Basic Aspects of Osteoblast Function

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Key Points

- The osteoblast lineage includes pluripotent precursors, preosteoblasts, osteoblasts, osteocytes, and bone lining cells.
- Osteoblasts are the cells responsible for formation of the collagen-rich bone matrix (osteoid) which becomes mineralized by the deposition and accumulation of mineral crystals.
- Mineralization of the bone matrix is regulated by proteins produced by the osteoblast lineage including alkaline phosphatase and non-collagenous proteins in the bone matrix.
- Osteoblast lineage cells also control the differentiation of osteoclasts through their production of receptor activator of NF-KB ligand (RANKL), macrophage colony-stimulating factor (M-CSF), and osteoprotegerin (OPG).
- Bone lining cells have the potential to be a source of osteoblast precursors.

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Introduction to the Osteoblast Lineage: Multiple Stage-Specific Functions

Osteoblasts are specialized mesenchymal-derived cells that produce and deposit the collagenous bone matrix and regulate the mineralization of that matrix by their production of additional non-collagenous proteins. The osteoblast lineage includes not only these bone-forming osteoblasts but also their pluripotent and lineage-committed precursors, bone lining cells, and matrix-embedded osteocytes (Fig. 1.1). Each of these stages of the osteoblast lineage has distinct functions, morphologies, particular locations relative to the bone surface, and increasingly well-defined markers of differentiation (noted on Fig. 1.1 and discussed below).

The different stages of osteoblast differentiation allow these cells to perform three major functions that determine skeletal structure (noted on Fig. 1.1 and discussed below): (1) production of bone matrix (osteoid), (2) regulation of osteoid mineralization by production of non-collagenous proteins, and (3) support of osteoclast formation. In addition, osteoblast lineage cells produce paracrine factors, such as IL-6 family cytokines, parathyroid hormone-related protein (PTHrP), and contact-dependent molecules such as EphrinB2, that regulate their own differentiation and activity [1–3]. Osteoblasts have also been suggested to act as "reversal" cells, allowing communication

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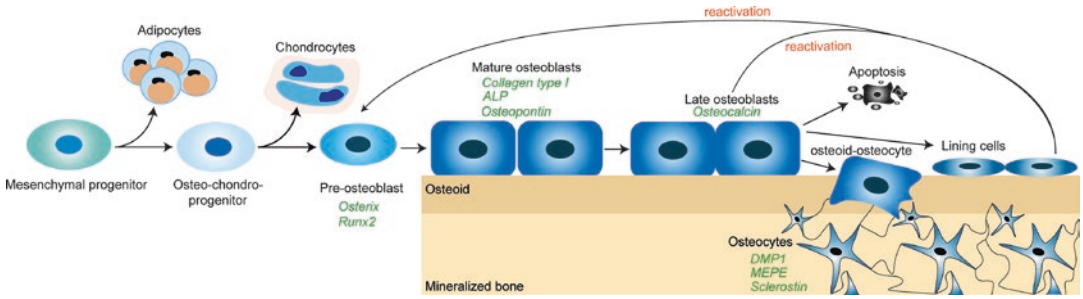

Fig. 1.1 Stages of the osteoblast lineage. The osteoblast lineage arises from pluripotent mesenchymal progenitors, capable of differentiating into adipocytes or into chondrocytes or osteoblasts. Commitment to the osteoblast lineage is determined by expression of transcription factors including Runx2 and osterix. Once osteoblasts become mature, they deposit collagen type I-rich matrix (osteoid) as a template for bioapatite mineral deposition and express alkaline phosphatase (ALP), osteopontin, and osteocalcin, proteins that regulate bone mineralization. Osteoblasts then undergo one of three fates: (1) apoptosis, (2) remain on the bone surface as bone lining cells, or (3) become embedded within their collagenous bone matrix as "osteoid-osteocytes," which then become terminally differentiated osteocytes. Osteocytes also regulate the mineralization of the bone matrix through their production of DMP-1, MEPE, and sclerostin. Bone lining cells appear to be capable of reactivation to become active osteoblasts or osteoblast precursors

between osteoclasts and osteoblasts, during the bone remodeling process [4]. The functions of the osteoblast lineage are not limited to the control of bone structure. They also regulate the hematopoietic stem cell niche [5, 6], contribute to hematopoietic malignancies [7], and to B cell homeostasis [8]. The osteoblast lineage also has endocrine functions in phosphate homeostasis [9] and glucose metabolism [10]. This chapter will focus on describing the stages of osteoblast differentiation and the functions of the lineage that regulate bone structure and bone matrix composition.

