Bone remodelling at a glance

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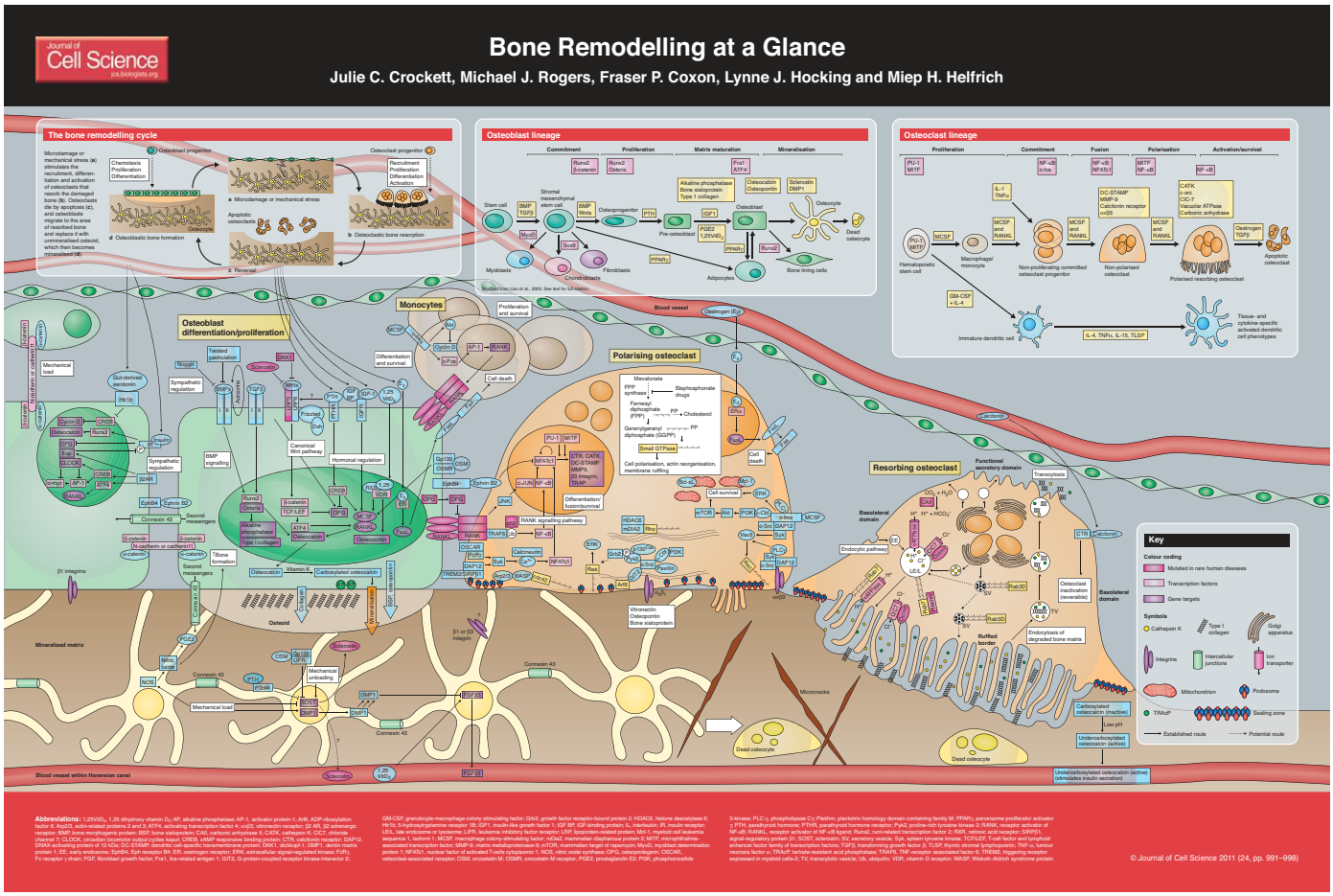
The bone remodelling cycle (see Poster panel "The bone remodelling cycle") maintains the integrity of the skeleton through the balanced activities of its constituent cell types. These are the bone-forming osteoblast, a cell that produces the organic bone matrix and aids its mineralisation (Karsenty et al., 2009); the bone-degrading osteoclast, a unique type of exocrine cell that dissolves bone mineral and enzymatically degrades extracellular matrix (ECM) proteins (Teitelbaum, 2007); and the osteocyte, an osteoblast-derived post-mitotic cell within bone matrix that acts as a mechanosensor and an endocrine cell (Bonewald and Johnson, 2008). A fourth cell type, the bone lining cell, is thought to have a specific role in coupling bone resorption to bone formation (Everts et al., 2002), perhaps by physically defining bone remodelling compartments (Andersen et al., 2009).

Molecular dissection of genetic disorders of highly increased or reduced bone mass has identified many of the crucial proteins controlling the activity of these bone cell types. This information has resulted in both novel ways to treat or diagnose more common bone disorders and a better understanding of the common genetic variants that lead to differences in bone density in the general population.

In this poster article, we illustrate the crucial signalling pathways involved in bone cell differentiation, function and survival, and describe how the coupled activities of the cells in bone are maintained through intercellular interactions. We pay particular attention to the factors and signalling processes that have been found to be indispensable for the maintenance of healthy bones through the study of rare genetic diseases of bone.

Osteoblasts: differentiation and function

Osteoblast differentiation is achieved by the concerted expression of a number of key transcription factors (see Poster panel "Osteoblast lineage"), and bone formation by osteoblasts is controlled both locally and systemically during bone modelling in development (Box 1) and throughout life. Studies of diseases associated with defects in bone formation, such as developmental limb disorders and high bone mass conditions, have demonstrated the crucial importance of local bone formation control by bone morphogenetic protein (BMP) (Cao and Chen, 2005) and wingless (Wnt) (Day et al., 2005) signalling pathways for osteoblast differentiation and function. In the adult, BMP2 can act as a potent stimulator of ectopic bone formation (Chen et al., 1997) and it is used clinically to enhance bone formation, for example, during fracture repair (Govender et al., 2002). BMP signalling through the recruitment and activation of



(See poster insert)

Box 1. Bone formation and function

During embryogenesis, long bones are formed initially as cartilage that becomes gradually replaced by bone, a process known as endochondral bone formation. By contrast, flat bones, such as the skull, are formed directly from mesenchymal condensation through a process called intramembranous ossification. During early childhood, both bone modelling (formation and shaping) and bone remodelling (replacing or renewing) occurs, whereas in adulthood bone remodelling is the predominant process to maintain skeletal integrity, with the exception of massive increases in bone formation that occur after a fracture. Most bones consist of a mixture of dense outer cortical bone and inner trabecular (spongy) bone, enabling the optimal compromise between strength and weight. In addition to providing support, attachment sites for muscles and protection for vulnerable internal organs, bone also provides a home for bone marrow and acts as a reservoir for minerals.

Osteoblasts produce bone by synthesis and directional secretion of type I collagen, which makes up over 90% of bone matrix protein. This, together with some minor types of collagen, proteoglycans, fibronectin and specific bone proteins, such as osteopontin, bone sialoprotein and osteocalcin, becomes the unmineralised flexible osteoid on which the osteoblasts reside. Rigidity of bone, which distinguishes it from other collagenous matrices, is provided by the bone mineral. Mineralisation is achieved by the local release of phosphate, which is generated by phosphatases present in osteoblast-derived, membrane-bound matrix vesicles within the osteoid. Together with the abundant calcium in the extracellular fluid, this results in nucleation and growth of crystals of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$]. The proportion of organic matrix to mineral (in adult human cortical bone approximately 60% mineral, 20% organic material, 20% water) is crucial to ensure the correct balance between stiffness and flexibility of the skeleton.

heterodimeric Smad proteins controls the expression of Runt-related transcription factor 2 (Runx2), also known as core binding factor alpha1 (cbfa1), a transcription factor indispensable for osteoblast differentiation (Ducy et al., 1997). The canonical Wnt signalling pathway is indispensable for osteoblast differentiation during skeletogenesis and continues to have important roles in mature osteoblasts (Box 2). Although the major function of circulating parathyroid hormone (PTH) is to regulate plasma calcium (see below), it also has an important role in bone formation and prevents osteoblast and osteocyte apoptosis. Intermittent administration of low levels of PTH increases osteoblast number, bone formation and bone mass, and is an established anabolic treatment for osteoporosis.

