



The Cells of Bone and Their Interactions

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Abstract

Bone tissue is comprised of a collagen-rich matrix containing non-collagenous organic compounds, strengthened by mineral crystals. Bone strength reflects the amount and structure of bone, as well as its quality. These qualities are determined and maintained by osteoblasts (bone-forming cells) and osteoclasts (bone-resorbing cells) on the surface of the bone and osteocytes embedded within the bone matrix. Bone development and growth also involves cartilage cells

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(chondrocytes). These cells do not act in isolation, but function in a coordinated manner, including co-ordination within each lineage, between the cells of bone, and between these cells and other cell types within the bone microenvironment. This chapter will briefly outline the cells of bone, their major functions, and some communication pathways responsible for controlling bone development and remodeling.

Keywords

Bone modeling · Bone remodeling · Coupling factors · Osteoblast · Osteoclast · Osteocyte

1 Osteoclasts

Osteoclasts are large, multinucleated cells responsible for bone resorption. Osteoclasts form when circulating mononuclear hematopoietic progenitor cells migrate to the bone surface, fuse with each other, and attach to the bone surface to form active osteoclasts. This process is mediated by the interaction of progenitor cells expressing receptor activator of NF-kappa B (RANK) with supporting cells expressing RANK ligand (RANKL) (Martin and Sims 2015). Fusion is triggered by dendritic cell-specific transmembrane protein (DC-STAMP) (Kukita et al. 2004) and osteoclast-specific transmembrane protein (OC-STAMP) (Miyamoto et al. 2012). Osteoclasts are also capable of generating new osteoclasts by fission of osteoclasts, where the released mononuclear or multinucleated osteoclast detaches before joining another osteoclast or osteoclast precursor to continue the resorptive process (Jansen et al. 2012).

Once formed and attached to the bone surface, osteoclasts are polarized cells with specific functional domains responsible for attachment, resorption, and release of resorption products (Fig. 1). Attachment to bone is mediated by the vitronectin receptor $\alpha_v\beta_3$ integrin which binds to arginine-glycine-aspartic acid (RGD) sequences in bone matrix proteins including osteopontin and bone sialoprotein (Ross and Teitelbaum 2005). These attachment sites, known as podosomes, form an actin-rich ring-like sealing zone surrounding and isolating the resorption lacunae from the rest of the local environment. Podosomes are rapidly assembled and disassembled, allowing the highly motile osteoclasts to move across the bone surface by partially detaching and reattaching (Horne et al. 2005).

Resorption makes use of the osteoclast's characteristic ruffled border which increases the surface area on which ion exchange can occur. To initiate resorption, osteoclasts acidify the lacunae to dissolve bone mineral (bioapatite) and matrix proteins. This is achieved by carbonic anhydrase II (Sly et al. 1985) which generates protons (H^+) and the vacuolar-type H^+ ATPase (V-ATPase) which pumps protons across the plasma membrane (Blair et al. 1989). Cellular equilibrium is maintained by passive chloride-bicarbonate exchangers (Teti et al. 1989) and chloride channel 7 (CLC-7), which exports chloride ions. Osteoclasts also express and release lysosomes and proteolytic enzymes, such as cathepsin K and matrix metalloproteinases (MMPs), which degrade the collagenous component of the bone

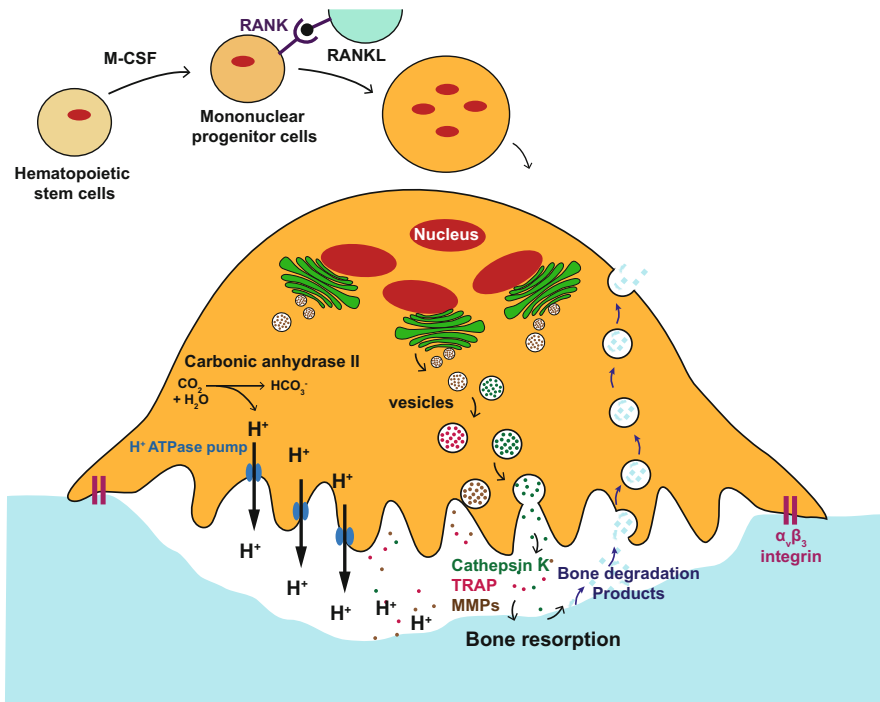


Fig. 1 Osteoclast formation and function. Multinucleated osteoclasts are formed by fusion of mononuclear precursors derived from hematopoietic stem cells; a process stimulated by macrophage colony-stimulating factor (M-CSF) and the interaction of receptor activator of NF-kappa B ligand (RANKL) provided by supporting cells (e.g., osteoblast lineage cells, T cells, etc.) with RANK on the surface of the osteoclast precursor. Multinucleated cells attach to the bone surface via $\alpha_v\beta_3$ integrin to form polarized osteoclasts with a sealing zone at the resorption area. The vacuolar-type H^+ ATPase in the ruffled border acidifies the resorption lacuna. Lysosomal proteases such as cathepsin K, tartrate-resistant acid phosphatase (TRAP), and matrix metalloproteinases (MMPs) are released by vesicle-mediated exocytosis to digest the organic matrix. Products of bone degradation are endocytosed in transcytotic vesicles, further digested inside the osteoclast, and released from the other side of the cell

matrix (Delaisse et al. 2003). Since bone resorption is a rapid energy-demanding process, osteoclasts have abundant mitochondria and a well-developed Golgi complex and endoplasmic reticulum.

