CALCIUM SIGNALLING AND CALCIUM TRANSPORT IN BONE DISEASE

H.C. Blair, **P.H. Schlesinger**, **Christopher L.-H. Huang**, and **M. Zaidi**
Departments of Pathology and of Cell Biology and Physiology, University of Pittsburgh and Veterans' Affairs Health System, Pittsburgh, PA 15261, Department of Physiology and Cell Biology, Washington University School of Medicine, St. Louis, MO 63110, Physiological Laboratory, University of Cambridge, Cambridge, CB2 3EG, United Kingdom, and The Bone Program, Mount Sinai School of Medicine, New York, New York 10029

Abstract

Calcium transport and calcium signalling mechanisms in bone cells have, in many cases, been discovered by study of diseases with disordered bone metabolism. Calcium matrix deposition is driven primarily by phosphate production, and disorders in bone deposition include abnormalities in membrane phosphate transport such as in chondrocalcinosis, and defects in phosphate-producing enzymes such as in hypophosphatasia. Matrix removal is driven by acidification, which dissolves the mineral. Disorders in calcium removal from bone matrix by osteoclasts cause osteopetrosis. On the other hand, although bone is central to management of extracellular calcium, bone is not a major calcium sensing organ, although calcium sensing proteins are expressed in both osteoblasts and osteoclasts. Intracellular calcium signals are involved in secondary control including cellular motility and survival, but the relationship of these findings to specific diseases is not clear. Intracellular calcium signals may regulate the balance of cell survival versus proliferation or anabolic functional response as part of signalling cascades that integrate the response to primary signals via cell stretch, estrogen, tyrosine kinase, and tumor necrosis factor receptors

Keywords

Hypophosphatasia; chondrocalcinosis; osteopetrosis; osteoporosis

---

Although bone is not considered a major calcium sensing organ in humans, the cells of bone tissue control over 99% of the human body's calcium content. The principal calcium sensors that regulate bone calcium uptake and release are in the parathyroid glands. Bone function is also modified by vitamin D and by calcium transport in the kidney and intestine. These indirect mechanisms of controlling bone calcium metabolism are beyond the scope of our considerations here. In spite of processing such massive quantities of the Ca^{2+} bone cells use calcium in their homeostatic control processes. The massive movement of calcium is carried out by specialized and regulated transporters. Defects in the transporters cause diseases with affect bone structure or function. Indeed, inborn errors have been very important in defining the calcium transport mechanisms in bone.

Additionally, calcium is used by bone forming and bone degrading cells as a secondary mediator of hormone and cytokine action. These actions include roles in intercellular communication within groups of osteoblasts, which are connected by gap junctions [Henriksen et al., 2006]. These osteoblast groups function in a coordinated fashion in bone synthesis and maintenance, and are collectively known as the osteon. Osteoblasts in these groups are connected by gap junctions which are capable of propagating signals in the cell groups, including calcium waves [Xia and Ferrier, 1992]. Calcium is also an important regulator of

cellular attachment, motility, endosome function [Piper and Luzio, 2004] and survival [Davies and Madesh, 2004]. These are all critical functions in bone bone degrading osteoclasts.

In this chapter, we will describe calcium transportand calcium regulation from the standpoint of bulk mineral transport, and then from the standpoint of cellular regulation. We will use inborn errors of metabolism where possible to illustrate the major calcium-related activities of the cells. After the framework of functional calcium transport is established, we will discuss calcium-dependent cellular regulatory functions.