The exact mechanisms involved in the anabolic effects of PTH on bone formation are not fully understood, but might involve Wnt signalling (Box 2) as well as insulin-like growth factor 1 (IGF-1). IGF-1, which is released by the liver in response to growth hormone, has a role in the commitment of mesenchymal stem cells to osteoprogenitor cells. IGF-1 also regulates osteoclastogenesis both directly, through the IGF receptor (IGFR) present on osteoclasts, and by upregulating the crucial osteoclast differentiation factor receptor activator of nuclear factor κB ligand (RANKL).

Another pathway by which osteoblast function is regulated is the sympathetic nervous system (Elefteriou et al., 2005). Sympathetic stimulation through the β_2 adrenergic receptor located on osteoblasts inhibits bone formation and increases bone resorption, thereby resulting in a reduction in bone mass.

Osteoclasts: differentiation and function

Osteoclasts are large, multinucleated cells that form through fusion of mononuclear precursors of the hematopoietic lineage (see Poster panel "Osteoclast lineage"). Osteoclast differentiation initially depends on signalling through c-fms, the receptor for macrophage colony stimulating factor (MCSF), in mononuclear precursor cells, which upregulates expression of RANK (Crockett et al., 2011). Its ligand, RANKL, is expressed in osteoblasts and stromal cells in response to PTH and stimulation by the active dihydroxy form of vitamin D_3 (1,25 Vit D_3) (Leibbrandt and Penninger, 2008). Signalling through RANK and c-fms in mononuclear precursors is the key driver of osteoclast formation. Together with co-stimulation by the immunoreceptor tyrosine-based activation motif (ITAM)-containing adaptors DAP12 (DNAX-activating protein of 12 kDa) and FcRγ (Fc receptor γ chain), this leads to activation of the transcription factors nuclear factor κB (NF-κB), activator protein 1 (AP-1) and nuclear factor of activated T-cells cytoplasmic 1 (NFATc1) (Humphrey et al., 2005). These in turn regulate expression of essential osteoclast genes, such as dendritic cell-specific transmembrane protein (DC-STAMP), tartrate-resistant acid phosphatase (TRAcP), cathepsin K, matrix metalloproteinase 9 (MMP-9) and β3 integrin, which allow the final differentiation and fusion of the precursors and function of the resulting multinucleated osteoclast.

Loss-of-function mutations of RANKL and RANK result in the high bone mass disease osteopetrosis because osteoclast formation is completely impaired (Table 1) (Guerrini et al., 2008; Sobacchi et al., 2007). Throughout the lifespan of the mature osteoclast (several weeks), continued signalling through c-fms and RANK is required for osteoclast survival. RANK signalling is tightly regulated by a decoy receptor for RANKL, osteoprotegerin (OPG), which is produced by osteoblasts and stromal cells, and prevents interaction of RANKL with RANK. These factors have been explored as potential therapeutic targets. Although anabolic treatment with OPG is no longer pursued, an antibody against RANKL, which is a powerful anti-catabolic, has recently been launched to treat diseases in which either osteoclast formation or osteoclast function is excessive (Rizzoli et al., 2010).

Osteoclast formation is upregulated in inflammatory conditions associated with bone loss, such as rheumatoid arthritis, through the synergistic action of pro-inflammatory cytokines, including tumour necrosis factor α (TNFα) and RANKL, and by the transdifferentiation of dendritic cells into osteoclasts (Rivollier et al., 2004). The immune system also regulates bone loss that is associated with osteoporosis. Oestrogen deficiency leads to upregulation of interleukin 7 (IL-7), which induces T-cell activation and a complex cascade of pathways all producing cytokines and reactive oxygen species, thereby resulting in increased RANKL and TNFα production (Weitzmann and Pacifici, 2007).

Osteoclast numbers in bone are controlled not only through formation, but also through the regulation of their lifespan. Normally, osteoclasts die by apoptosis, a process that involves signalling pathways that include extracellular-signal-regulated kinase (ERK), the serine/threonine protein kinase Akt and mammalian target of rapamycin (mTOR), which regulate the expression of apoptotic factors, such as B-cell lymphoma-extra large ($Bcl X_L$), the BH3-only family member Bim and myeloid cell leukaemia sequence 1 (Mcl-1) (Akiyama et al., 2003; Xing and Boyce, 2005; Bradley et al., 2008; Sutherland et al., 2009). However, osteoclast survival is thought to be increased in pathological conditions that are associated with increased osteoclast numbers, such as Paget's disease of bone (Chamoux et al., 2009).

Pathways leading to osteoclast activation and initiation of bone resorption

Bone resorption is the process of osteoclast-mediated destruction of bone matrix. When

osteoclasts are activated to resorb (see "Polarising osteoclast" in the Poster), they polarise and form distinct and unique membrane domains, including the sealing zone (SZ), the ruffled border (RB) and the functional secretory domain (FSD) (Mulari et al., 2003). Importantly, osteoclasts generate these domains only when they are in contact with mineralised matrix and do not form an RB when cultured in vitro on plastic or glass (Saltel et al., 2004). Osteoclast polarisation involves rearrangement of the actin cytoskeleton to form an F-actin ring that comprises a dense continuous zone of highly dynamic podosomes (Luxenburg et al., 2007), thereby isolating an area of membrane that develops into the RB. The αvβ3-integrin (the vitronectin receptor) mediates the attachment of podosomes to the ECM through the formation of a signalling complex consisting of the tyrosine kinases c-Src, proline-rich tyrosine kinase 2 (PYK2) and spleen tyrosine kinase (Syk), as well as a number of scaffold proteins, including the E3 ligase c-Cbl, paxillin and the Crk-associated substrate (CAS) family member $p130^{Cas}$. This leads to activation of signalling pathways that involve phosphoinositide 3-kinase (PI3K) and phospholipase Cγ (PLCγ) (which are also activated by c-fms and complement αvβ3 signalling), and activation of the small GTPases Rac and Cdc42 by guanine nucleotide-exchange factors (GEFs) such as Vav3 (Novack and Faccio, 2009), combined with deactivation of ADP-ribosylation factor 6 (Arf6) through its GTPase-activating protein (GAP) GIT2 (G-protein-coupled receptor kinase-activator 2) (Heckel et al., 2009). Together with changes in the activity of Rho and downstream effects on microtubule acetylation and stabilisation, the combined action of these pathways promotes actin and microtubule reorganisation, thereby leading to the formation of the SZ and subsequently the RB. Whereas integrin αvβ3 continues to mediate signalling between the ECM and the cytoskeleton during resorption, it is likely that the tight adhesion to bone in the SZ is mediated through other proteins such as CD44 (Lakkakorpi et al., 1991; Chabadel et al., 2007).

Pathways involved in continued bone resorption: role of the ruffled border

Maintenance of the RB is essential for osteoclastic bone resorption (see "Resorbing osteoclast" in the Poster). The RB is a highly convoluted membrane that forms as a result of directed transport of late endosomes and/or lysosomes, and serves to deliver the proteins involved in the resorption process. These are the vacuolar-type H^+-ATPase (V-ATPase), whose role is to acidify the space beneath the RB (the resorption lacuna), thus enabling dissolution of bone mineral, and the cysteine protease cathepsin K, which degrades type I collagen. The chloride-proton antiporter ClC-7 (Graves et al., 2008) acts in concert with the V-ATPase at the RB (as on lysosomes) by transporting chloride ions into the resorption lacuna (Kornak et al., 2001). Loss-of-function mutations of all these proteins and Ostm1, a subunit of ClC-7, are the underlying basis of most cases of the high bone mass disease osteoclast-rich osteopetrosis (Table 1). In this type of osteopetrosis, osteoclasts form normally, but are unable to generate an RB and do not resorb (Villa et al., 2009; Lange et al., 2006). The trafficking of lysosomal and endosomal components that results in the formation of the RB, together with the presence of V-ATPase, ClC-7 and other lysosomal proteins at the RB (Palokangas et al., 1997), indicates that this unusual membrane domain is more akin to an intracellular lysosomal membrane than to a plasma membrane (Salo et al., 1996). Accordingly, formation of the RB is dependent on the lysosomal small GTPase Rab7 (Zhao et al., 2001). Another Rab family GTPase, Rab3D, is also necessary, but localises to a poorly characterised secretory compartment that is distinct from the Rab7-regulated lysosomal compartment (Pavlos et al., 2005).