During resorption, bone matrix degradation products are taken up from resorption lacunae by the osteoclast in transcytotic vesicles; they are then digested further within the cell and released by vesicular exocytosis at the cell surface opposite to the bone surface (Nesbitt and Horton 1997; Salo et al. 1997). One degradation product released is C-terminal telopeptide of type I collagen (CTX-1), which is measured in the serum as a bone resorption marker.

Bone resorption levels are determined both by the number and activity of osteoclasts. Reduced bone resorption, regardless of whether it is due to a reduction

in osteoclast number, osteoclast enzyme activity, or osteoclast motility, leads to high bone mass, termed osteopetrosis (Soriano et al. 1991; Sly et al. 1985). The study of these conditions led to the development of pharmacological agents to reduce bone resorption (anti-resorptives) in osteoporosis (Lacey et al. 2012).

The function of osteoclasts is not limited to bone resorption. Osteoclasts also regulate bone formation as discussed in Sect. 6.

2 The Osteoblast Lineage and Matrix-Producing Osteoblasts

Osteoblasts produce bone's collagen matrix and regulate its mineralization. The osteoblast lineage includes matrix-producing osteoblasts, their pluripotent and lineage-committed precursors, bone lining cells, and matrix-embedded osteocytes; each stage has distinct functions, morphologies, locations relative to the bone surface, and increasingly well-defined markers (Fig. 2).

The osteoblast lineage arises from pluripotent skeletal stem cells (SSC) which can also differentiate into chondrocytes, adipocytes, and hematopoiesis-supporting stromal cells (Gehron Robey and Riminucci 2020); their lineage is determined by

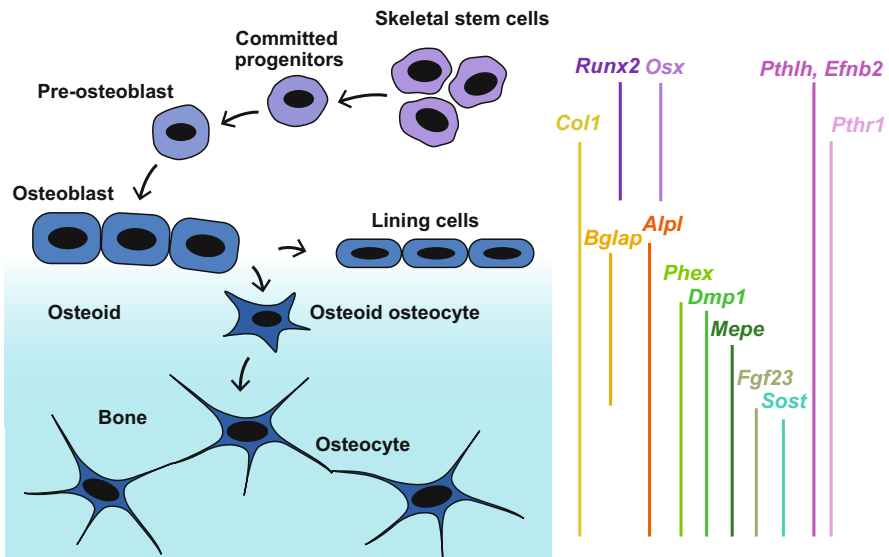


Fig. 2 Osteoblast/osteocyte lineage differentiation and gene expression. At early stages of commitment to the osteoblast lineage, expression of *Runx2* and *Osx* is high. Matrix-producing osteoblasts express PTH receptor (*Pth1r*), alkaline phosphatase (*Alpl*), and collagen type I (*Col1*). Osteocalcin (*Bglap*) is expressed by mature osteoblasts and newly embedded osteocytes close to bone-forming surfaces. Osteoid osteocytes and mature osteocytes express higher levels of *Phex*, *Mepe*, and *Dmp1*. Levels of *Fgf23* and *Sost* (sclerostin) are expressed at highest levels by the most mature osteocytes. Some genes, such as parathyroid hormone-related protein (*Pthlh*) and EphrinB2 (*Efnb2*), are expressed at similar levels through all stages of osteoblast differentiation. There are no known specific markers of bone lining cells

cytokines, hormones, growth factors, epigenetic factors, cell-matrix interactions, and direct cell-cell interactions within the lineage, each regulates expression of cell fate-specific transcription factors. Many of these promote commitment of SSC to the osteoblast lineage, but two are essential: runt-related transcription factor 2 (RUNX2) and osterix (OSX) (Ducy et al. 1999; Nakashima et al. 2002). RUNX2 stimulates osteoblast progenitor cell differentiation into preosteoblasts expressing low levels of collagen type I and alkaline phosphatase. OSX stimulates subsequent differentiation to mature osteoblasts producing high levels of collagen type I, alkaline phosphatase, and osteocalcin. Other transcription factors including activating transcription factor 4 (ATF4) (Yang et al. 2004), activator protein 1 (AP-1) (Sabatakos et al. 2000), and CCAAT/enhancer-binding proteins β and δ (C/EBP β and C/EBP δ) (Gutierrez et al. 2002) also promote the transition to matrix-producing osteoblasts.

Mature matrix-producing osteoblasts normally appear as a single layer of aligned cuboidal cells on the bone surface, forming tight junctions with adjacent osteoblasts, but they also form clusters within mesenchymal condensations and can deposit bone on other surfaces, such as mineralized cartilage. Bone is a heterogeneous compound material; it includes a mineral phase containing bioapatite and an organic matrix comprising largely type I collagen (~90%), non-collagenous proteins (~5%), small amounts of lipid (~2%), proteoglycans, and water (Fratzl et al. 2004). Osteoblasts deposit the organic component of bone matrix and non-collagenous proteins including growth factors such as transforming growth factor- β (TGF- β), insulin-like growth factor-1 (IGF-1), and mineralization regulators, such as osteocalcin. For this high level of protein production, active osteoblasts have a prominent Golgi complex and abundant rough endoplasmic reticulum.

