratzl-Zelman N, Valenta A, Roschger P, et al. Decreased bone turnover and deterioration of bone structure in two cases of pycnodysostosis. *J Clin Endocrinol Metab* 2004;**89:** 1538–47.

11. Sacchetti B, Funari A, Michienzi S, et al. Self-renewing osteoprogenitors in bone marrow sinusoids can organise a hematopoietic microenvironment. *Cell* 2007;**131:**324–36.

12. Mukherjee S, Raje N, Schoonmaker JA, et al. Pharmacologic targeting of a stem/progenitor population *in vivo* is associated with enhanced bone regeneration in mice. *J Clin Invest* 2008;**118:**491–504.

13. Rosen CJ, Klibanski A. Bone, fat, and body composition: evolving concepts in the pathogenesis of osteoporosis. *Am J Med* 2009;**122**(5):409–14.

14. Hamrick MW, Ferrari SL. Leptin and the sympathetic connection of fat to bone. *Osteoporos Int* 2008;**19:**905–12.

15. Reid IR. Relationships between fat and bone. *Osteoporos Int* 2008;**19:**595–606.

16. Pittas AG, Harris SS, Eliades M, Stark P, Dawson-Hughes B. Association between serum osteocalcin and markers of metabolic phenotype. *J Clin Endocrinol Metab* 2009;**94:**827–32.

17. Gonzalez EA, Lund RJ, Martin KJ, et al. Treatment of a murine model of high-turnover renal osteodystrophy by exogenous BMP-7. *Kidney Int* 2002;**61:**1322–31.

18. Aubin JE. Advances in the osteoblast lineage. *Biochem Cell Biol* 1998;**76:**899–910.

19. Yin T, Li L. The stem cell niches in bone. *J Clin Invest* 2006;**116:**1195–201.

20. Johnson ML, Gong G, Kimberling W, Recker SM, Kimmel DB, Recker RB. Linkage of a gene causing high bone mass to human chromosome 11 (11q12-13). *Am J Hum Genet* 1997;**60:**1326–32.

21. Krishnan V, Bryant HU, Macdougald OA. Regulation of bone mass by Wnt signaling. *J Clin Invest* 2006;**116:**1202–9.

22. Jackson A, Vayssiere B, Garcia T, et al. Gene array analysis of Wnt-regulated genes in C3H10T1/2 cells. *Bone* 2005;**36:**585–98.

23. Robling AG, Niziolek PJ, Baldridge LA, et al. Mechanical stimulation of bone in vivo reduces osteocyte expression of SOST/sclerostin. *J Biol Chem* 2008;**283:**5866–75.

24. Gilsanz V, Wren TA, Sanchez M, Dorey F, Judex S, Rubin C. Low-level, high-frequency mechanical signals enhance musculoskeletal development of young women with low BMD. *J Bone Miner Res* 2006;**21:**1464–74.

25. Riggs BL, Khosla S, Melton LJ, 3rd. Sex steroids and the construction and conservation of the adult skeleton. *Endocr Rev* 2002;**23:**279–302.

26. Yadav VK, Ryu JH, Suda N, et al. LRP-5 controls bone formation by inhibiting serotonin synthesis in the duodenum. *Cell* 2008;**135:**825–37.

27. Ziere G, Dieleman JP, van der Cammen TJ, Hofman A, Pols HA, Stricker BH. Selective serotonin reuptake inhibiting antidepressants are associated with an increased risk of nonvertebral fractures. *J Clin Psychopharmacol* 2008;**28:**411–17.