The bone resorption process creates a high concentration of degraded collagen fragments, in addition to calcium and phosphate, within the resorption lacuna, which are endocytosed by osteoclasts and then transported through the cell and released at the FSD (Nesbitt et al., 1997; Salo et al., 1997) before finally reaching the bloodstream. During transcytosis, these collagen fragments are further proteolytically degraded by cathepsin K (Yamaza et al., 1998) and TRAcP, an osteoclast-specific enzyme that is activated by cathepsin K (Ljusberg et al., 2005). These enzymes are probably endocytosed from the resorption lacuna together with the collagen fragments, although it remains unclear how TRAcP is initially targeted to the RB. The transcytotic pathway might additionally play a role in maintenance of the RB membrane by balancing exocytic and endocytic events (Stenbeck, 2002).

Many of the processes involved in osteoclast polarisation and vesicular trafficking that enable bone resorption are regulated by small GTPases such as Rac, Rho and Rabs (Coxon and Taylor, 2008), which require post-translational modification by isoprenylation to localise correctly in the cell and to exert their specific function. Indeed, disruption of the key enzymes involved in isoprenylation in osteoclasts by bisphosphonates and related compounds leads to effective inhibition of bone resorption (Coxon et al., 2006). By virtue of their mineral-binding property, bisphosphonates specifically target to bone, where they are released and preferentially taken up by resorbing osteoclasts during dissolution of the mineral, explaining their remarkable selectivity for this cell type in vivo (Coxon et al., 2006). Bisphosphonate drugs have been in clinical use for several decades to treat diseases associated with excessive bone resorption, such as Paget's disease of bone, cancer-induced bone disease and for osteoporosis.

Box 2. Wnt signalling in bone remodelling

Canonical Wnt signalling is a key pathway in bone formation. The activation of β-catenin through the Wnt co-receptors low-density lipoprotein receptor-related proteins 5 and 6 (LRP5, LRP6) and Frizzled results in the upregulation of transcription factors that are crucial for osteoblast differentiation. Gain-of-function or loss-of-function mutations within LRP5 are associated with high and low bone mass phenotypes in humans, respectively (Boyden et al., 2002; Little et al., 2002; Gong et al., 2001). However, as osteoblast-specific expression of gain-of-function mutations in mice results in a high bone mass phenotype (Babij et al., 2003) and LRP5 also regulates synthesis of serotonin, a systemic negative regulator of bone mass, in the duodenum (Yadav et al., 2008; Yadav et al., 2009), the relative contribution of osteoblasts versus duodenal LRP5 to the regulation of bone mass is under debate.

Non-canonical Wnt signalling mediates the commitment of mesenchymal stem cells to the osteoblast lineage by preventing the expression of peroxisome proliferator activated receptor-γ (PPARγ), which is required for adipocyte differentiation (Takada et al., 2007). Osteoporosis and reduced levels of circulating oestrogen are associated with a switch that favours adipocytic over osteoblastic development (Rosen et al., 2009).

There is evidence for cross-talk between PTH and Wnt signalling, because binding of PTH to its receptor recruits and phosphorylates LRP6, which leads to stabilisation of β-catenin. In addition, the endogenous inhibitor of Wnt signalling Dickkopf 1 (DKK1) prevents Wnt-dependent PTH effects (Wan et al., 2008; Guo et al., 2010). In differentiated osteoblasts, canonical Wnt signalling also stimulates OPG and inhibits RANKL expression, thereby negatively regulating osteoclast formation (Glass et al., 2005).

Table 1. New treatments for osteoporosis that have been developed following identification of bone-disease-causing mutations

Mutated protein	Normal function	Bone disease resulting from loss of function (phenotype)	Osteoporosis therapy: licensed[a] in development[b]	Reference for therapy
RANKL	Pro-catabolic: crucial cytokine required for osteoclast formation, function and survival	Osteoclast-poor osteopetrosis (high bone mass)	Anti-RANKL antibody (Denosumab)[a] Anti-catabolic	(Rizzoli et al., 2010)
Cathepsin-K	Pro-catabolic: proteolytic enzyme released by osteoclasts that degrades collagen matrix	Pycnodysostosis (high bone mass)	Cathepsin-K inhibitor (Odanacatib)[a] Anti-catabolic	(Pérez-Castrillón et al., 2010
V-ATPase	Pro-catabolic: proton pump on ruffled border of resorbing osteoclasts to create acidic environment to dissolve bone mineral	Osteoclast-rich osteopetrosis (high bone mass)	V-ATPase inhibitors[b] Anti-catabolic	(Huss and Wieczorek, 2009)
ClC-7 and Ostm1	Pro-catabolic: proton-chloride antiporter on ruffled border of resorbing osteoclasts, essential to maintain electroneutrality. Ostm1 is a subunit of this antiporter	Osteoclast-rich osteopetrosis (high bone mass)	ClC-7 inhibitors[b] Anti-catabolic	(Schaller et al., 2005)
LRP5	Pro-anabolic: co-receptor for canonical Wnt signalling pathway, promotes osteoblast differentiation (inhibited by DKK-1)	Osteoporosis pseudoglioma syndrome (low bone mass)	Anti-DKK-1 antibodies[b] Anabolic	(Glantschnig et al., 2010)
Sclerostin	Anti-anabolic: secreted by osteocytes and inhibits osteoblast differentiation	Sclerosteosis and Van Buchem disease (both high bone mass)	Anti-sclerostin antibodies[b] Anabolic	(Li et al., 2009)

The anti-catabolic and pro-anabolic therapies described here are also licensed or in development for the treatment of other diseases. Denosumab (Amgen) has been licensed for use in the treatment of metastatic bone disease and future indications are likely to include rheumatoid arthritis, psoriatic arthritis and multiple myeloma. Anti-DKK antibodies (Novartis) are in phase I/II clinical trials for the treatment of multiple myeloma. The use of anti-sclerostin antibodies as an anabolic factor to improve fracture healing is being investigated and is showing promising results in animal models (Paszty et al., 2010).

Osteocytes: formation and function

Osteocytes are the most abundant bone cell type, accounting for 95% of all bone cells. These cells are osteoblasts that have been spared apoptosis at the end of a bone formation cycle and have become incorporated into the bone matrix (see Poster panel "Osteoblast lineage"), where they can have a lifespan of decades. During entombment into the bone matrix (paradoxically also called osteocyte birth), osteoblasts profoundly change their morphology, losing over 70% of cell organelles and cytoplasm, and acquiring a stellar shape with 50 or more thin extensions (termed osteocyte processes) that connect with other osteocytes and also remain connected with osteoblasts on the bone surface (Rochefort et al., 2010). The resulting osteocyte network provides microporosity in the mineralised bone. Osteocyte bodies are contained within spaces referred to as lacunae, whereas their connected processes are contained within channels (termed canaliculi) – together they make up the lacunar–canalicular network.