Osteoblast Differentiation and the Stages of the Osteoblast Lineage

Osteoblast Precursors

The osteoblast lineage arises from pluripotent mesenchymal progenitors. In vitro, these cells can be induced to differentiate into other mesenchymal origin cells such as chondrocytes, adipocytes, myoblasts, or fibroblasts [11] (Fig. 1.1). In vivo, bone marrow-derived mesenchymal progenitors have a more restricted future, being capable of differentiating into chondrocytes, osteoblasts, and adipocytes [12]. The location of these cells in the marrow has been refined by cell lineage-tracing studies (using genetically altered mice with fluorescent tags that are retained throughout differentiation) to be in close association with vascular structures [13]. This provided support for much earlier studies proposing that the pericyte, a cell found wrapped around endothelial cells, can behave as an osteoblast progenitor [14, 15]. Pericytes in different tissues appear to behave in an organ-specific manner, dictated by their anatomy and position; only bone marrow-residing pericytes appear capable of becoming osteoblasts [12]. This illustrates the importance of the microenvironment in determining differentiation. For more details, the reader is directed to a recent focused review on the identity of osteoblast progenitor populations [16].

The source of osteoblast progenitors is not restricted to the bone marrow pericytes. During embryonic bone development, perichondrial cells were identified as precursors giving rise to osteoblasts on trabecular bone [17]. This has been confirmed by lineage-tracing studies, which also identified these precursors as entering the marrow space with invading blood vessels and thereby contributing to both bone development and fracture healing [18]. Similar observations have been made that differentiated hypertrophic

chondrocytes at the growth plate can "transdifferentiate" into osteoblasts during development and fracture healing [19], again confirming much earlier in vitro work [20]. Lineage tracing studies have also suggested that bone lining cells [21] and recently embedded osteocytes [22] can act as osteoblast precursors, although the latter remain highly controversial. It is likely that lining cells are already committed to the lineage, rather than having the potential to differentiate in chondrocytes or adipocytes. This suggests that there are multiple sources of osteoblast progenitors in vivo, with differentiation that is both context- and location-specific.

Commitment of precursors to the osteoblast lineage is controlled by the expression of a range of transcription factors. Absolutely essential for the commitment to the preosteoblast stage are runt-related transcription factor 2 (Runx2) and osterix [23, 24]. Other transcription factors including activating transcription factor 4 (ATF4) [25], activator protein 1 (AP-1) [26], and CCAAT/enhancer-binding proteins β and δ (C/EBPβ and C/EBPδ) [27] promote the transition to matrix-producing osteoblasts.

Since osteoblasts and adipocytes are derived from common precursors, many of these transcription factors also inhibit mesenchymal progenitor commitment to adipogenesis [26, 28]. Alternatively, transcription factors such as peroxisome proliferation-activated receptor γ (PPARγ) [29] and CCAAT/enhancer-binding protein α (C/EBPα) [30] promote differentiation into adipocytes. This inverse relationship between osteoblast and adipocyte differentiation was first observed in cell culture [31]. This has also been described in vivo in genetically altered mouse models, either where high osteoblast numbers are associated with low marrow adipocyte volume [26] or where low osteoblast numbers are associated with high marrow adipocyte volume [32–34]. Similar reciprocal regulation has been made in animal models of ovariectomy-induced bone loss [35]. There are exceptions to this, such as the C3H/HeJ mouse strain which has high bone mass [36] and high marrow adiposity [37]. Reciprocal regulation of osteoblasts and adipocytes has also been observed clinically: increased marrow adiposity is associated with age-related osteoporosis [38]. Understanding the relationships between osteoblast and adipocyte commitment remains an area of active research, since it may allow the development of additional treatments to increase bone mass.

The osteoblast precursor can also give rise to chondrocytes; this is important in the context of developmental and pediatric bone growth, and fracture healing, and may be of relevance for methods to repair joint cartilage. The osteoblast commitment transcription factors Runx2 and osterix not only promote osteoblast commitment but also stimulate the final stage of chondrocyte differentiation prior to vascular invasion in endochondral ossification [39–41]. Reciprocal regulation of chondrogenesis versus osteoblastogenesis from the same common precursor has also been suggested [42], as described above for adipocytes, but mechanisms controlling this have not yet been identified.

Matrix-Forming Osteoblasts

Mature matrix-forming osteoblasts are characterized by a cuboidal morphology and are located in groups with extensive cell-cell contact [43–45]. Osteoblasts are also located in close apposition to the bone surface; this indicates that as they differentiate to this stage, these cells must migrate, probably in groups to the bone surface, likely in response to coupling factors produced by osteoclasts or other cells within the basic multicellular unit [46–48]. There are two exceptions to this. During skeletal development, osteoblasts can form bone de novo (without a surface to work on), and during endochondral ossification, calcified cartilage serves as a template on which osteoblasts deposit bone.