Prior to mineralization, the collagen-containing matrix substance is called osteoid; thus, osteoblasts do not produce “bone,” but osteoid matrix, which is later mineralized to form bone. Osteoid is deposited in one of the two forms: woven or lamellar. Woven bone is deposited during bone development and fracture healing; it contains disordered, seemingly randomly oriented collagen fibers and osteocytes. In contrast, lamellar bone is highly organized, with parallel collagen fibers oriented in perpendicular planes in adjacent lamellae (Giraud-Guille 1988), adding strength. The collagen fibers are also stabilized and strengthened by inter- and intramolecular cross-links (Oxlund et al. 1995). Procollagen type I N-terminal propeptide (PINP) is a specific marker of collagen deposition and is used as a serum bone formation marker. Defects in collagen composition, collagen fibril assembly, cross-linking, or posttranslational modifications lead to skeletal fragilities, including those observed in osteogenesis imperfecta (Forlino and Marini 2016).

After osteoid is deposited, osteoblasts have three possible fates: they may undergo apoptosis, be trapped within the bone matrix and differentiate into osteocytes (Sect. 3), or remain on the bone surface as bone lining cells. The latter are characterized by flat nuclei and reduced capacity to produce protein but, like other cells of the osteoblast lineage, lining cells retain connections with each other via gap junctions (Miller et al. 1989). Bone lining cells can act as osteoblast precursors (Matic et al. 2016), and bone anabolic agents, such as PTH, convert murine quiescent bone lining cells into active osteoblasts (Dobnig and Turner 1995;

Kim et al. 2017). In addition, bone lining cells retract from the bone surface to allow access by osteoclasts for remodeling (Sect. 7).

The osteoblast lineage also regulates osteoclast differentiation by producing RANKL and OPG (see Sect. 9). This communication between bone-forming and bone-resorbing cells ensures an integrated response of bone tissue to systemic, local, or mechanical stimuli. Osteoblast lineage cells also act as “reversal” cells during bone remodeling (Sect. 7).

3 Osteocytes

Osteocytes form when osteoblasts are embedded within the osteoid matrix during bone formation. Osteocytes do not divide and may live for decades in the bone matrix, with their life span determined by the rate of bone remodeling. Osteocytes are the most abundant cells in the skeleton comprising 90–95% of all bone cells, a total of ~42 billion in the adult human skeleton (Buenzli and Sims 2015). They are now understood to be central regulators of bone strength by signaling to cells on the bone surface (Dallas et al. 2013) and by modifying their local environment (Blank and Sims 2019; Tsourdi et al. 2018).

The mechanism by which some osteoblasts become entrapped in the matrix is not yet known, but as they mature into osteocytes, their morphology changes including an increase in dendritic length and a reduction in cell body size (Dallas et al. 2013). The osteocyte cell body and processes reside in fluid-filled spaces within bone matrix called lacunae and canaliculi, respectively, to form the lacunocanalicular network. Osteocyte lacunar shape depends on the bone type and region. In woven bone, lacunae are irregular, spherical, and randomly oriented, while in lamellar bone, lacunae are ellipsoid and aligned with the direction of the collagen fibers (Marotti et al. 1985). Osteocyte dendritic processes make intercellular contacts with each other, with osteoblasts and lining cells on the bone surface, and they have been reported to reach into the nearby vascular canals (Dallas et al. 2013). The network is highly complex – within osteocytes alone, there are 3.7 trillion connections, and the network is very densely arranged (with cell numbers in the order of 19,000–28,500 per mm³) making up a large volume of the bone matrix itself (Buenzli and Sims 2015). This suggests the network could be an appealing target for therapeutic intervention. The large bone surface area of the osteocyte lacunocanalicular network (215 m²) provides ample space for bone mineral exchange and regulation (Buenzli and Sims 2015). However, there is only ~85 nm canalicular space surrounding each process (Varga et al. 2015; Buenzli and Sims 2015). This limits the size of therapeutics able to pass through the lacunocanalicular network to act on osteocytes.

Gene expression also changes during the osteoblast-to-osteocyte differentiation. Osteocalcin expression is higher in less mature osteocytes close to bone-forming surfaces (Sims et al. 1997), while expression of phosphate-regulating endopeptidase homolog X-linked (PHEX), matrix extracellular phosphoglycoprotein (MEPE), dentin matrix acidic phosphoprotein 1 (DMP1), and sclerostin increases as osteocytes differentiate. Such osteocyte markers regulate bone formation (sclerostin

(Li et al. 2005; Van Bezooijen et al. 2005)), mineralization (DMP1), phosphate homeostasis (PHEX, MEPE, FGF23), and dendrite formation (E11) (Dallas et al. 2013). Osteocytes also control bone mass and quality through the controlled release of cytokines and cytokine receptors including tumor necrosis factor- α (TNF- α) (Bakker et al. 2009), oncostatin M (Walker et al. 2010), soluble IL-6 receptor (McGregor et al. 2019), and IL-1 (Bakker et al. 2009).

4 The Process of Bone Mineralization: Control by Osteoblasts and Osteocytes

After osteoid is deposited, it becomes hardened by deposition of calcium-phosphate mineral under the control of osteoblasts and osteocytes. This has two phases: a primary, rapid initiation (which lasts for 1–3 days), followed by slower, gradual secondary mineralization (Fuchs et al. 2008). The latter phase may continue for several years, until a maximal level of mineralization is reached or until that portion of bone is remodeled (Sect. 7).

The initiation of primary mineralization is controlled, at least in part, by non-collagenous proteins produced by osteoblasts on the bone surface, since defects in late-stage osteoblast differentiation delay its initiation (Takyar et al. 2013). These proteins include osteocalcin, MEPE, PHOSPHO-1, and alkaline phosphatase (Houston et al. 2004; Ling et al. 2005; Gowen et al. 2003; Poole et al. 2005). In the absence of these factors, initiation is slow, and the mineralization front is diffuse, as observed in hypophosphatemic rickets or osteomalacia.

Secondary mineralization is controlled by later osteoblasts and osteocytes (Vrahnas et al. 2019; Blank and Sims 2019) and their expression of proteins such as DMP1, MEPE, ectonucleotide pyrophosphatase/phosphodiesterase (ENPP1), and PHEX (Kalajzic et al. 2004; Dallas and Bonewald 2010; Paic et al. 2009). There is naturally some overlap between these functions.