Like the neuronal network, the osteocyte network processes and transmits signals from their site of origin to a distant site where an effect is required. Specifically, the osteocyte network senses mechanical forces on bone, for example, by compression or stretching of the bone matrix during locomotion, and transmits this signal through its network to ultimately influence the activities of osteoblasts and osteoclasts on the bone surface. It was initially thought that osteocytes respond exclusively to mechanical stimuli, but it is now clear that they also sense metabolic signals. Osteocyte death (as evidenced by the presence of empty osteocyte lacunae) is increased after oestrogen withdrawal, unloading of bone and during ageing (Manolagas and Parfitt, 2010), conditions that are associated with lower bone mass due to increased remodelling. These observations, combined with evidence that experimental ablation of osteocytes in young mice leads to rapid induction of bone resorption (Tatsumi et al., 2007), suggest strongly that living osteocytes have an important role in negatively regulating osteoclastic resorption, although the precise signals they send to inhibit osteoclasts are not yet known. The effects of osteocytes on osteoblasts are twofold: in response to sensing mechanical effects on bone, they positively regulate osteoblasts through the production of messengers, such as nitric oxide and prostaglandin E2, and they negatively regulate osteoblasts through secretion of sclerostin (discussed further below) (Rochefort et al., 2010).

Bone cells and bone matrix

Bone cells, like any connective tissue cells, live in close contact with the abundant ECM, which has a key role in regulating their proliferation, differentiation and activation through a variety of adhesion molecules, as discussed below.

Osteoblast–matrix interactions

Osteoblasts stably interact with matrix through integrins. β1 integrins (α1β1, α2β1 and α5β1) seem the most abundant (Helfrich et al., 2008) and have a crucial role in organising the cells on the developing bone surface during osteoid production (Zimmerman et al., 2000). Osteoblasts also express a range of cell–cell adhesion molecules, particularly cadherins, which have a role in osteoblast differentiation and function (Civitelli et al., 2002; Marie, 2002). Coupling between cells in the osteoblast lineage is further mediated by gap junctions and hemichannels, particularly the junctions formed by connexin 43 (Civitelli, 2008). These allow exchange of ions and small molecules, for example, ATP, nitric oxide and prostaglandins.

Osteoclast–matrix interactions

Osteoclasts migrate over mineralised trabecular surfaces and tunnel through cortical bone, and therefore have only an intermittent relationship with the matrix. At times, they form tight adhesive interactions with bone as described above, but they are also highly motile, even during active resorption. Osteoclasts use mainly αvβ3 and α2β1 integrins to interact with the ECM (Helfrich et al., 2008). They bind to collagen through β1 integrins, whereas bone-specific or bone-enriched RGD-containing proteins, such as bone sialoprotein and osteopontin, are bound through β3 integrin (Helfrich et al., 1992; Helfrich et al., 1996). As migrating cells, mature osteoclasts do not express cadherins, but it has been suggested that cadherins have a role during osteoclast differentiation to facilitate intimate contact with

stromal cells that express essential growth factors (Mbalaviele et al., 2006).

Osteocyte–matrix interactions

Specific interaction points between osteocyte integrins and the matrix lining the lacunae and canaliculi might be crucial in generating and amplifying signals that are induced by tissue deformation (Wang et al., 2007); roles for β1 or β3 integrins have been suggested (Litzenberger et al., 2010; McNamara et al., 2009). Live-cell imaging studies have shown that a population of osteocytes near the surface of bone is surprisingly motile, suggesting that the formation of the osteocyte network might be more actively controlled by the cells involved than initially thought (Dallas and Bonewald, 2010).

Coupling bone formation to bone resorption

During bone remodelling, bone formation is tightly coupled to bone resorption, and direct contacts between osteoclasts and osteoblasts have been proposed to maintain this relationship. Recently, the ephrin B (EphB) receptors (the largest class of receptor tyrosine kinases) and their ephrinB ligands have been implicated in this coupling. These receptor–ligand interactions activate bidirectional signalling, where interaction between ligand and receptor induces signalling in both the receptor-expressing and the ligand-expressing cells. Here, 'forward' signalling from receptor EphB4 present on osteoblasts activates a RhoA-dependent pathway to enhance osteoblast differentiation, whereas the 'reverse' signalling from its ligand ephrinB2, which is expressed by osteoclasts, downregulates c-Fos and NFATc1 to inhibit osteoclast function (Matsuo, 2010). The result of this bidirectional signalling might affect the switch from bone resorption to bone formation. EphB4 and ephrinB2 also signal between cells that belong to the osteoblast lineage and could thus have additional positive effects on bone formation during bone remodelling (Martin et al., 2010).

In addition, a number of soluble factors have been implicated in the coupling between bone formation and bone resorption, including factors that are released from bone matrix during resorption, such as transforming growth factor β (TGF-β), factors that are secreted by osteoclasts, including cardiotrophin-1, TRAcP and glutamate (Walker et al., 2008; Karsdal et al., 2007; Coxon and Taylor, 2008), and osteoblast-derived factors, including oncostatin M (Walker et al., 2010).

Systemic regulation of bone remodelling

Osteoclastic bone resorption is controlled systemically by four main hormones: calcitonin, PTH, vitamin D_3 (1,25 Vit D_3) and oestrogen. Secretion of the first three is driven by the need to control the serum calcium level within precise physiological limits (i.e. 2.2–2.6 mM), with bone acting as a mineral reservoir for this homeostasis. Calcitonin acts through its receptors that are expressed specifically on osteoclasts and directly inhibits osteoclastic resorption (Zaidi et al., 2002). By contrast, PTH binds to its receptors that are expressed on osteoblasts and bone marrow stromal cells, in which, through signalling by cAMP responsive element binding protein (CREB), it activates expression of MCSF and RANKL, thereby indirectly stimulating osteoclastic bone resorption. An important non-skeletal action of PTH is to stimulate increased renal reabsorption of calcium, which, together with increased resorption to mobilise calcium from bone, restores physiological serum calcium levels (Talmage and Elliott, 1958). PTH also stimulates the production of 1,25 Vit D_3 from a circulating inactive precursor. 1,25 Vit D_3, in turn, facilitates calcium absorption from the gut and the kidney, and also positively regulates bone resorption indirectly through the 1,25 Vit D_3 and retinoid X receptors in osteoblasts, which increases RANKL and MCSF expression.

The major role for oestrogen in the skeletal system is as a bone-sparing hormone that acts through receptors expressed by both osteoclasts and osteoblasts. This sex hormone is crucial in the control of osteoclast lifespan, and can cause pre-osteoclast and osteoclast apoptosis through Fas and Fas ligand signalling. Therefore, loss of oestrogen in women after the menopause results in increased osteoclast formation and survival (Krum et al., 2008; Nakamura et al., 2007). Oestrogen also blocks osteoclast function indirectly through effects on the immune system and has a role in regulating the response of bone to mechanical stimulation (Zaman et al., 2006).

Bone as an endocrine organ

A recently emerged role for bone is that of an endocrine organ. Firstly, the osteoblast-derived protein osteocalcin was identified as a positive regulator of pancreatic insulin secretion (Lee et al., 2007). Osteocalcin expression is upregulated by insulin signalling in osteoblasts through downregulation of Twist, an inhibitor of Runx2 (Fulzele et al., 2010), whereas insulin-dependent downregulation of OPG in osteoblasts stimulates osteoclasts and lowers the pH of the bone ECM, which is required for activation of osteocalcin before it enters the circulation (Hinoi et al., 2008; Ferron et al., 2010).

Secondly, a new role for osteocytes in regulating bone metabolism has recently come to light. Osteocytes synthesise fibroblast growth factor 23 (FGF23), which plays a key role in phosphate homeostasis by acting on the parathyroid gland and the kidney to reduce circulating phosphate levels. FGF23 production is stimulated by 1,25 Vit D_3 and acts, in turn, by reducing 1,25 Vit D_3 levels. Two other osteocyte-expressed proteins, phosphate regulating endopeptidase homolog, X-linked (PHEX) and dentin matrix acidic phosphoprotein 1 (DMP1), are thought to negatively regulate of FGF23 in the osteocyte (Quarles, 2008).