At the electron microscope level, matrix-forming osteoblasts exhibit abundant endoplasmic reticulum, in line with their major function as factories for production of type I collagen, the main component of the osteoid matrix (see below). Matrix-producing osteoblasts also express a range of non-collagenous proteins. These include proteins involved in regulating the

incorporation of mineral into the osteoid matrix (alkaline phosphatase [49], osteocalcin [50], and osteopontin [50]) and receptors that regulate their response to factors influencing their further differentiation and function, such as receptors for IL-6 family cytokines [33, 51] or the receptor used by parathyroid hormone (PTH) and PTH-related protein (PTHrP), PTH1R [52]. The mechanisms of matrix production and mineralization will be discussed below.

When their production of osteoid matrix is complete, mature osteoblasts undergo one of three fates: [1] remain on the surface of bone as less metabolically active bone lining cells, [2] die by apoptosis, or [3] become entrapped within the osteoid matrix and, as the osteoid is mineralized, further differentiate to become osteocytes (Fig. 1.1).

Osteocytes

Osteocytes are embedded within the bone matrix during the process of bone formation, and through their extensive dendritic processes and their fluid-filled network of communicating channels, they sense and respond to mechanical strain and microdamage to bone. They are the most abundant cells in bone by far, forming a highly complex cellular communication network through the bone matrix with a total of ~3.7 trillion connections throughout the adult skeleton [53].

How osteoblasts become embedded into the bone matrix remains unknown. The manner in which osteoblasts become osteocytes has been described as "encased," "buried," and "merged" into the matrix suggesting that the manner of transformation may depend on the type of bone formed [54]. It is possible that the type of bone being made (woven vs lamellar) or mode of ossification as well as location (periosteal/endocortical/trabecular) can determine how an osteoblast becomes embedded into the matrix. There are no specific signals made by the osteoblast that have been found to directly control this process. When an osteoblast transitions into the recently secreted matrix (osteoid) to become an osteocyte (Fig. 1.1) they are termed "osteoid-osteocytes" [55]. The most striking difference between osteoblasts and osteoid-osteocytes is the morphological change that occurs during this transition. The cuboidal morphology of the osteoblast changes into a less cuboidal cell which eventually transforms into a smaller cell body with many dendritic cellular projections characteristic of osteocytes. Upon mineralization of the osteoid, the ultrastructure of the osteocyte changes in line with its reduced protein-production capacity, including reduced endoplasmic reticulum and Golgi apparatus [56].

Differentiated osteocytes reside within lacunae in the bone matrix and form an extensive intercellular network throughout the bone matrix and regulate both bone formation and resorption. Cell contact is a notable feature of this network [53], as is the ability of these cells to sense and respond to mechanical load and microdamage [57]. In addition to controlling osteoblast activity on the bone surface by the release of local factors such as sclerostin [58], and oncostatin M [33], osteocytes regulate mineralization of the bone matrix by expressing factors such as dentin matrix protein 1 (DMP-1) [59] and matrix extracellular phosphoglycoprotein (MEPE) [60] and act in an endocrine manner to control phosphate homeostasis by their release of fibroblast growth factor 23 (FGF23) [61] (refer also to Chap. 3 (Basic Aspects of Osteocyte Function)).

Bone Lining Cells

Osteoblasts that do not become terminally differentiated osteocytes or undergo apoptosis remain on the bone surface to become flattened bone lining cells. Lining cells are characterized by flat nuclei and the ability to synthesize only small amounts of protein and, like other cells of the osteoblast lineage, connect with each other via gap junctions [62].

Although long regarded as a protective cell population covering the bone surface that is "resting," or "quiescent", bone lining cells, like osteoblasts, express receptors for endocrine and paracrine agents. Their contraction from the bone surface in response to PTH [63] was suggested to

allow osteoclasts access to the bone surface [64]. It has been suggested that this lifting of the bone lining cell layer occurs not only in response to PTH treatment but also at the commencement of bone remodeling to generate a temporary canopy [65]. Such a canopy was previously suggested as a mechanism that encloses the bone remodeling activity, separating it from the rest of the bone marrow microenvironment [66], thereby providing a controlled locale in which osteoblast lineage cells, osteoclasts, and other contributing marrow cells, may exchange factors. This canopy is also closely associated with blood vessels, which can thereby readily provide both osteoblast and osteoclast precursors for the bone remodeling process [67, 68].