1. BULK CALCIUM TRANSPORT BY THE OSTEOBLAST AND RELATED CELLS

The osteoblast is a mesenchymal stem cell derivative [Pittenger et al., 1999]. Specialization of the osteoblast allows it to produce an extracellular matrix of type I collagen and accessory proteins including osteocalcin [Young 2003], which are present in minor quantities. The type I collagen is very dense and heavily crosslinked, creating an extremely high tensile strength. To provide strength to the skeleton, it comprises approximately 10% of the dry mass of bone. Osteocalcin is a calcium-binding low molecular weight protein, its calcium binding properties conferred by post-translational modification to produce γ–carboxyglutamate [Bugel, 2005; Weber, 2001]. Osteocalcin is important in proper calcification of the matrix. Dozens of other accessory proteins found in bone matrix also play important, but secondary, roles in bone structure. The major component of bone matrix is bone mineral, which provides its resistance to compression, and in combination with type I collagen produces a skeleton of great strength and durability. However, the bone matrix is also amassive depot of minerals [Neuman and Neuman, 1958]. This includes over 99% of the body's calcium, but the calcium-balancing anions, mainly PO_4^{3-}, are also important and, in the presence of a chronic acid load, amount to approximately 1.5 moles of available base equivalents for each calcium [Cho et al., 2003]. When necessary the skeleton is sacrificed to maintain the pH of the extracellular fluid [Bushinsky, 2001; Carano et al., 1993].

How the osteoblast deposits mineral has long been an interesting mystery. Details of the process are still unclear, although some general principals can be derived from diseases of mineralization which point to the central mechanisms. The chemistry of bone mineral, hydroxyapatite, requires that any mechanism of bone formation include a supply of Ca^{2+} and $H_2PO_4^-$ and some way to dispose of 1.4 H+ per each Ca^{2+} deposited.

$$10Ca + 6H_2PO_4^- + 2H_2O \leftrightarrow Ca_{10}(H_2PO_4)_6(OH)_2 + 14H^+$$

<div align="right">Equation 1</div>
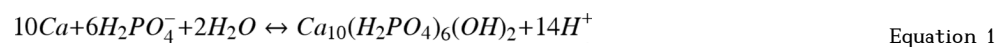
The need for local control of extracellular ion composition and pH buffering is reflected in the multicellular structure that regulates bone formation in the air-breathing vertebrates, which is called the "osteon". This is an extended group of gap-junction connected cells that are embedded in the bone matrix, together with a layer of osteoblasts "covering" the bone surface at the interface of the osteon with the bone marrow (Figure 1). This multicellular structure is of key importance. That the surface osteoblasts are connected to the earlier generations of osteoblasts (called osteocytes), which have become embedded in the matrix produced by the cells, allows the unit to be regulated by interaction with marrow cells and serum components. It has long been known that the access of water and ions to bone matrix is very restricted [Deakins and Burt, 1944;Neuman and Neuman, 1958]. Thus the bone matrix is entirely surrounded by cells, which control the movement of constituent proteins of bone matrix and also of the ions which are deposited to produce bone mineral. Therefore it is mainly byactive or facilitated transport that constituents in the extracellular fluid can reach the matrix. Note, for example, that tetracycline or calcein will accumulate very specifically in the mineralizing

layer of bone at the surface of an activeosteon, while bone surfaces deep within the osteon are unlabeled. When the osteon dies, as in osteonecrosis following high-dose glucocorticoid exposure, the cell-matrix surfaces can be labeled by tetracycline in an osteon specific geometry [Eberhardt et al., 2001]. The control of this cellular array surrounding bone matrix requires both connexin43 and purinergic receptors [Civitelli et al., 1993; A. Hoebertz et al., 2003].

The mechanism of bulk calcium transport for bone formation is partly established from experimental work and is partly conjectural (Figure 1). The source of the calcium is obviouslythe extracellular fluid, and its transport through the active surface osteoblasts of a bone forming osteon is clearly regulated. However, the molecular mechanism is poorly understood. There is insufficient Ca^{2+}-ATPase activity for mineralizationto be supported by direct transport [Gay and Lloyd, 1995], and this would be, energetically, very inefficient as well. It was long hypothesized that there wasa calbindin-dependent calcium ferry [Balmain et al., 1989], but this is either not to be the case, or the calcium buffer system for facilitated transport involves a unique mechanism not yet described, in that calbindin-negative osteoblasts lose 25% of their calcium binding capacity but transport calcium at a normalrate [Turnbull et al., 2004]. Osteoblasts express abundant sodium-calcium exchangers, however, in a pattern likely to correlate with bulk mineral transport [Stains et al., 2002], so it is probably early to discard the calcium ferry hypothesis entirely, and it is more likely that a calbindin-independent mechanism of some sort exists.