Finally, through studying patients with the rare hereditary high bone mass conditions sclerosteosis and Van Buchem disease, the *SOST* gene that encodes sclerostin, an inhibitor of bone formation, has been discovered (Balemans and Van Hul, 2004). Sclerostin is synthesised exclusively by bone cells that are in contact with mineral, that is, late-stage osteoblasts and osteocytes. Expression of sclerostin is inhibited by PTH and oncostatin M (Walker et al., 2010), and by mechanical loading of bone (Robling et al., 2008). Sclerostin acts as an inhibitor of Wnt signalling (Box 2). Owing to its exclusive expression in bone, sclerostin has become a key target for development of novel bone anabolics; anti-sclerostin antibodies are currently in phase 2 clinical trials (Table 1).

Mechanical regulation of bone remodelling

Mechanical force is a key regulator of bone remodelling and of bone architecture in general (Jacobs et al., 2010). It influences bone metabolism not only locally (e.g. resulting in a bigger bone in the serving arm of a professional tennis player), but also systemically (as illustrated by the profound bone loss in astronauts experiencing zero gravity and in immobilised patients). Whole-animal studies have shown dramatic responses to mechanical stimuli at the tissue level and in vitro studies have confirmed that individual bone cells, such as osteocytes and osteoblasts, are able to sense and respond to mechanical forces. Early signals produced in response to mechanical stimuli include nitric oxide, prostaglandins and Wnt signalling proteins (Bonewald and Johnson, 2008). In osteocytes, β-catenin rapidly translocates to the nucleus after exposure to fluid shear stress, suggesting activation of Wnt signalling or cadherin-mediated signalling (Huesa et al., 2009; Norvell et al., 2004; Santos et al., 2010). However, the precise mechanical stimulus that is sensed by bone cells in vivo and

Box 3. Genetic contributors to osteoporosis

Identification of the molecules that are essential for bone cell formation or cause inherited disorders of osteoclast function has led to a better molecular understanding of the common bone disorder osteoporosis. This age-related low bone mass condition, most prominent in women following the menopause, can be assessed by measuring bone mineral density (BMD). BMD varies naturally within the population, and peak bone mass and the rate of bone loss in later life are determined by the interplay between multiple genetic and environmental factors. Common genetic variants associated with BMD have been reported for components of several of the signalling pathways mentioned above, including those activated by RANKL, Wnt-LRP, TGF-β and BMPs, as well as cytoskeletal scaffolding proteins, GAPs and endosomal transporters. In addition, genetic variation also affects nuclear hormone receptors, for example, vitamin D receptor and oestrogen receptor, and the ECM (Kiel et al., 2007; Rivadeneira et al., 2009).

However, the contribution of the individual genetic variants that have been identified to date to the overall variation in BMD and bone loss is typically small (Rivadeneira et al., 2009). Therefore, their combined effects will be the important factor in determining the risk of osteoporotic fractures. Additionally, in the majority of cases, the functional variants of these genes have not yet been identified, thus preventing an accurate estimation of the actual risk or a meaningful assessment of their combined effects at a molecular or cellular level. There is also evidence for site-specific effects of genetic variants. Such localised effects undoubtedly occur through interactions with the local environment – for example under different loading conditions – but also concur with the evidence for intrinsic differences in bone cell functions between different skeletal sites (Everts et al., 2009).

the signal produced as a result remain unclear. Despite this, the profound anabolic effects of mechanical stimulation of bone have prompted the development of mechanical therapies to increase bone mass. The presence of microcracks in bone affects mechanosensing and is currently considered a crucial driver of the remodelling response by initiating osteoclastic resorption (Cardoso et al., 2009).

Conclusions

Over the past "Bone and Joint Decade", the progress made in understanding bone remodelling, both through experimental approaches and by uncovering the molecular basis of inherited bone disease, has been truly spectacular. The vast amount of new knowledge has already been translated into novel therapeutic approaches for the treatment of common bone diseases and is leading to the development of better biomarkers to monitor response to treatment. There is the exciting possibility that soon we will be able to understand the genetic predisposition for age-related bone loss (Box 3), develop better screening methods to identify those most at risk and use genetic information to decide on the most appropriate treatment. All this will require intricate knowledge of bone anatomy, bone cell function and bone remodelling to inform our understanding of the functional consequences of such genetic differences.

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References

Akiyama, T., Bouillet, P., Miyazaki, T., Kadono, Y., Chikuda, H., Chung, U. I., Fukuda, A., Hikita, A., Seto, H., Okada, T. et al. (2003). Regulation of osteoclast apoptosis by ubiquitylation of proapoptotic BH3-only Bcl-2 family member Bim. *EMBO J.* **22**, 6653-6664.

Andersen, T. L., Sondergaard, T. E., Skorzynska, K. E., gnaes-Hansen, F., Plesner, T. L., Hauge, E. M., Plesner, T. and Delaisse, J. M. (2009). A physical mechanism for coupling bone resorption and formation in adult human bone. *Am. J. Pathol.* **174**, 239-247.

Babij, P., Zhao, W., Small, C., Kharode, Y., Yaworsky, P. J., Bouxsein, M. L., Reddy, P. S., Bodine, P. V., Robinson, J. A., Bhat, B. et al. (2003). High bone mass in mice expressing a mutant LRP5 gene. *J. Bone Miner. Res.* **18**, 960-974.

Balemans, W. and Van Hul, W. (2004). Identification of the disease-causing gene in sclerosteosis-discovery of a novel bone anabolic target? *J. Musculoskelet. Neuronal Interact.* **4**, 139-142.

Bonewald, L. F. and Johnson, M. L. (2008). Osteocytes, mechanosensing and Wnt signaling. *Bone* **42**, 606-615.

Boyden, L. M., Mao, J., Belsky, J., Mitzner, L., Farhi, A., Mitnick, M. A., Wu, D., Insogna, K. and Lifton, R. P. (2002). High bone density due to a mutation in LDL-receptor-related protein 5. *N. Engl. J. Med.* **346**, 1513-1521.

Bradley, E. W., Ruan, M. M. and Oursler, M. J. (2008). Novel pro-survival functions of the Kruppel-like transcription factor Egr2 in promotion of macrophage colony-stimulating factor-mediated osteoclast survival downstream of the MEK/ERK pathway. *J. Biol. Chem.* **283**, 8055-8064.

Cao, X. and Chen, D. (2005). The BMP signalling and in vivo bone formation. *Gene* **357**, 1-8.

Cardoso, L., Herman, B. C., Verborgt, O., Laudier, D., Majeska, R. J. and Schaffler, M. B. (2009). Osteocyte apoptosis controls activation of intracortical resorption in response to bone fatigue. *J. Bone Miner. Res.* **24**, 597-605.

Chabadel, A., Banon-Rodriguez, I., Cluet, D., Rudkin, B. B., Wehrle-Haller, B., Genot, E., Jurdic, P., Anton, I. M. and Saltel, F. (2007). CD44 and beta3 integrin organize two functionally distinct actin-based domains in osteoclasts. *Mol. Biol. Cell* **18**, 4899-4910.

Chamoux, E., Couture, J., Bisson, M., Morissette, J., Brown, J. P. and Roux, S. (2009). The p62 P392L mutation linked to Paget's disease induces activation of human osteoclasts. *Mol. Endocrinol.* **23**, 1668-1680.

Chen, D., Harris, M. A., Rossini, G., Dunstan, C. R., Dallas, S. L., Feng, J. Q., Mundy, G. R. and Harris, S. E. (1997). Bone morphogenetic protein 2 (BMP-2) enhances BMP-3, BMP-4, and bone cell differentiation marker gene expression during the induction of mineralized bone matrix formation in cultures of fetal rat calvarial osteoblasts. *Calcif. Tissue Int.* **60**, 283-290.

Civitelli, R. (2008). Cell-cell communication in the osteoblast/osteocyte lineage. *Arch. Biochem. Biophys.* **473**, 188-192.

Civitelli, R., Lecanda, F., Jørgensen, N. R. and Steinberg, T. H. (2002). Intercellular junctions and cell-cell communication in bone. In *Principles of Bone Biology* (eds. J. P. Bilezikan, L. Raisz and G. A. Rodan), pp. 287-302. San Diego: Academic Press.

Coxon, F. P. and Taylor, A. (2008). Vesicular trafficking in osteoclasts. *Semin. Cell Dev. Biol.* **19**, 424-433.