In addition to forming a canopy, bone lining cells are capable of reactivation to form active matrix-producing osteoblasts. This was first hypothesized when intermittent PTH administration increased osteoblast number on the bone surface without increasing osteoblast proliferation [69]. This mechanism has now been verified by lineage-tracing studies where intermittent administration of PTH reactivated quiescent lining cells to mature osteoblast in vivo [70]. Such reactivation of lining cells has also been demonstrated after mechanical loading [71] and after treatment with anti-sclerostin, a therapeutic stimulus of bone formation [72]. This reactivation is in addition to the proposal that these cells form a proliferating progenitor population during adulthood [21] and may provide a more rapidly inducible partially differentiated source of osteoblast precursors.

Bone Formation: Osteoid Production and its Mineralization

Bone is a heterogenous compound material. The mineral phase, in the form of modified hydroxyapatite (bioapatite) crystals, contributes about two-thirds of its weight. The remaining organic matrix consists largely of type I collagen (~90%) [73, 74], with small amounts of lipid (~2%), ~5% non-collagenous proteins, proteoglycans, and water [75]. Non-collagenous proteins within the bone matrix include substances that act as signaling molecules (such as transforming growth factor β (TGFβ) and insulin-like growth factor 1 (IGF1)) and substances that regulate mineralization (such as osteocalcin and DMP-1).

While a range of cell types are capable of depositing mineral, particularly in cell culture conditions or in pathological circumstances (such as vascular calcifications), it is only the osteoblast that is capable of bone formation. Osteoblasts are responsible for the deposition of bone matrix on a range of surfaces and in a number of different contexts. During endochondral bone formation, osteoblasts deposit bone on a cartilage template. This process occurs both in skeletal development and in fracture healing. In these instances, osteoblasts attach to the cartilage template and deposit osteoid, which becomes mineralized, according to processes described below. During intramembranous bone development, bone is formed directly by mesenchymal precursors with no underlying template. This process occurs largely during skeletal development, particularly of the calvarial bones, and occurs during the formation of the periosteal collar at the diaphysis (midshaft) of bones that form by endochondral ossification. During bone remodeling, bone mass is maintained by osteoblasts that form sufficient bone to replace bone that was recently removed by osteoclasts. In contrast, during bone growth, periosteal expansion occurs by modeling, where osteoblasts form bone on a bone surface that has not been previously resorbed. There are also pathological conditions, where bone is formed in locations where it is not normally found, e.g., in heterotopic ossifications in the muscle in the context of injury [76] or in rare genetic conditions [77]. In all of these processes, bone formation occurs as follows.

Osteoblasts do not produce "bone" *per se*, but synthesize a collagen-rich osteoid matrix. The osteoid matrix serves as a template for the subsequent deposition of mineral in the form of bioapatite which contributes to the hardness of bone. The balance between osteoid and mineral content determines bone strength: essentially, the collagen provides flexibility, while the mineral provides hardness. The process of mineralization is