The only essential components of the mineral deposition mechanism that are fairly certain at this time relate to phosphate. Even for phosphate, alternative mechanisms are proposed, which are not mutually exclusive but probably function in parallel, in the regulation of different aspects of skeletal calcium transport, and to some extent provide redundancy that allows many mineral transport disorders to be survivable. Alkaline phosphatase activity is essential to produce phosphate. Its substrate is pyrophosphate. In the absence of the alkaline phosphatase, normally highly expressed as an ectoenzyme by osteoblasts, there is little matrix mineralization and very high serum pyrophosphate accumulation occurs [Whyte et al., 1995]. The pyrophosphate is, to a major extent, produced by a nucleoside pyrophosphatase, PC-1 [Lotz et al., 1995; Hessle et al., 2002]. This gene is also expressed in other organs, where polymorphisms may be related to pathology, but mutations in the bone are not described. A multipass transmembrane protein related to progressive ankylosis (fusion of joints), ANK, is also major additional source of pyrophosphate [Ho, Johnson, and Kingsley, 2000]. It is a pyrophosphate transporter, so presumably the source of the pyrophosphate is intracellular although the biochemical pathway is unknown. Mutations in its human homolog, ANKH, cause a group of diseases including craniometaphysial dysplasia and chondrocalcinosis [Reichenberger et al., 2001; Nurnberg et al., 2001; Williams et al., 2002; Pendleton et al., 2002].

2. A PROTON CONNECTION

The third component required for bone formation, removal of protons, is essential but it is frequently ignored in considerations of mineralization. High concentrations of phosphate and calcium at neutral pH will form an initial precipitate, but mineral formation is quickly limited as the pH falls below 5.6 [Neuman and Neuman, 1958]. From the hydroxyapatite formation equation (Equation 1) it is clear that ~1.5 moles of H^+ is produced by the combination of $H_2PO_4^-$, Ca^{2+} and H_2O. The exact stoichiometry depends on the pH at the deposition site, which determines the ratio of HPO_4^{2-} to $H_2PO_4^-$; this detail is excluded, for clarity, from Equation 1, where only the predominant phosphate ion, $H_2PO_4^-$, is shown; the phosphoric acid K_2 is 6.70. On the other hand, PO_4^{3-} and H_3PO_4 are of no importance biologically since the phosphoric acid K_1 is 1.96 and K_3 is 12.32.

The deposition of limited quantities of hydroxyapatite in extracellular matrix has been observed without bounding cells. Cartilage calcification is such a case where local pH control and Ca^{2+} are dependent upon diffusion and the rate of mineral deposition is driven by phosphate presentation. Chondrocytes produce alkaline phosphatase that generates the required phosphate, but cartilage is not delimited by any cellular structures and transfer of Ca^{2+} and H^+ is by diffusion from extracellular fluid.

Bone mineral, on the other hand, is deposited rapidly along a line of new bone within an osteon where the matrix is completely enclosed by cells. The calcium deposition is carefully orchestrated and precise, requiring regulated Ca^{2+} and H^+ transport (Figure 1). The existence of local alkalinization at the site of mineral deposition was long ago noted in using a pH indicator [Cretin, 1951]. We noted in genescreen studies of human osteoblasts that many subunits of the vacuolar-type H^+-ATPase are highly expressed, but no clear vectorial localization of the pump (hypothetically in the opposite orientation as in the osteoclast, discussed below) has been observed. Other possibilities include an H^+-pyrophosphatase, which could make the requisite (outward) acid gradient as well as producing pyrophosphate for mineral formation, but such transporters have not been found in metazoans.