During primary and secondary mineralization, many changes occur within the matrix as it matures. Mineral accumulates (Fuchs et al. 2008, 2011), mineral crystal size increases, its structure becomes more ordered, and carbonate is incorporated into the bioapatite lattice (Vrahnas et al. 2016). The collagen fibers become more cross-linked (Paschalis et al. 2004) and more compact (Vrahnas et al. 2016, 2018), and water content reduces (Granke et al. 2015). There is much variation in the degree of these changes at the tissue level because basic multicellular unit (BMU)-based remodeling (Sect. 7) leads to bone heterogeneity (Boivin and Meunier 2002; Roschger et al. 2003). A high bone remodeling rate results in lower total mineralization since secondary mineralization of the BMU is truncated by bone resorption (Boivin and Meunier 2002; Paschalis et al. 1997). In contrast, when remodeling is slow, for example, with anti-resorptive therapies, more bone completes secondary mineralization uninterrupted and reaches the maximal mineralization level, leading to a higher average level of mineralization throughout the bone (Fuchs et al. 2011).

The processes by which mineral is deposited and accumulates within osteoid remain poorly defined. It commences with the budding of matrix vesicles from

cells facing the matrix (osteoblasts and possibly osteocytes) which attach to the surrounding collagen matrix. The vesicles accumulate ions outside the cell and rupture, releasing mineral into the surrounding matrix (Anderson 1967). This amorphous mineral forms ordered crystals through a nucleation process driven by contact with collagen (Rohde and Mayer 2007; Stanford et al. 1995). Matrix vesicles were originally reported to lack mineral and to accumulate poorly crystalline mineral only after budding from the cell and becoming immobilized in the collagen matrix (Anderson 1995). This remains the predominant view (Dillon et al. 2019), but others have reported the presence of mineral in matrix vesicles prior to their release (Rohde and Mayer 2007; Azari et al. 2008; Stanford et al. 1995). Approaches to treat hypophosphatasia and X-linked hypophosphatemia have focused on this process.

5 Chondrocytes

While not “bone cells” per se, chondrocytes (cells embedded in cartilage matrix) contribute to longitudinal bone growth and joint health in adults, so it is important to have a basic understanding of them when considering pharmacological effects on the skeleton.

In embryonic development, chondrocytes drive the initial stages of endochondral ossification (see Sect. 6). Their programmed proliferation, differentiation, cartilage matrix production, and eventual hypertrophy define the cartilaginous model of the skeleton to be formed. After ossification commences, continued proliferation, differentiation, and hypertrophy of chondrocytes at the epiphyseal growth plates facilitate longitudinal growth of the pediatric and adolescent skeleton, which ends with the removal of the growth plates in the adult skeleton. Growth plate chondrocytes also influence the early stages of vascularization and bone deposition during bone growth (Poulton et al. 2012), and this may determine adult bone mass, as well as bone size. Many factors influencing osteoblast differentiation and bone formation also regulate chondrocyte activity (e.g., parathyroid hormone-related protein (PTHrP), TGF- β , BMP2, Wnt/ β -catenin signaling, fibroblast growth factor, growth hormone, and the insulin-like growth factors (IGFs), reviewed in detail elsewhere (Hartmann and Yang 2020)); this may relate to their derivation from a common precursor cell. These effects should be kept in mind, particularly in pediatric pharmacology.

Chondrocytes also form the cartilage matrix covering the ends of long bones at the joints. Here, cartilage protects subchondral bone (bone underlying the cartilage) from mechanical stress, and the close proximity of cartilage and bone provides biochemical and molecular cross talk between these two tissues. Since cartilage is avascular, bone tissue provides nutrition to support chondrocyte metabolism, and chondrocytes in turn may provide factors able to promote osteoblast activity. This is reviewed in detail elsewhere (Findlay and Atkins 2014).

6 Osteogenesis and Bone Modeling

The cells of bone (osteoclasts, osteoblasts, and osteocytes) and cartilage (chondrocytes) form the skeleton during embryogenesis, control its growth in childhood, and manage its renewal in adult life. The shape and size of bone, and its organization into compact cortical bone at the periphery, with the inner trabecular network require tight coordination within and between each cell lineage. During embryogenesis, the skeleton forms initially when mesenchymal progenitor cells aggregate at sites where bone is to be formed. These bones form by two ossification processes: intramembranous and endochondral.

In intramembranous ossification, the progenitors gather and differentiate directly into osteoblasts; these deposit osteoid, which forms membranous bone, including the flat bones of the skull, mandible, maxilla, and clavicles. These bones grow by further osteoblastic differentiation of mesenchymal cells at the periphery (periosteum) of the newly forming bone. This process requires tight regulation of osteoblast differentiation for the skull to form normally; for example, in mice and humans with defective osteoblast differentiation due to lack of the transcription factors *Runx2* and *Osterix*, early cessation of skull growth results in cleidocranial dysplasia (Ducy et al. 1997; Mundlos et al. 1997; Otto et al. 1997; Lee et al. 1997).

The majority of the skeleton forms by endochondral ossification; this also requires tight control of cellular differentiation and communication between multiple cell types. In this process, mesenchymal progenitors first differentiate to chondrocytes and form a cartilage template of the future bone, which is gradually replaced with mineralized bone (Fig. 3). As the chondrocytes proliferate and deposit cartilage, the template grows, and chondrocytes at the center become hypoxic and enter hypertrophy; this contributes to longitudinal expansion (Cooper et al. 2013). As the cells enter hypertrophy, their gene expression pattern changes, leading to three major changes: mineralization of the surrounding cartilage matrix, vascular invasion, and entry of osteoclast precursors. This is followed by resorption of the mineralized cartilage, which expands the marrow cavity, leading to a new morphology where the long bone houses two growth plates, in mirror image, separated by the primary ossification center. As the bone continues to grow, mineralization and vascular invasion occur in the two remaining cartilaginous ends of the bones, forming the secondary ossification centers.

Chondrocyte proliferation and subsequent hypertrophic expansion at the two growth plates continues longitudinal bone growth. As this occurs, mineralized cartilage remnants from the resorptive process form a template on which osteoblasts deposit new bone to form trabecular bone (Mackie et al. 2011). At the diaphyseal perichondrium, cortical bone forms by the coalescence of trabecular bone with a thin periosteum formed by intramembranous ossification (Rauch 2012). In humans, the growth plates close and are fully resorbed in adulthood. In rodents, while the growth plates remain, they are essentially inactive as bones do not continue to lengthen.