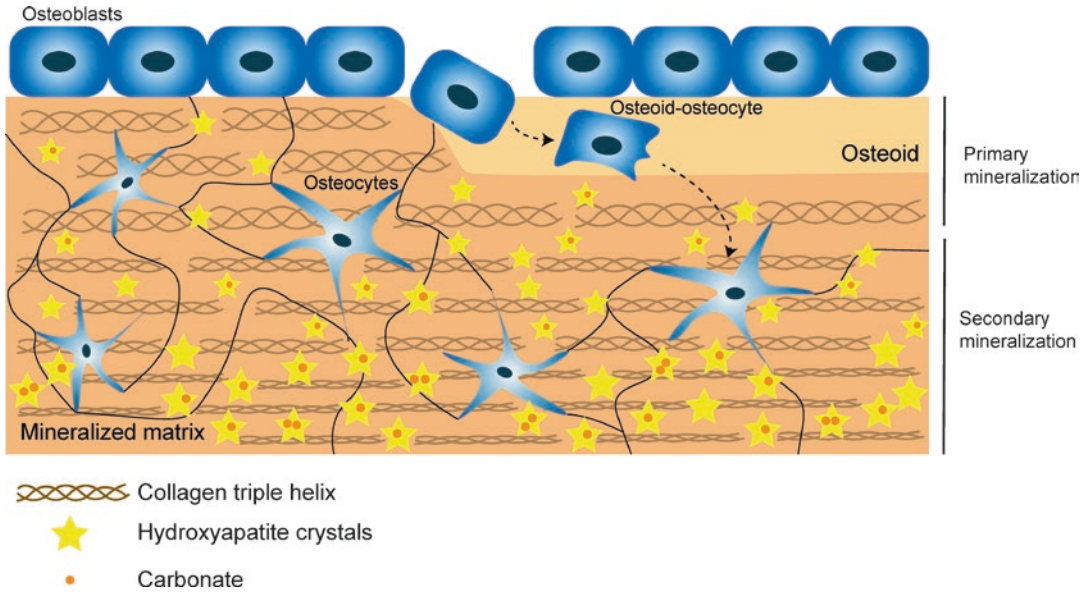

Fig. 1.2 The process of bone matrix production and mineralization. Mature osteoblasts on the bone surface deposit newly formed matrix, known as osteoid (1), largely comprised of type I collagen (a triple helical structure). After collagen deposition, the matrix becomes progressively mineralized by the accumulation of hydroxyapatitic bioapatite crystals (2). This mineralization process has two phases. Within ~5–10 days, osteoid undergoes rapid primary mineralization, and over subsequent weeks, months, and years, secondary mineralization occurs. The bioapatite crystals grow and accumulate carbonate in the more mature regions of bone, and collagen fibers become more condensed (compact) presumably due to steric hindrance caused by the presence of the growing crystals

controlled by non-collagenous proteins produced by late-stage osteoblasts and osteocytes. We will describe each of these processes in turn (Fig. 1.2).

Osteoid Deposition

When osteoid is deposited by osteoblasts, it has two potential forms depending on its collagen orientation and speed of production: woven and lamellar bone. During bone development and fracture healing, woven bone is deposited rapidly: this substance contains disordered, seemingly randomly oriented collagen fibers. In contrast, lamellar bone is highly organized. Fibers are more slowly deposited, predominantly oriented longitudinally, and create a defined, ordered structure [78]. Collagen fibers in lamellar bone are oriented in perpendicular planes in adjacent lamellae [79], adding strength of the substance. The loose structure and random orientation of woven bone suggest that it is mechanically weaker than lamellar bone. This has been tested in human fetal bone, where younger, more woven bone was associated with lower elasticity and lower resistance to penetration (microhardness) [80].

How osteoblasts are instructed to form either woven or lamellar bone is not known, but ultra-high voltage electron microscopy studies suggest that even during lamellar bone formation, collagen fibers are deposited sparsely and randomly, but as the osteoblast becomes more distant due to further deposition, the fibres begin to reoirent parallel to the direction of growth and become thicker [81]. This suggests that as-yet unidentified events after initial collagen secretion may be responsible for the woven or lamellar nature of bone. Adding to these observations, live cell imaging of osteoblasts engineered to deposit fluorescent-labeled collagen has recently revealed that osteoblasts constantly move during the collagen assembly process, and actively exert forces on the fibrils, physically shaping the collagen

matrix and potentially guiding the formation of osteocyte lacunae [82].

Type I collagen comprises a triple-helix structure of two α1 and one α2 polypeptide chain [83]. In osteoblasts, single pro-α chains are synthesized in the endoplasmic reticulum, which assemble into procollagen triple helices, and are released by exocytosis into the extracellular space, where the N- and C-termini are cleaved, allowing the formation of fibrils [84]. Multiple intracellular posttranslational modifications, including hydroxylation of proline and lysine residues, and glycosylation, stabilize the collagen triple helical structure [85]. After secretion, collagen fibers are stabilized and bone is strengthened further by the formation of inter- and intra-molecular cross-links, through the action of lysyl oxidase [86]. Other modifications such as advanced glycation adversely affect the mechanical properties of the bone matrix, particularly during ageing [87]. Defects not only in the proteins coding collagen itself but also in the many different aspects of collagen fibril assembly, including collagen folding, secretion, cross-linking, and posttranslational modifications, have been described in the diverse family of skeletal fragilities observed in osteogenesis imperfecta [88].

Matrix Mineralization

After collagen is deposited, it becomes progressively mineralized by the accumulation of bioapatite crystals. This mineralization process has two phases. Within ~5–10 days, osteoid undergoes rapid primary mineralization, and over subsequent weeks, months, and years, secondary mineralization occurs [89]. During primary mineralization, the tissue usually reaches ~50–70% of its final mineral content [90, 91]. During secondary mineralization, mineral continues to accumulate at a slower rate [92], the crystals beome larger [89], and carbonate is substituted for phosphate groups within the matrix [93, 94]. In addition, as mineral is deposited, the surrounding collagen fibers of bone also change, becoming more compact, possibly in response to the growing crystals [93, 94] (Fig. 1.2).