3. CALCIUM-DEPENDENT CELLULAR REGULATION IN THE OSTEOBLAST AND IN RELATED CELLS

As noted in the introduction, the bone is not a classical calcium sensing organ. However, osteoblasts express a G-protein coupled calcium receptor, GPRC6A [Pi et al., 2005], and purinergic receptors that mediate calcium uptake or regulation in the osteoblast [Hoebertz et al., 2003]. The osteoblast may thus play a role in adaptation to abnormal extracellular calcium such as in diseases where the parathyroid calciostat is unable to maintain extracellular calcium activity within its normal narrow limits, and knockout of the calcium sensing receptor does impair calcium homeostasis independently of PTH [Kos et al., 2003]. This mechanism will, however, require further study. Activating mutations of the calcium sensing receptor are associated with Bartter's syndrome [Watanabe et al., 2002], which is a renal calcium reabsorption defect, and no osteoblast-related phenotype is known. Purinergic receptors are known to be present on osteoblasts and ATP or UTP coordinate osteoblast activity in bone remodeling [Hoebertz et al., 2002]. Similarly, in the mineralization of otoliths purines play an important role in mineral deposition by otoconial epithelial cells [Suzuki et al., 1997]. From mice defective in vestibular function a new and unique family of membrane proteins has been identified, the otopetrins [Hurle et al., 2001]. These membrane proteins are essential for otolith mineralization but are found in many tissues. We have studied the expression of otopetrin 1 in cultured cells and are studying it as a potential novel regulator of P2Y and P2X activity. Although the function of otopetrins in osteoblasts has not been studied, they are essential for mineralization in otolith formation [Hurle et al., 2003; Hughes et al., 2004].

As in most cells, osteoblasts express several calcium channels. The clearest role of calcium channels in functional osteoblast regulation is for L-type voltage-sensitive channels. These channels mediate changes in osteoblast intracellular calcium that vary with major osteoblast regulatory agents including vitamin D and PTH [Li et al., 1997; Gu et al., 2001; Ryder et al., 2001; Bergh et al., 2006]. Pharmacological inhibitor studies of L-type calcium channels show mixed results in assays of osteoblast differentiation [Zahanich et al., 2005; Nishiya et al., 2002]. However, the L-type calcium channels are probably essential intermediates in osteoblast intercellular calcium signals [Jorgensen et al., 2003]. Additional calcium channels may be involved in metabolic coupling to osteoblastic intracellular calcium, including the ryanodine receptor, purinergic receptors, and IP3Rs [Sun et al., 2002; Jorgensen et al, 2003]. There are also sodium/calcium channels such as ENaC that may respond to membrane swelling in the osteoblast [Kizer et al., 1997]. These transporters may be important, and some associations

with cytokine response are known [Kirkwood, et al., 1997; Bradford et al., 2000]. However, physiological mechanisms are for the most part not clear.

Osteoblast intracellular calcium is regulated by membrane stretch or shear stress and by other mechanisms [Kamioka et al., 2006]. Further, the absence of stretch causes atrophy. This effect is important, with acute and severe bone loss caused by disuse or unweighting [Bikle and Halloran, 1999]. Stretch-induced calcium transients, probably via L-type calcium channels, are important in the coupling of bone flexion to the activity and proliferation of osteoblasts and related cells [Hughes-Fulford, 2004]. Skeletal flexion also promotes chondrocyte growth [Drescher et al., 2003]. Osteoblastic cellular responses include cell proliferation [Riddle et al., 2006], as well as diverse, generally anabolic, effects on differentiationand activity.

It is likely that the calcium signal is secondary to potassium-dependent depolarization [Wiltink et al., 1994; Gofa and Davidson, 1996; Hattori et al., 2001; Jorgensen et al., 2003]. Stretch-activated potassium channelactivity has been demonstrated [Duncan and Misler 1989; Ypey et al., 1992; Davidson, 1993]. This may be due to two-pore domain potassium channels [Chen et al., 2005; Hughes et al., 2006], although the mechanism is not firmly established. Other channels have been proposed, including activation by focal adhesion kinaseor outwardly-rectifying BK potassium channels [Rezzonico et al., 2003]. Stretch-activated channels are, in turn, co-regulated with cell attachment-related proteins. These may, in addition to the ion signals, modify the cell attachment and attachment-related kinases. This has been demonstrated in chondrocytes [Lee et al., 2000], and a similar mechanism in osteoblasts probably modifies the focal adhesion kinase [Boutahar et al., 2004].