Changes in bone shape continue as the skeleton adapts during growth and in adulthood. Growth continues to be mediated by bone formation on expanding external surfaces (such as the periosteum) and resorption on internal expanding

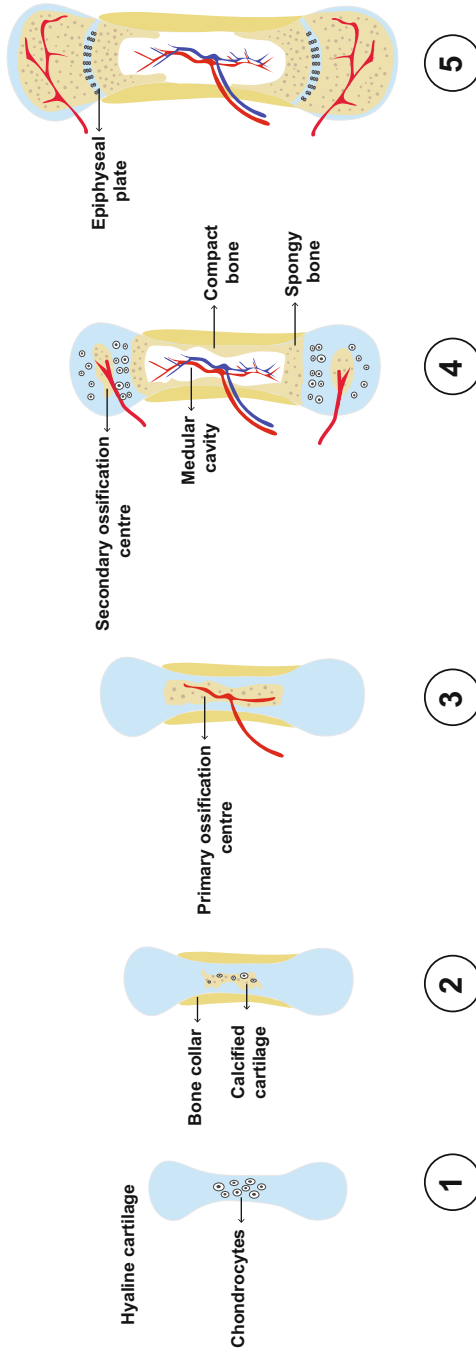


Fig. 3 Endochondral ossification. During skeletal development, long bones are formed by endochondral ossification. Chondrocytes at the center of the growing cartilage model of bone become hypertrophic (1) and produce calcified matrix (2). Blood vessels invade the spaces remaining from the death of chondrocytes and hematopoietic progenitors into the tissue. These differentiate into osteoblasts and osteoclasts, forming the primary ossification center (3), which enlarges with the activity of osteoclasts and eventually becomes the medullary cavity. Cartilage continues to grow at the ends of the bone (epiphyses) and forms a secondary ossification center through the same process (4). When fetal skeletal development is completed, cartilage remains only at the joint surface and between the diaphysis and epiphysis (referred as the epiphyseal growth plate) (5). Epiphyseal plate chondrocytes drive the longitudinal growth of bones until puberty, when their proliferation ceases.

surfaces (such as the endocortical surface). In such situations, there is not direct communication between osteoblasts and osteoclasts, but these processes must be coordinated, perhaps by signals through the osteocyte network, termed osteo-transmitters (Johnson et al. 2015). When bone formation and resorption occur on different surfaces, and lead to changes in bone shape, this is termed “modeling.”

7 Bone Remodeling

Another process involving coordination of osteoclasts and osteoblasts is the renewal of cortical and trabecular bone throughout life by remodeling. This process occurs in small regions of bone asynchronously throughout the skeleton, allowing gradual renewal of the entire skeleton over time. Bone resorption and formation occur in sequence on the same surface in the remodeling cycle; this contrasts with modeling, in which the activities occur on different surfaces. Remodeling has five well-defined phases: (1) activation of remodeling, (2) bone resorption, (3) a reversal phase, (4) bone formation, and (5) quiescence (Fig. 4). The process by which growth plate cartilage is converted to bone during growth (Sect. 6) is analogous to bone remodeling, since it includes sequential cycles of bone resorption and formation. For bone remodeling to maintain bone mass, the amount of bone formed by osteoblasts must be matched to the amount resorbed by osteoclasts. If unbalanced, bone mass changes (e.g., greater resorption than formation leads to bone loss). A better understanding of coupling, both on the surface of bone and in the generation of new trabecular bone at the growth plate, will aid in developing agents to build bone mass by shifting the balance toward bone formation for osteoporosis therapy.

Activation of bone remodeling likely occurs when osteocytes respond to bone damage by apoptosis by releasing signals to nearby cells (Schaffler et al. 2014). This attracts osteoclast precursors which fuse to form multinucleated cells, attach, and resorb bone; the signals terminating resorption are not known. During the subsequent reversal phase (Baron 1977), the newly exposed surface is cleaned by reversal cells, now understood to be mononuclear osteoblast lineage cells (Everts et al. 2002). Recently it has been proposed that small osteoclasts reside among the reversal cells and continue bone resorption but at a slower rate (Lassen et al. 2017). Toward the end of the reversal phase, osteoblast precursors accumulate on and above the bone surface and differentiate into bone-forming osteoblasts (Lassen et al. 2017). These cells form new bone in the pit left by the resorbing osteoclasts. Following bone formation, lining cells cover the new bone surface which becomes quiescent, but mineralization in the underlying bone continues.

Remodeling occurs both on trabecular bone surfaces and in the cutting cones of osteonal cortical bone. In the latter case, osteoclasts resorb a tunnel into the bone, which is refilled by osteoblasts; the sequence is the same in both processes. Here we will review basic principles and examples and refer the reader to recent reviews for the details of the many communication pathways involved (Sims and Martin 2015, 2020).