The final stage of mineralization achieved in the bone substance varies locally within the bone matrix and depends on the species, sex, age, and anatomical location of the bone [95]. Mineralization involves the release of matrix vesicles, which are cell-derived extracellular membrane-enclosed particles of poorly crystalline bioapatite mineral [96, 97]. The mineral crystals become ordered (a process termed nucleation) by a process driven by contact with collagen, local availability of calcium and phosphate, and by apatite nucleators such as DMP-1 and osteopontin [98, 99]. The importance of phosphate-regulating proteins is clearly illustrated by the association of human and murine genetic insufficiencies in phosphate regulators with impaired bone mineralization [100–102].

Mineralization initiation, accrual, and crystal maturation are controlled, not only by apatite nucleators but also by a range of multifunctional non-collagenous proteins secreted by mature osteoblasts and osteocytes. Osteoblasts and osteocytes express proteins that support mineralization such as alkaline phosphatase, PHOSPHO1, phosphate-regulating neutral endopeptidase, X-linked (PHEX), and bone sialoprotein/integrin-binding sialoprotein. Osteoblasts and osteocytes also express proteins that inhibit mineralization, such as osteocalcin [103], MEPE, and PC-1 (*Enpp1*) [104]. An illustration of the fine control exerted by osteoblasts on mineralization is their ability to control local levels of inorganic phosphate through alkaline phosphatase (ALP) and plasma cell membrane glycoprotein-1 (PC-1). Hydroxyapatite nucleation depends on a high ratio of inorganic phosphate (P_i), which promotes mineralization, to inorganic pyrophosphate (PP_i), which inhibits it. Alkaline phosphatase (ALP) positively regulates this balance by hydrolyzing PP_i to form the P_i required for hydroxyapatite crystal nucleation; insufficiency of ALP leads to poor mineralization, as observed in individuals with hypophosphatasia [100]. In contrast, PC-1 inhibits mineralization by producing inorganic pyrophosphate; insufficiency of PC-1 therefore leads to excessive mineralization [104].

The Osteoblast Lineage Supports Osteoclast Formation, Attachment, and Bone Resorption

The function of the osteoblast lineage is not restricted to bone formation. Osteoblast lineage cells also control the differentiation of osteoclasts, the cells responsible for bone resorption. There are three major ways in which cells of the osteoblast lineage carry out this role: (1) by producing RANKL and OPG in response to paracrine and endocrine agents, (2) by releasing chemoattractants that draw osteoclast precursors to the bone surface, and (3) by preparing the bone surface for osteoclast attachment. We will discuss each of these actions in turn.

Production of RANKL and OPG

A range of locally acting cytokines, including interleukin-11 (IL-11), prostaglandin E_2, PTHrP, and oncostatin M, stimulate osteoclast formation, but do not achieve this by direct action on osteoclast precursor themselves. Instead, these agents, and endocrine factors like PTH and 1,25-dihydroxyvitamin D, stimulate osteoclast formation indirectly, by acting on osteoblast lineage cells to stimulate expression of RANKL and CSF-1 (M-CSF), two regulatory molecules that are both required for osteoclastogenesis [105–110]. It is the interaction of RANKL with its receptor (RANK), expressed on the cell surface of mononuclear hemopoietic osteoclast precursors, that triggers osteoclast formation (Fig. 1.3).

The necessity for RANKL and RANK for osteoclastogenesis was demonstrated by the generation of genetically altered mice that lack either RANKL or RANK and exhibited a lack of osteoclasts and severe osteopetrosis [111, 112]. Osteoblast lineage cells also express a soluble protein that is a non-signaling decoy receptor for RANKL, known as osteoprotegerin (OPG). OPG acts as a “brake” on osteoclast differentiation by blocking the interaction of RANKL and RANK [113, 114], and through modulation of RANKL and OPG expression, osteoblasts can precisely regulate the formation of osteoclasts.