While stretch activation of calcium signals involves interaction with attachment proteins, not all stretch-related signals require calcium. All mechanical stimuli activate ERK1/2, with downstream upregulation of pro-growth transcription factors, such as c-fos [Ruwhof and van der Laarse, 2000]. The integrin β subunit is essential for ERK1/2 activation and downstream signals in response to mechanical stimulation [MacKenna et al., 1998]. However, the cation channels may be dispensable for most of the gene expression effects [Sadoshima et al., 1992]. On the other hand, in connected osteoblasts and osteocytes, calcium is a key intercellular signal.

Calcium fluxes in osteoblasts may have anabolic effects or may promote apoptosis, depending on the context of the signal. Survival mechanisms triggered along with cell stretch, includingPI-3-kinase activity and phosphorylation of Akt [Danciu et al., 2003], are probably involved in the promotion of cell survival and anabolic effects in stretch related calcium signals. The IP3R receptor family may be important in changes of nuclear calcium transport with senescence [Huang et al., 2000]. On the other hand, induction of calcium via NAD+ and the RyR induces apoptosis [Romanello et al., 2001]. This is not surprising in that this type of calcium signal is unrelated, as far as is known, to adhesion-related survival signals. Further, the RyR is a critical regulator of nuclear calcium in osteoblasts [Adebanjo et al., 1999]. The anabolic/apoptotic decision may also be dependent on the type ofcalcium channel, and on the magnitude and location of the calcium current.

Abnormal calcium transport undoubtedly contribute to bone defects but specific examples are poorly described. For the major calcium transporters, significant defects are lethal in embryonic life, such as in the Cav1.2 L-type calcium channel [Seisenberger et al., 2000]. Interestingly, for the closely related Cav1.2 and Cav1.3 L-type calcium channels, which are likely to be the principal calcium channels in stretch-related calcium fluxes in osteoblasts, the Cav1.3 channel may partially compensate for loss of function of Cav1.2 [Xu et al., 2003]. There are mutations of Cav1.2 with cardiac defects and a variety of other developmentalabnor-malities including syndactyly (fusion of fingers), but no clear bone phenotype at the level of bone matrix structure

[Splawski et al., 2005]. In other cases, knockouts are survivable, but, as in the IP3R1 knockout [Hirota et al., 1998], with complex defects including runting; specific skeletal changes have not been characterized.

4. BULK CALCIUM TRANSPORT BY THE OSTEOCLAST

The osteoclast is unique in mobilizing massive quantities of calcium from mineralized tissue. Dissolving hydroxyapatite requires the addition of protons, just as deposition of hydroxyapatite liberates acid (see Equation 1). To allow acidification, the osteoclast produces an isolated micro-compartment on the bone surface. This is achieved by close apposition to the matrix via adhesion of αv integrins to matrix RGD peptides, with β3 the major complementary subunit [Miyauchi et al., 1991]. Inside the osteoclast the cytoskeleton [Akisaka et al., 2006], and transport activities [Vaananen et al., 2000; Schlesinger et al., 1994] are reorganized to support the resorption compartment.

The key metabolic activity within this sealed compartment is acid transport. It is driven by a V-type H+-ATPase [Blair et al., 1989]. This ATPase is comprised of two major subassemblies, membrane (Vo) and cytoplasmic (V1). The Vo component consists of a hydrogen channel, 17 kDa, and large, 116 kDa, protein with several transmembrane domains. This protein is crucial for membrane insertion, and four homologous genes encode variants of it [Nishi & Forgac, 2002]. The isoform TCIRG1 (ATP6i; A3) is amplified specifically in osteoclasts [Li et al., 1999; Mattsson et al., 2000]. Defects in this protein are common causes of osteopetrosis in the human [Blair et al., 2004]. Heterogeneity in TCIRG1 expression and function may also affect bone density [Carn et al., 2002]. The V1 assembly is essential to life. The proton pump mechanism is inferred by analogy to the homologous mitochondrial F-ATPase, which produces ATP from proton gradients rather than vice versa. It was described in detail in the 1980s and 90s [Boyer, 1997]. The F1 or V1 assemblies are nano-motors that couple ATP hydrolysis to electrogenic H+ translocation [Finbow and Harrison, 1997]. The F and V-ATPases are electrogenic and transport H^+ without counter-ions. Thus, counter-transport of cations or co-transport of anions is required for H^+ transport in meaningful quantity, as for mineral removal. And the osteoclast must move an amazing amount of acid, since bone mineral requires addition of ~1.5 moles of H^+ per mole of calcium removed at pH 7.4, and the osteoclast can degrade approximately its own volume in bone mineral per day. Indeed, the quality of cultured osteoclasts in vitro is relatively easy to judge from rapid acidification of the medium completely out of proportion from the quantity of cells [Carano et al., 1993].