Coxon, F. P., Thompson, K. and Rogers, M. J. (2006). Recent advances in understanding the mechanism of action of bisphosphonates. *Curr. Opin. Pharmacol.* **6**, 307-312.

Crockett, J. C., Mellis, D. J., Scott, D. I. and Helfrich, M. H. (2011). New knowledge on critical osteoclast formation and activation pathways from study of rare genetic diseases of osteoclasts: focus on the RANK/RANKL axis. *Osteoporos. Int.* **1**, 1-20.

Dallas, S. L. and Bonewald, L. F. (2010). Dynamics of the transition from osteoblast to osteocyte. *Ann. N. Y. Acad. Sci.* **1192**, 437-443.

Day, T. F., Guo, X., Garrett-Beal, L. and Yang, Y. (2005). Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. *Dev. Cell* **8**, 739-750.

Ducy, P., Zhang, R., Geoffroy, V., Ridall, A. L. and Karsenty, G. (1997). Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. *Cell* **89**, 747-754.

Elefteriou, F., Ahn, J. D., Takeda, S., Starbuck, M., Yang, X., Liu, X., Kondo, H., Richards, W. G., Bannon, T. W., Noda, M. et al. (2005). Leptin regulation of bone resorption by the sympathetic nervous system and CART. *Nature* **434**, 514-520.

Everts, V., Delaisse, J. M., Korper, W., Jansen, D. C., Tigchelaar-Gutter, W., Saftig, P. and Beertsen, W. (2002). The bone lining cell: its role in cleaning Howship's lacunae and initiating bone formation. *J. Bone Miner. Res.* **17**, 77-90.

Everts, V., de Vries, T. J. and Helfrich, M. H. (2009). Osteoclast heterogeneity: lessons from osteopetrosis and inflammatory conditions. *Biochim. Biophys. Acta* **1792**, 757-765.

Ferron, M., We, J., Yoshizawa, T., Del Fattore, A., DePinho, R. A., Teti, A., Ducy, P. and Karsenty, G. (2010). Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism. *Cell* **23**, 296-308.

Fulzele, K., Riddle, R. C., DiGirolamo, D. J., Cao, X., Wan, C., Chen, D., Faugere, M. C., Aja, S., Hussain, M. A., Bruning, J. C. et al. (2010). Insulin receptor signaling in osteoblasts regulates postnatal bone acquisition and body composition. *Cell* **142**, 309-319.

Glantschnig, H., Hampton, R. A., Lu, P., Zhao, J. Z., Vitelli, S., Huang, L. Haytko, P., Cusick, T., Ireland, C. Jarantow, S. W. et al. (2010). Generation and selection of novel fully human monoclonal antibodies that neutralize Dickkopf-1 (DKK1) inhibitory function in vitro and increase bone mass in vivo. *J. Biol. Chem.* **285**, 40135-40147.

Glass, D. A., 2nd, Bialek, P., Ahn, J. D., Starbuck, M., Patel, M. S., Clevers, H., Taketo, M. M., Long, F., McMahon, A. P., Lang, R. A. et al. (2005). Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. *Dev. Cell* **8**, 751-764.

Gong, Y., Slee, R. B., Fukai, N., Rawadi, G., Roman-Roman, S., Reginato, A. M., Wang, H., Cundy, T., Glorieux, F. H., Lev, D. et al. (2001). LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. *Cell* **107**, 513-523.

Govender, S., Csimma, C., Genant, H. K., Valentin-Opran, A., Amit, Y., Arbel, R., Aro, H., Atar, D., Bishay, M., Börner, M. G. et al. (2002). BMP-2 evaluation in surgery for tibial trauma (BESTT) study group. Recombinant human bone morphogenetic protein-2 for treatment of open tibial fractures: a prospective, controlled, randomized study of four hundred and fifty patients. *J. Bone Joint Surg. Am.* **84**, 2123-2134.

Graves, A. R., Curran, P. K., Smith, C. L. and Mindell, J. A. (2008). The Cl^-/H^+ antiporter ClC-7 is the primary chloride permeation pathway in lysosomes. *Nature* **453**, 788-792.

Guerrini, M. M., Sobacchi, C., Cassani, B., Abinun, M., Kilic, S. S., Pangrazio, A., Moratto, D., Mazzolari, E., Clayton-Smith, J., Orchard, P. et al. (2008). Human osteoclast-poor osteopetrosis with hypogammaglobulinemia due to TNFRSF11A (RANK) mutations. *Am. J. Hum. Genet.* **83**, 64-76.

Guo, J., Liu, M., Yang, D., Bouxsein, M. L., Saito, H., Galvin, R. J., Kuhstoss, S. A., Thomas, C. C., Schipani, E., Baron, R. et al. (2010). Suppression of Wnt signaling by Dkk1 attenuates PTH-mediated stromal cell response and new bone formation. *Cell Metab.* **11**, 161-171.

Heckel, T., Czupalla, C., Expirto Santo, A. I., Anitei, M., Arantzazu Sanchez-Fernandez, M., Mosch, K., Krause, E. and Hoflack, B. (2009). Src-dependent repression of ARF6 is required to maintain podosome-rich sealing zones in bone-digesting osteoclasts. *Proc. Natl. Acad. Sci. USA* **106**, 1451-1456.

Helfrich, M. H., Nesbitt, S. A., Dorey, E. L. and Horton, M. A. (1992). Rat osteoclasts adhere to a wide range of RGD (Arg-Gly-Asp) peptide-containing proteins, including the bone sialoproteins and fibronectin, via a β3 integrin. *J. Bone Miner. Res.* **7**, 335-343.

Helfrich, M. H., Nesbitt, S. A., Lakkakorpi, P. T., Barnes, M. J., Bodary, S. C., Shankar, G., Mason, W. T., Mendrick, D. L., Väänänen, H. K. et al. (1996). Beta 1 integrins and osteoclast function: involvement in collagen recognition and bone resorption. *Bone* **19**, 317-328.

Helfrich, M. H., Stenbeck, G., Nesbitt, M. A. and Horton, M. A. (2008). Integrins and adhesion molecules. In *Principles of Bone Biology* (eds J. P. Bilezikan, L. G. Raisz and T. J. Martin), pp. 385-424. San Diego, Academic Press, Elsevier.

Hinoi, E., Gao, N., Jung, D. Y., Yadav, V., Yoshizawa, T., Myers, M. G., Jr, Chua, S. C., Jr, Kim, J. K., Kaestner, K. H. and Karsenty, G. (2008). The sympathetic tone mediates leptin's inhibition of insulin secretion by modulating osteocalcin bioactivity. *J. Cell Biol.* **183**, 1235-1242.

Huesa, C., Helfrich, M. H. and Aspden, R. M. (2009). Parallel-plate fluid flow systems for bone cell stimulation. *J. Biomech.* **43**, 1182-1189.

Humphrey, M. B., Lanier, L. L. and Nakamura, M. C. (2005). Role of ITAM-containing adapter proteins and their receptors in the immune system and bone. *Immunol. Rev.* **208**, 50-65.

Huss, M. and Wieczorek, H. (2009). Inhibitors of V-ATPases: old and new players. *J. Exp. Biol.* **212**, 341-346.

Jacobs, C. R., Temiyasathit, S. and Castillo, A. B. (2010). Osteocyte mechanobiology and pericellular mechanics. *Annu. Rev. Biomed. Eng.* **12**, 369-400.

Karsdal, M. A., Martin, T. J., Bollerslev, J., Christiansen, C. and Henriksen, K. (2007). Are nonresorbing osteoclasts sources of bone anabolic activity? *J. Bone Miner. Res.* **22**, 487-494.

Karsenty, G., Kronenberg, H. M. and Settembre, C. (2009). Genetic control of bone formation. *Annu. Rev. Cell Dev. Biol.* **25**, 629-648.

Kiel, D. P., Demissie, S., Dupuis, J., Lunetta, K. L., Murabito, J. M. and Karasik, D. (2007). Genome-wide association with bone mass and geometry in the Framingham Heart Study. *BMC. Med. Genet.* **8 Suppl. 1**, S14.