RANKL is expressed at all stages of osteoblast differentiation, including in precursors, matrix-producing osteoblasts, bone lining cells, and osteocytes [115]. RANKL production is not exclusive to osteoblast lineage cells. T-cells and natural killer (NK)-cells also express RANKL and are capable of promoting osteoclast formation [116, 117]. It appears that expression of RANKL by T-cells is dispensable for normal bone development and maintenance [118]. In contrast, in mice that lack RANKL in the osteoblast lineage, severe osteopetrosis is observed [119]. However, the most important stage in osteoblast differentiation for production of RANKL is not known, and whether the key source of RANKL is the osteocyte, the bone lining cell, or the preosteoblast remains controversial [21, 119–122]. One important concept to consider is that direct contact between the RANKL-expressing osteoblast lineage cells and the RANK-expressing haemopoietic osteoclast precursors is absolutely required for osteoclast formation in vitro [123, 124], and the same situation is likely to be true in vivo (Fig. 1.3). While recombinant soluble RANKL certainly promotes osteoclast formation from precursors in vitro [125], and in vivo [126], there remains no convincing evidence that soluble RANKL, produced by osteoblast lineage cells, can substitute for the membrane form, nor is there any convincing evidence of a physiological role for circulating RANKL. This means it is important to consider the location of the osteoblast lineage cells most likely to support osteoclast formation. Cells in the marrow, or in direct contact with it, such as osteoblast precursors and bone lining cells, rather than embedded osteocytes, are more likely to come into contact with osteoclast precursors, and therefore more likely to support osteoclast formation in normal remodeling. It has been difficult to understand how osteocytes, from within the matrix, could control RANKL availability to osteoclast precursors in the bloodstream through a contact-dependent mechanism although it has been suggested that osteocyte processes extend into the marrow space [127]. However, even

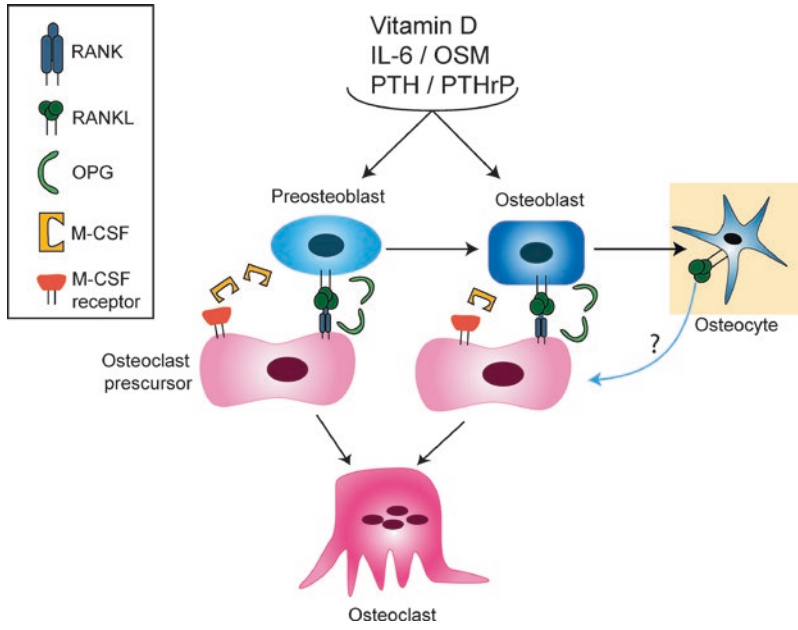

Fig. 1.3 The osteoblast lineage supports osteoclastogenesis. Osteoblast lineage cells control the differentiation of osteoclasts in response to paracrine and endocrine agents and locally acting cytokines such as vitamin D, interleukin-6 (IL-6), oncostatin M (OSM), and parathyroid hormone (PTH) / parathyroid hormone-related protein (PTHrP). These agents and factors act on the osteoblast lineage to stimulate expression of RANKL and M-CSF which each promote osteoclast formation. M-CSF is soluble. Receptors for both RANKL and M-CSF are expressed on the cell surface of mononuclear hemopoietic osteoclast precursors. Direct contact between membrane-bound RANKL and its membrane-bound receptor (RANK) triggers osteoclast formation. Osteoblast lineage cells also express a decoy receptor for RANKL, known as osteoprotegerin (OPG), which blocks the interaction of RANKL and RANK. Through their modulation of RANKL and OPG expression, osteoblasts can precisely regulate the formation of osteoclasts. Osteocytes also express RANKL, but the mechanism by which this reaches the osteoclast precursors remains undefined

when osteocytes were cultured in direct contact with osteoclast precursors and stimulated with appropriate stimuli, only binucleated "osteoclasts" formed [120].