Thus, calcium mobilization is dependent on and requires a cotransport of ions to balance the electrogenic proton pump. Studies of isolated osteoclast vesicles made it clear that this cotransport includes primarily chloride [Blair et al., 1991]. A Cl⁻ channel was isolated from the avian osteoclast ruffled border [Schlesinger et al., 1997], which is a homologue of a human intracellular chloride channel 5 (CLIC5) belonging to a family of proteins which form chloride channels in their membrane conformation [Heiss & Poustka, 1997; Ashley, 2003]. These proteins are structurally related to the omega family of glutathione S-transferases, and are required for development in *C. elegans* [Berry et al., 2003]. However, trans-genic mice deficient in an unrelated and also widely expressed chloride transporter, CLCN7 [Brandt and Jentsch, 1995], are osteopetrotic [Cleiren et al., 2001; Kornak et al., 2001]. Polymorphisms in CLCN7 are associated with osteopet-rosis in several families as reported in a number of studies [Blair et al., 2004]. However, CLCN7 is a chloride-proton antiporter [Picollo & Pusch, 2005; Scheel, et al., 2005] rather than a chloride channel. This complicates the model for osteo-clastic acid secretion a bit in that a chloride-proton antiporter will not transport chloride without a significant H^+ gradient [Diewald et al., 2002: Accardi et al., 2005]. In an elegant experiment correcting CLCN7 expression in osteoclasts rescued bone metabolism and uncovered an underlying lysosomal defect [Kasper et al., 2005]. Thus it is clear that multiple Cl⁻ transporters

play important roles in osteo-clast function [Jentsch et al., 2005]. The CLIC family of intracellular proteins, which are chloride channels, have been identified with acidification in osteoclasts for some time [Blair & Schlesinger, 1990: Schlesinger et al., 1997]. Recently, CLIC5, has been directly implicated in osteoclast bone resorption and H^+ transport [Edwards et al., 2006]. In combination the CLCN7 exchanger and CLIC5 provide a H^+ leak and charge neutralization that are important in acidification [Grabe & Oster, 2001].

This would extend our model of balanced HCl transport for mineral dissolution, but additional studies are required to understand the integration of this model [Blair et al., 2002]. There is a pervasive cytoskeletal-src dependence of proper targeting for the ion transporters of osteoclasts [Zuo et al., 2006: Tehrani et al., 2006: Abu-Amer et al., 1997: Soriano et al., 1991]. The actin-directed disposition of CLIC protein has also been observed in microvilli of placental cells [Berryman et al., 2004]. In osteoclasts the coordinated disposition of V-ATPase and CLIC required for full expression of the bone resorption phenotype [Edwards et al., 2006]. It is clear that much of the osteoclasts organization exists to support the massive acid secretion for bone calcium solubilization.

To complete bulk calcium transport, the high calcium solution at the osteoclast attachment [Silver et al., 1988] must be moved to and diluted in the extracellular space. Some calcium may be released when the osteoclast detaches, occurring under normal circumstances at intervals of roughly one day, but the volume of solublized bone producing calcium and phosphate is too great for this to be the sole mechanism. Studies using confocal imaging and labeled matrix showed that the bulk transport of calcium and other degraded membrane components by vacuolar transcytosis through the osteoclast plays a role in this process [Nesbitt and Horton, 1997; Salo et al., 1997]. However, there is also good evidence for calcium binding proteins and an epithelial calcium channel, TRPV5, that support a calcium-ferry mechanism [van der Eerden et al., 2005]. The osteoclast has several other calcium transport proteins, including a Ca^{2+} ATPase [Bekker and Gay, 1990], which is expressed highly, as expected for a cell which may undertake significant cytoplasmic calcium transcytosis. Contrariwise, knockout of the TRPV5 calcium channel does not cause osteopetrosis, although rickets and hyperparathy-roidism occur [Renkema et al., 2005]. The massive calcium movement occurring in bone resorption undoubtedly requires that the osteoclast have a mechanism for protecting cytoplasmic and organelle calcium content even if the bulk transport is vesicular. Therefore understanding the parallel contributions of transcytosis and cytoplasmic transport will require a quantitative analysis of their relative contributions.