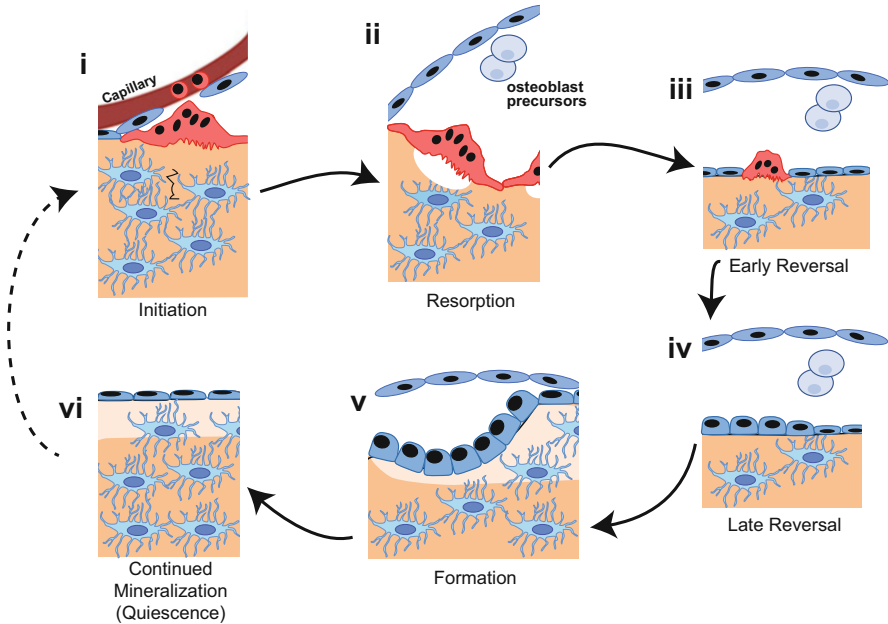


Fig. 4 The bone remodeling cycle. Bone remodeling is initiated (i) when osteocytes sense microdamage within the bone and signal to the bone surface, resulting in lifting of bone lining cells, egress of osteoclast precursors from the vasculature, and the attachment of multinucleated osteoclasts to the bone surface. Bone resorption (ii) follows, occurring within the space outlined by the canopy, and during bone resorption, osteoclasts also provide signals to osteoclast precursors within the remodeling space. The reversal phase follows. During the early reversal phase (iii), mononuclear osteoblast lineage cells cover the bone surface and, with small osteoclasts, complete the process of resorption. In the late reversal phase (iv), osteoblast progenitors accumulate on the bone surface, until there are sufficient for the bone formation phase to commence (v). After the osteoblasts have refilled the pit, bone mineralization continues during the “quiescence” phase (vi) until a maximum level is reached or until the cycle recommences

8 Coupling Factors: Signals Between Osteoclasts and the Osteoblast Lineage

Since bone remodeling occurs on a single surface, there must be local signals to control transitions between each phase. The coupling of bone resorption to subsequent bone formation has been studied extensively, leading to the identification of multiple classes of coupling mechanisms. These include (1) factors released from the bone matrix during osteoclast-mediated resorption (matrix-derived factors); (2) factors produced and expressed by osteoclasts themselves (secreted, released by exocytosis, or expressed on the cell surface); and (3) non-osteoclast-mediated mechanisms. Although most coupling factors released or expressed by osteoclasts described to date stimulate bone formation, they may also have inhibitory effects.

Release of non-collagenous factors from the bone matrix by active osteoclasts was the first mechanism of coupling described. During bone resorption, the acid pH generated by osteoclasts releases and activates proteins such as TGF- β and IGF-1 from the bone matrix (Howard et al. 1981). Since there is a time delay between bone resorption and subsequent formation, these factors must act on early osteoblast precursors to promote their recruitment and migration to the bone surface. Differentiation of these cells into osteoblasts and the level of bone formation they exert are likely controlled by later processes during the remodeling cycle (Sims and Martin 2014). The importance of released matrix-bound factors is highlighted by low bone formation levels in patients and mouse models with “osteoclast-poor” osteopetrosis (Sobacchi et al. 2007; Frattini et al. 2007; Grigoriadis et al. 1994).

However, in “osteoclast-rich” osteopetrosis, where bone resorption is impaired but osteoclasts are still present, bone formation is normal or even increased, rather than being reduced (Sobacchi et al. 1993; Marzia et al. 2000; Gil-Henn et al. 2007; Pennypacker et al. 2009; Del Fattore et al. 2006). Osteoclasts therefore also express coupling factors independent of their resorptive activity. This is one reason why anti-resorptive approaches to reduce osteoclast formation (such as RANKL inhibition) may have different effects on bone formation to anti-resorptives that block osteoclast activity. This was noted when experimental cathepsin K inhibitors, which blocked bone resorption without blocking osteoclast formation, retained osteoblast activity when used in animal models (reviewed in Sims and Ng 2014).

Many coupling factors synthesized by osteoclasts have now been identified, such as cardiotrophin-1 (Walker et al. 2008), sphingosine-1-phosphate (Pederson et al. 2008; Ryu et al. 2006), and contact-dependent molecules, such as EphrinB2 (Zhao et al. 2006) and semaphorin D (Negishi-Koga et al. 2011). None are uniquely expressed by osteoclasts (Sims and Martin 2015, 2020). It should also be noted that while it is plausible *in vitro* for membrane-bound factors to access mature osteoblasts, this is unlikely *in vivo* due to the time delay between bone resorption and formation during remodeling (Sims and Martin 2014). Osteoclasts are more likely to come into contact with osteoblast precursors, bone lining cells on the bone surface or in the remodeling canopy, or osteoblast lineage cells in the reversal phase.

Osteoclasts may also stimulate bone formation during remodeling by releasing microvesicles containing coupling factors (Ekstrom et al. 2013). These have been reported to stimulate osteoblast differentiation by their expression of RANK (Ikebuchi et al. 2018) and to inhibit osteoblast activity by providing micro-RNAs (Li et al. 2016). Although it is hard to know how these microvesicles could be provided to osteoblast precursors in a controlled manner, this is an intriguing possibility.

Coupling can also be regulated by osteoclast-independent mechanisms. One example is the size and shape of the resorption pit: osteoblast lineage cells, once attracted to the resorbed bone surface, can “sense” changes in topography and fill the space left by the resorbing osteoclast (Gray et al. 1996). While osteoclast-derived coupling factors may attract precursor cells to the surface, the topography of the bone itself regulates the matrix-depositing activity of the osteoblasts. Osteoblasts must sense the spatial limits and inform each other (via gap junctions or cell-contact-

dependent communication processes such as EphrinB2:EphB4 signaling) of when the space has been filled (Tonna and Sims 2014).