RANKL production by osteoblast-lineage cells is also stimulated by microdamage within the bone matrix. Microdamage or microcracks are small defects in the bone matrix that occur in both pathological conditions and with normal skeletal loading [128]. Experimental loading, which causes a higher level of microdamage, initiates bone resorption [129], and indeed, resorption and replacement of the bone compromised by this damage is one of the important mechanical functions of bone remodeling [128]. It has been suggested that the microdamage site "steers" those osteoclasts already functioning on the bone surface toward the site of damage [130]. Microdamage within the bone is sensed by osteocytes, which are terminally differentiated osteoblasts that reside within the bone matrix, and sense changes in pressure within the matrix. Anatomical studies of rat bone in which microcracks were induced by ex vivo loading demonstrated that osteocytes located near to microcracks are more likely to be apoptotic compared to sites more distant to the microcrack [131]. Mechanical loading of human bone ex vivo and of rat bone in vivo increases osteocyte apoptosis [132, 133], and osteocytes surrounding the dying cell increase their production of RANKL to initiate resorption [134]. In support of this, short-term deletion of osteocytes in vivo resulted in a rapid increase in expression of RANKL mRNA in the bone, presumably by osteoblast lineage cells, and an increase in osteoclast formation [135].

Another factor produced by the osteoblast lineage and required for osteoclast formation is CSF-1/M-CSF [136, 137]. Together, RANKL and CSF-1 are all that is required to support osteoclast formation from bone marrow precursors in vitro. Just as observed in RANKL null mice, mutant mice lacking CSF-1 also exhibit severe osteopetrosis due to lack of osteoclast formation [138]. While RANKL is membrane bound and acts to promote osteoclast precursor fusion, CSF-1 is secreted by osteoblasts and promotes osteoclast precursor proliferation [139].

Release of Chemoattractants

Another mechanism by which osteoblasts control osteoclast differentiation is by controlling the movement of osteoclast precursors toward each other (allowing fusion) and to the bone surface (allowing attachment) through their release of chemoattractants. These factors may be deposited in the bone matrix itself during bone formation; they may be released by active osteoblasts or may be released from apoptotic osteocytes. Some bone matrix-derived factors, suggested to act as chemoattractants for monocytic osteoclast precursors, include osteocalcin, fetuin-A, and collagen-I fragments [140]. Thus, attraction of osteoclast precursors to the bone surface may be determined by the specific content of the bone to be resorbed; this is supported by studies of ageing bone. As bone ages, collagen-I is isomerized, and aged bone, which has a higher ratio of α/β collagen isomers, supports the formation of many more osteoclasts in vitro than younger bone [141], supporting a role for matrix constituents, deposited by osteoblasts, in the control of osteoclast formation.

Production of a range of chemokines (including stromal-derived factor-1 (SDF-1/CXCL12); chemokine-ligands 3, 5, and 7 (CCL3, CCL5, CCL7) [142]; chemokine (C-X-C motif) ligand 1 (CXCL1) [143]; and monocyte chemoattractant protein-1 (MCP-1) [144]) by osteoblast-lineage cells is stimulated by osteoclastogenic factors including the cytokines interleukin-1β (IL-1β), tumor necrosis factor α (TNF-α), and PTHrP. Such factors have been shown in vitro to act on osteoclast precursors (monocyte macrophages) to stimulate their chemotaxis and fusion [143, 145, 146], and it is likely that they have similar roles in vivo.

Preparing the Bone Surface for Osteoclast Attachment and Resorption

To commence resorption, the multinucleated osteoclast attaches to the bone matrix via the interaction of integrins with arginine-glycine-aspartic acid (RGD) sequences in non-collagenous matrix proteins including osteopontin and bone sialoprotein [147]. These proteins were laid down by osteoblasts during the previous cycle of bone formation. So, at some distance, it could be said that osteoblasts regulate osteoclast attachment by their control of the bone matrix itself. Intriguingly, mice lacking bone sialoprotein or osteopontin demonstrate, respectively, reduced osteoclast surface and reduced response to osteoclastogenic stimuli [148, 149]. However, this appears to be an indirect result of the reduced osteoblast numbers (and therefore reduced osteoblast-derived RANKL and M-CSF), or a requirement for intracellular osteoclastic osteopontin [150], rather than it relating to attachment to the bone matrix. Further work is required to determine how the bone matrix itself regulates osteoclast attachment; however, it should be noted that this is unlikely to be a method that precisely controls bone resorption, given the time delay between bone formation and subsequent resorption; more likely it is a mechanism that may exist in different types of bone that are responsible for biological variation in the level of bone resorption.

Concluding Remarks

The osteoblast lineage includes a range of cell types: multipotent precursors, matrix-producing osteoblasts, osteocytes, and bone lining cells; each of these stages of the lineage has distinct functions which we are only beginning to fully

understand. The most well-known role of the osteoblast lineage is the production of bone matrix and the control of its mineralization by non-collagenous proteins. The osteoblast lineage controls both the progression of differentiation of its own lineage and the formation of osteoclasts, the cells that resorb bone. As such the lineage is central to the control of bone mass, both by forming it and by controlling its destruction.

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