Kornak, U., Kasper, D., Bosl, M. R., Kaiser, E., Schweizer, M., Schulz, A., Friedrich, W., Delling, G. and Jentsch, T. J. (2001). Loss of the ClC-7 chloride channel leads to osteopetrosis in mice and man. *Cell* **104**, 205-215.

Krum, S. A., Miranda-Carboni, G. A., Hauschka, P. V., Carroll, J. S., Lane, T. F., Freedman, L. P. and Brown, M. (2008). Estrogen protects bone by inducing Fas ligand in osteoblasts to regulate osteoclast survival. *EMBO J.* **27**, 535-545.

Lakkakorpi, P. T., Horton, M. A., Helfrich, M. H., Karhukorpi, E. K. and Väänänen, H. K. (1991). Vitronectin receptor has a role in bone resorption but does not mediate tight sealing zone attachment of osteoclasts to the bone surface. *J. Cell Biol.* **115**, 1179-1186.

Lange, P. F., Wartosch, L., Jentsch, T. J. and Fuhrmann, J. C. (2006). ClC-7 requires Ostm1 as a beta-subunit to support bone resorption and lysosomal function. *Nature* **440**, 220-223.

Lee, N. K., Sowa, H., Hinoi, E., Ferron, M., Ahn, J. D., Confavreux, C., Dacquin, R., Mee, P. J., McKee, M. D., Jung, D. Y. et al. (2007). Endocrine regulation of energy metabolism by the skeleton. *Cell* **130**, 456-469.

Leibbrandt, A. and Penninger, J. M. (2008). RANK/RANKL: regulators of immune responses and bone physiology. *Ann. N. Y. Acad. Sci.* **1143**, 123-150.

Li, X., Ominsky, M. S., Warmington, K. S., Morony, S., Gong, J., Cao, J., Gao, Y., Shalhoub, V., Tipton, B., Haldankar, R. et al. (2009). Sclerostin antibody treatment increases bone formation, bone mass, and bone strength in a rat model of postmenopausal osteoporosis. *J. Bone Miner. Res.* **24**, 578-588.

Lian, J. B., Stein, G. S. and Aubin, J. E. (2003). Bone formation: maturation and functional activities of osteoblast lineage cells. In *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, 5th edn (ed. M. J. Favus), pp. 13-28. Washington, DC: American Society for Bone and Mineral Research.

Little, R. D., Carulli, J. P., Del Mastro, R. G., Dupuis, J., Osborne, M., Folz, C., Manning, S. P., Swain, P. M., Zhao, S. C., Eustace, B. et al. (2002). A mutation in the LDL receptor-related protein 5 gene results in the autosomal dominant high-bone-mass trait. *Am. J. Hum. Genet.* **70**, 11-19.

Litzenberger, J. B., Kim, J. B., Tummala, P. and Jacobs, C. R. (2010). Beta1 integrins mediate mechanosensitive signaling pathways in osteocytes. *Calcif. Tissue Int.* **86**, 325-332.

Ljusberg, J., Wang, Y., Lang, P., Norgard, M., Dodds, R., Hultenby, K., Ek-Rylander, B. and Andersson, G. (2005). Proteolytic excision of a repressive loop domain in tartrate-resistant acid phosphatase by cathepsin K in osteoclasts. *J. Biol. Chem.* **280**, 28370-28381.

Luxenburg, C., Geblinger, D., Klein, E., Anderson, K., Hanein, D., Geiger, B. and Addadi, L. (2007). The architecture of the adhesive apparatus of cultured osteoclasts: from podosome formation to sealing zone assembly. *PLoS One* **2**, e179.

Manolagas, S. C. and Parfitt, A. M. (2010). What old means to bone. *Trends Endocrinol. Metab.* **21**, 369-374.

Marie, P. J. (2002). Role of N-cadherin in bone formation. *J. Cell Physiol.* **190**, 297-305.

Martin, T. J., Allan, E. H., Ho, P. W., Gooi, J. H., Quinn, J. M., Gillespie, M. T., Krasnoperov, V. and Sims, N. A. (2010). Communication between EphrinB2 and EphB4 within the osteoblast lineage. *Adv. Exp. Med. Biol.* **658**, 51-60.

Matsuo, K. (2010). Eph and ephrin interactions in bone. *Adv. Exp. Med. Biol.* **658**, 95-103.

Mbalaviele, G., Shin, C. S. and Civitelli, R. (2006). Cell-cell adhesion and signaling through cadherins: connecting bone cells in their microenvironment. *J. Bone Miner. Res.* **21**, 1821-1827.

McNamara, L. M., Majeska, R. J., Weinbaum, S., Friedrich, V. and Schaffler, M. B. (2009). Attachment of osteocyte cell processes to the bone matrix. *Anat. Rec. (Hoboken)* **292**, 355-363.

Mulari, M., Vääräniemi, J. and Väänänen, H. K. (2003). Intracellular membrane trafficking in bone resorbing osteoclasts. *Microsc. Res. Tech.* **61**, 496-503.

Nakamura, T., Imai, Y., Matsumoto, T., Sato, S., Takeuchi, K., Igarashi, K., Harada, Y., Azuma, Y., Krust, A., Yamamoto, Y. et al. (2007). Estrogen prevents bone loss via estrogen receptor alpha and induction of Fas ligand in osteoclasts. *Cell* **130**, 811-823.

Nesbitt, S. A. and Horton, M. A. (1997). Trafficking of matrix collagens through bone resorbing osteoclasts. *Science* **276**, 266-273.

Norvell, S. M., Alvarez, M., Bidwell, J. P. and Pavalko, F. M. (2004). Fluid shear stress induces beta-catenin signaling in osteoblasts. *Calcif. Tissue Int.* **75**, 396-404.

Novack, D. V. and Faccio, R. (2009). Osteoclast motility: putting the brakes on bone resorption. *Ageing Res. Rev.* **10**, 54-61.

Palokangas, H., Mulari, M. and Väänänen, H. K. (1997). Endocytic pathway from the basal plasma membrane to the ruffled border membrane in bone-resorbing osteoclasts. *J. Cell Sci.* **110**, 1767-1780.

Paszty, C., Turner, C. H. and Robinson, M. K. (2010). Sclerostin: a gem from the genome leads to bone-building antibodies. *J. Bone Miner. Res.* **25**, 1897-1904.

Pavlos, N. J., Xu, J., Riedel, D., Yeoh, J. S., Teitelbaum, S. L., Papadimitriou, J. M., Jahn, R., Ross, F. P. and Zheng, M. H. (2005). Rab3D regulates a novel vesicular trafficking pathway that is required for osteoclastic bone resorption. *Mol. Cell. Biol.* **25**, 5253-5269.

Pérez-Castrillón, J. L., Pinacho, F., De Luis, D., Lopez-Menendez, M. and Dueñas Laita, A. (2010). Odanacatib, a new drug for the treatment of osteoporosis: review of the results in postmenopausal women. *J. Osteoporos.* pii: 401581.

Quarles, L. D. (2008). Endocrine functions of bone in mineral metabolism regulation. *J. Clin. Invest.* **118**, 3820-3828.

Rivadeneira, F., Styrkarsdottir, U., Estrada, K., Halldorsson, B. V., Hsu, Y. H., Richards, J. B., Zillikens, M. C., Kavvoura, F. K., Amin, N., Aulchenko, Y. S. et al. (2009). Twenty bone-mineral-density loci identified by large-scale meta-analysis of genome-wide association studies. *Nat. Genet.* **41**, 1199-1206.

Rivollier, A., Mazzorana, M., Tebib, J., Piperno, M., Aitsiselmi, T., Rabourdin-Combe, C., Jurdic, P. and Servet-Delprat, C. (2004). Immature dendritic cell transdifferentiation into osteoclasts: a novel pathway sustained by the rheumatoid arthritis microenvironment. *Blood* **104**, 4029-4037.

Rizzoli, R, Yasothan, U. and Kirkpatrick, P. (2010). Denosumab. *Nat. Rev. Drug Discov.* **9**, 591-592.