The formation of a cellular canopy over the active BMU during the initiation of remodeling was proposed many years ago (Rasmussen and Bordier 1974) and identified much later in human biopsies (Hauge et al. 2001). Bone lining cells, confirmed as osteoblast lineage cells by immunohistochemistry (Kristensen et al. 2013), are suggested to lift from the bone surface at the start of the remodeling cycle to form a compartment that moves with the osteoclast during resorption. This zone provides an isolated space for coupling to occur and connects with the vasculature, providing a route for osteoclast precursors, including partially differentiated “quiescent osteoclast precursors” (Mizoguchi et al. 2009) to enter the remodeling space. Capillaries associated with the canopy also provide a mechanism for ingress of other cells, including mesenchymal precursors (Eghbali-Fatourehchi et al. 2007) and immune and endothelial cells (see below).

The reversal phase may also mediate coupling independent of osteoclasts. Mononuclear cells lining the bone surface during the reversal phase activate matrix metalloproteinases to clean collagen remnants from the resorption pits and signal to osteoblasts to act in that space (Everts et al. 2002); this is controversial since osteoblasts even form bone in areas in which pits have been made mechanically (Gray et al. 1996). The lining cells express osteoblast lineage markers (Delaisse 2014) and become progressively more active as they accumulate (Lassen et al. 2017), suggesting a reversal phase during which osteoblast differentiation continues until a critical mass of mature osteoblasts is reached for matrix formation to commence.

9 RANKL/OPG as an Example of Signals from the Osteoblast Lineage to Osteoclasts

Communication between bone cells is not restricted to actions from osteoclasts to the osteoblast lineage. The osteoblast lineage also supports osteoclast formation and the process of bone resorption. The most intensely studied pathway is the production of RANKL and OPG by the osteoblast lineage, but the stage of osteoblast differentiation responsible remains controversial.

RANKL (gene name: *TNFSF11*) is a member of the TNF superfamily, expressed by a wide range of cells, including osteoblasts, osteocytes, hypertrophic chondrocytes, T cells, mammary gland epithelial cells, and stromal cells (Martin and Sims 2015); many of these reside within the local bone environment. RANKL stimulates osteoclast differentiation when it binds to RANK on hematopoietic progenitors and induces fusion of osteoclast precursors (Sect. 1, Fig. 1). RANKL binding to RANK is restricted by a decoy soluble receptor osteoprotegerin (OPG; gene name: *TNFRSF11B*), which is thereby a strong physiological inhibitor of osteoclast formation. Early studies in gene knockout mice showed RANKL is essential for osteoclastogenesis and OPG could inhibit it; mice overexpressing OPG (Simonet et al. 1997) and mice lacking RANKL (Kong et al. 1999; Dougall

et al. 1999) both showed severe osteopetrosis. These discoveries led to the development of denosumab, an anti-RANKL monoclonal antibody now used for the treatment of osteoporosis (Lacey et al. 2012).

Elevated expression of RANKL stimulates osteoclast formation both in pathology and in normal remodeling. One example of its role in pathology is inflammatory arthritis, where T cells express elevated RANKL levels under the influence of inflammatory cytokines (Horwood et al. 1999). Pharmacologic inhibition of RANKL or of osteoclastogenesis therefore blocks both pathological joint erosion and the systemic bone loss associated with the inflammation (Romas et al. 2002; Sims et al. 2004).

RANKL-inducing cytokines, such as oncostatin M, are also required for physiological regulation of remodeling (Sims 2016). These locally produced cytokines stimulate osteoclast formation, not by acting directly on osteoclast precursors, but acting on by osteoblast lineage cells (Tamura et al. 1993; Jimi et al. 1996). The same is true for systemic factors like PTH and 1,25-dihydroxyvitamin-D₃. These early *in vitro* studies used a mixed stromal cell population including immature and mature osteoblast lineage cells. Importantly, the interaction of RANK and RANKL was contact-dependent (Tamura et al. 1993; Jimi et al. 1996), indicating it is membrane-bound RANKL, and not any soluble form, that induces osteoclastogenesis. Very recent mouse genetic studies have confirmed membrane-bound, and not soluble, RANKL is required for physiological osteoclastogenesis *in vivo* (Asano et al. 2019).

More recently, osteopetrosis due to impaired osteoclastogenesis was detected in mice with deletion of RANKL in the entire osteoblast lineage (Xiong et al. 2011), confirming the importance of RANKL in the osteoblast lineage indicated by those early stromal cell culture studies. Surprisingly, RANKL deletion in late osteoblasts and osteocytes also led to a reduction in osteoclastogenesis and high bone mass (Xiong et al. 2015; Nakashima et al. 2011). This suggested both early osteoblasts and osteocytes might support osteoclast formation by producing RANKL. The ability of osteocytes to support osteoclastogenesis has been controversial, since there is little evidence of direct contact between osteocytes and osteoclast precursors. However, even when cultured in contact with osteoclast precursors *in vitro* osteocytes do not fully support osteoclast formation (Chia et al. 2015; McGregor et al. 2019), and it must be remembered that the phenotype of the osteocyte-specific knockout is much less severe than that of the osteoblast-lineage knockout. This suggests that the physiological role of osteocyte-derived RANKL is less critical than RANKL derived from less differentiated osteoblasts and their precursors.

Although this is the most studied pathway, the osteoblast lineage also uses RANKL-independent mechanisms to regulate bone resorption, including signals to initiate remodeling (Schaffler et al. 2014) and to stimulate osteoblast precursor proliferation (Van Wesenbeeck et al. 2002; Wiktor-Jedrzejczak et al. 1990), chemo-attractants to enlist osteoclast precursors to the bone surface (Onan et al. 2009), and factors modulating mature osteoclast activity (Wong et al. 1999).

10 Signals Within the Osteoblast Lineage Regulate Bone Formation

The regulation of remodeling is not restricted to coupling factors and the RANKL/OPG system; many factors expressed in the osteoblast lineage also work within the lineage to regulate bone formation and resorption.

A key example of communication within the osteoblast lineage is sclerostin. Its importance is clear since genetic suppression of sclerostin, either in mouse models or in humans, leads to profoundly elevated bone mass (Balemans et al. 2001, 2002; Li et al. 2009). Sclerostin is synthesized and secreted by osteocytes yet acts at earlier stages of osteoblast differentiation to inhibit bone formation. This is achieved by binding of sclerostin to lipoprotein receptor-related protein (LRP) 4/5/6 and thereby antagonizing the Wnt/ β -catenin signaling pathway earlier in the osteoblast lineage (Baron and Kneissel 2013). The profound high bone mass phenotypes of individuals with suppressed sclerostin expression led to the development of pharmacological agents to inhibit sclerostin action as treatments for osteoporosis and other conditions of skeletal destruction (Cosman et al. 2016). Sclerostin also appears to stimulate osteoclastogenesis (at least indirectly), since patients treated with anti-sclerostin exhibited decreased CTX1 levels (Cosman et al. 2016). The mechanism by which this occurs remains somewhat unclear: while mice lacking β -catenin in osteoblasts have reduced osteoclast formation and elevated OPG levels (Glass et al. 2005), sclerostin treatment of osteocytes in vitro suppresses OPG expression (Wijenayaka et al. 2011). Regardless of mechanism, if anti-sclerostin acts as a mixed anabolic and anti-catabolic agent, this is likely to improve its efficacy for low bone mass.

The osteoblast lineage also secretes locally acting proteins that stimulate bone formation, such as PTHrP (Ansari et al. 2018; Miao et al. 2005) and the IL-6 family cytokine oncostatin M (Walker et al. 2010). Both are secreted by osteoblasts and osteocytes, both promote bone formation by suppressing sclerostin and by promoting osteoblast progenitor proliferation and commitment to the osteoblast lineage. Furthermore, when acting early in the osteoblast lineage, both promote osteoclastogenesis by stimulating expression of RANKL.

Osteoblasts also express contact-dependent molecules regulating osteoblast differentiation. Examples of this include gap junction molecules such as connexin 43, which promotes osteoblast survival and function (Stains et al. 2014; Plotkin and Bellido 2013), and membrane-bound EphrinB2. Although the latter is expressed at all stages of the lineage, its function differs depending on the stage of differentiation. Early in the committed lineage, EphrinB2 promotes the late stages of osteoblast differentiation (Takyar et al. 2013) and initiation of osteoid mineralization (Tonna et al. 2014). In contrast, EphrinB2 expression in osteocytes limits secondary mineralization, without affecting initiation of osteoid mineralization (Vrahnas et al. 2019).

11 Interactions Between Bone Cells and Other Cells and Tissues in and Beyond Bone

Bone cells act as local regulators to maintain bone tissue homeostasis and to influence nearby tissues such as the marrow, vasculature, and skeletal muscle. Within bone itself, endochondral ossification and remodeling depend on contributions from the vasculature (see above) and the neuronal system (Idelevich and Baron 2018). Not only this, but a wide range of cells present in the BMU, including macrophages and T cells, regulate osteoblast differentiation (Sims and Martin 2014). The osteoblast lineage in turn regulates the hematopoietic stem cell niche (Calvi et al. 2003; Askmyr et al. 2009) and contributes to hematopoietic malignancies (Raaijmakers et al. 2010) and B cell homeostasis (Wu et al. 2008).

Cross talk between bone and muscle is also vital for skeletal development and maintenance. A well-studied example of this is the response of osteocytes to mechanical load and the anabolic effect of exercise on the skeleton, particularly during bone growth and development. However, the interaction is not limited to mechanical effects. Osteoblasts release muscle-active cytokines (myokines), and muscle also produces bone-active cytokines (Johnson et al. 2014; Brotto and Bonewald 2015).

The osteoblast lineage also acts as an endocrine system to regulate the function of other organs (Fig. 5). Through the release of FGF23, osteocytes act on the kidney

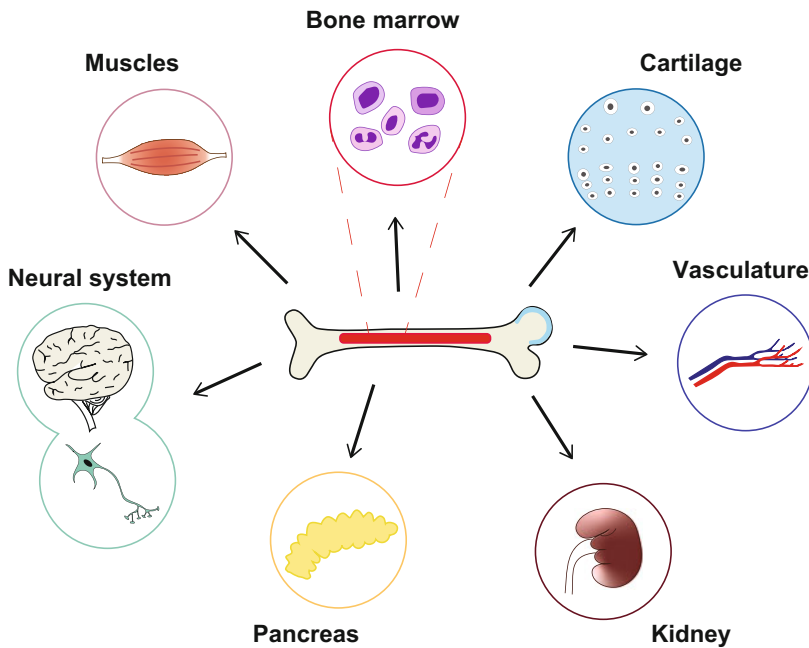


Fig. 5 An overview of the diversity of interactions regulating bone structure. Bone tissue interacts with other cells and tissues in their close proximity, such as cartilage, blood vessels, marrow cells, nerves, and muscles. It also affects distant organs, such as the pancreas, brain, and kidney, by producing and secreting different factors into circulation

to regulate phosphate homeostasis (Fukumoto and Martin 2009), and release of osteocalcin by the osteoblast lineage has been implicated in functions as diverse as glucose metabolism, fertility, memory, and the fight-or-flight response (Berger et al. 2019; Karsenty 2017). These effects and the molecules responsible for transmitting them must be considered when using pharmacological agents to modify bone cell function.

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