Robling, A. G., Niziolek, P. J., Baldridge, L. A., Condon, K. W., Allen, M. R., Alam, I., Mantila, S. M., Gluhak-Heinrich, J., Bellido, T. M., Harris, S. E. et al. (2008). Mechanical stimulation of bone in vivo reduces osteocyte expression of Sost/sclerostin. *J. Biol. Chem.* **283**, 5866-5587.

Rochefort, G. Y., Pallu, S. and Benhamou, C. L. (2010). Osteocyte: the unrecognised side of bone tissue. *Osteoporos. Int.* **21**, 1457-1469.

Rosen, C. J., Ackert-Bicknell, C., Rodriguez, J. P. and Pino, A. M. (2009). Marrow fat and the bone microenvironment: developmental, functional, and pathological implications. *Crit. Rev. Eukaryot. Gene Expr.* **19**, 109-124.

Salo, J., Metsikko, K., Palokangas, H., Lehenkari, P. and Väänänen, H. K. (1996). Bone-resorbing osteoclasts reveal a dynamic division of basal plasma membrane into two different domains. *J. Cell Sci.* **109**, 301-307.

Salo, J., Lehenkari, P., Mulari, M., Metsikko, K. and Väänänen, H. K. (1997). Removal of osteoclast bone resorption products by transcytosis. *Science* **276**, 270-273.

Saltel, F., Destaing, O., Bard, F., Eichert, D. and Jurdic, P. (2004). Apatite-mediated actin dynamics in resorbing osteoclasts. *Mol. Biol. Cell* **15**, 5231-5241.

Santos, A., Bakker, A. D., Zandieh-Doulabi, B., de Blieck-Hogervorst, J. M. and Klein-Nulend, J. (2010). Early activation of the beta-catenin pathway in osteocytes is mediated by nitric oxide, phosphatidyl inositol-3 kinase/Akt, and focal adhesion kinase. *Biochem. Biophys. Res. Commun.* **391**, 364-369.

Schaller, S., Henriksen, K., Sørensen, M. G. and Karsdal, M. A. (2005). The role of chloride channels in osteoclasts: ClC-7 as a target for osteoporosis treatment. *Drug News Perspect.* **18**, 489-495.

Sobacchi, C., Frattini, A., Guerrini, M. M., Abinun, M., Pangrazio, A., Susani, L., Bredius, R., Mancini, G., Cant, A., Bishop, N. et al. (2007). Osteoclast-poor human osteopetrosis due to mutations in the gene encoding RANKL. *Nat. Genet.* **39**, 960-962.

Stenbeck, G. (2002). Formation and function of the ruffled border in osteoclasts. *Semin. Cell Dev. Biol.* **13**, 285-292.

Sutherland, K. A., Rogers, H. L., Tosh, D. and Rogers, M. J. (2009). RANKL increases the level of Mcl-1 in osteoclasts and reduces bisphosphonate-induced osteoclast apoptosis in vitro. *Arthritis Res. Ther.* **11**, R58.

Takada, I., Mihara, M., Suzawa, M., Ohtake, F., Kobayashi, S., Igarashi, M., Youn, M. Y., Takeyama, K., Nakamura, T., Mezaki, Y. et al. (2007). A histone lysine methyltransferase activated by non-canonical Wnt signalling suppresses PPAR-gamma transactivation. *Nat. Cell Biol.* **9**, 1273-1285.

Talmage, R. V. and Elliott, J. R. (1958). Removal of calcium from bone as influenced by the parathyroids. *Endocrinology* **62**, 717-722.

Tatsumi, S., Ishii, K., Amizuka, N., Li, M., Kobayashi, T., Kohno, K., Ito, M., Takeshita, S. and Ikeda, K. (2007). Targeted ablation of osteocytes induces osteoporosis with defective mechanotransduction. *Cell Metab.* **5**, 464-475.

Teitelbaum, S. L. (2007). Osteoclasts: what do they do and how do they do it? *Am. J. Pathol.* **170**, 427-435.

Villa, A., Guerrini, M. M., Cassani, B., Pangrazio, A. and Sobacchi, C., (2009). Infantile malignant, autosomal recessive osteopetrosis: the rich and the poor. *Calcif. Tissue Int.* **84**, 1-12.

Walker, E. C., McGregor, N. E., Poulton, I. J., Pompolo, S., Allan, E. H., Quinn, J. M., Gillespie, M. T., Martin, T. J. and Sims, N. A. (2008). Cardiotrophin-1 is an osteoclast-derived stimulus of bone formation required for normal bone remodeling. *J. Bone Miner. Res.* **23**, 2025-2032.

Walker, E. C., McGregor, N. E., Poulton, I. J., Solano, M., Pompolo, S., Fernandes, T. J., Constable, M. J., Nicholson, G. C., Zhang, J. G., Nicola, N. A., et al. (2010). Oncostatin M promotes bone formation independently of resorption when signaling through leukemia inhibitory factor receptor in mice. *J. Clin. Invest.* **120**, 582-592.

Wan, M., Yang, C., Li, J., Wu, X., Yuan, H., Ma, H., He, X., Nie, S., Chang, C. and Cao, X. (2008). Parathyroid hormone signaling through low-density lipoprotein-related protein 6. *Genes Dev.* **22**, 2968-2979.

Wang, Y., McNamara, L. M., Schaffler, M. B. and Weinbaum, S. (2007). A model for the role of integrins in flow induced mechanotransduction in osteocytes. *Proc. Natl. Acad. Sci. USA* **104**, 15941-15946.

Weitzmann, M. N. and Pacifici, R. (2007). T cells: unexpected players in the bone loss induced by estrogen deficiency and in basal bone homeostasis. *Ann. N.Y. Acad. Sci.* **1116**, 360-375.

Xing, L. and Boyce, B. F. (2005). Regulation of apoptosis in osteoclasts and osteoblastic cells. *Biochem. Biophys. Res. Commun.* **328**, 709-720.

Yadav, V. K., Ryu, J. H., Suda, N., Tanaka, K. F., Gingrich, J. A., Schutz, G., Glorieux, F. H., Chiang, C. Y., Zajac, J. D., Insogna, K. L. et al. (2008). Lrp5 controls bone formation by inhibiting serotonin synthesis in the duodenum. *Cell* **135**, 825-837.

Yadav, V. K., Oury, F., Suda, N., Liu, Z. W., Gao, X. B., Confavreux, C., Klemenhagen, K. C., Tanaka, K. F., Gingrich, J. A., Guo, X. E. et al. (2009). A serotonin-dependent mechanism explains the leptin regulation of bone mass, appetite, and energy expenditure. *Cell* **138**, 976-989.

Yamaza, T., Goto, T., Kamiya, T., Kobayashi, Y., Sakai, H. and Tanaka, T. (1998). Study of immunoelectron microscopic localization of cathepsin K in osteoclasts and other bone cells in the mouse femur. *Bone* **23**, 499-509.

Zaidi, M., Inzerillo, A. M., Moonga, B. S., Bevis, P. J. and Huang, C. L. (2002). Forty years of calcitonin-where are we now? A tribute to the work of Iain Macintyre, FRS. *Bone* **30**, 655-663.

Zaman, G., Jessop, H. L., Muzylak, M., De Souza, R. L., Pitsillides, A. A., Price, J. S. and Lanyon, L. L. (2006). Osteocytes use estrogen receptor alpha to respond to strain but their ERalpha content is regulated by estrogen. *J. Bone Miner. Res.* **21**, 1297-1306.

Zhao, H., Laitala-Leinonen, T., Parikka, V. and Vaananen, H. K. (2001). Downregulation of small GTPase Rab7 impairs osteoclast polarization and bone resorption. *J. Biol. Chem.* **276**, 39295-39302.

Zimmerman, D., Jin, F., Leboy, P., Hardy, S. and Damsky, C. (2000). Impaired bone formation in transgenic mice resulting from altered integrin function in osteoblasts. *Dev. Biol.* **220**, 2-15.

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