5. CALCIUM-DEPENDENT CELLULAR REGULATION IN THE OSTEOCLAST

5.1. Ca^{2+} Signalling in Osteoclasts

Osteoclasts show a cell membrane Ca^{2+} sensor function: this is not unexpected from the increases in ambient $[Ca^{2+}]$ to levels as high as 8–20 mM [Silver et al., 1988] following hydroxyapatite dissolution by osteoclastic activity. Thus, such extracellular $[Ca^{2+}]$ alterations alter bone resorptive activity in isolated rat osteoclasts [Malgaroli et al., 1989, Zaidi et al., 1989] following rapid and sustained changes in micro-spectrofluometrically determined cytosolic $[Ca^{2+}]$, cell retraction ('R' effect) and longer-term inhibition of enzyme release and bone resorption [Datta et al., 1989a correct to 1989, Zaidi et al., 1989, Moonga et al., 1990; Zaidi, 1990]. These findings suggest an existence of long and short term feedback mechanisms on both enzyme release [Zaidi et al., 1989; Moonga et al., 1990] and osteoclastic bone resorptive activity [Datta et al., 1989] controlled by the increases in extracellular $[Ca^{2+}]$ that result from the latter process.

Cell physiological studies using fura-2 fluorescence to measure increases in free cytsolic $[Ca^{2+}]$ suggested that such signals likely arise both through release of intracellularly stored

Ca^{2+} and extracellular Ca^{2+} entry. Thus, ionomycin applications elicited cytosolic $[Ca^{2+}]$ transients in osteoclasts exposed to Ca^{2+}-free external solutions that fully recovered to baseline, persisted following surface membrane potential manipulations but disappeared following repeated ionophore application as would have been expected by progressive depletion of Ca^{2+} stores. Restoration of extracellular $[Ca^{2+}]$ then elicited cytosolic $[Ca^{2+}]$ overshoots consistent with the capacitative Ca^{2+} entry reported in other cells. In contrast Ca^{2+} transients in osteoclasts studied in Ca^{2+}-containing bathing solutions decayed to sustained levels and persisted despite repeated ionophore application. [Shankar et al., 1994].

5.2. Evidence for a Surface Membrane Ca^{2+} Receptor (CaR)

Neither the increase in cytosolic $[Ca^{2+}]$ nor the associated cell retraction or inhibition of bone resorption appear to involve voltage-dependent L-type Ca^{2+} channels. These were largely insensitive to both dihydropyridine or phenylalkylamine Ca^{2+} channel specific reagents even under depolarizing conditions [Datta et al., 1990; see also: Zaidi et al., 1990]. The available evidence favours specialist cellular mechanisms that sense ambient $[Ca^{2+}]$ that parallel situations described in a number of other cell types, including CT-secreting thyroid parafollicular cells, parathyroid hormone (PTH)-secreting chief cells of the parathyroid gland [Brown et al., 1993], gastrointestinal enterocytes [Gama et al., 1997; Pazianas et al., 1995] renin-secreting renal juxtaglomerular and proximal tubular cells [Riccardi et al., 1995], neurones [Quinn et al., 1997], cytotrophoblasts [Lundgren et al., 1994], keratinocytes [Zaidi, 1990; Brown, 1991] and testicular Leydig cells [Adebanjo et al., 1998a].

Such a hypothesis would similarly suggest for the osteoclast an existence of *specific surface membrane* Ca^{2+} receptors (CaR) sensitive to higher, millimolar, $[Ca^{2+}]$ changes than some of the other examples suggested above. Nevertheless, one could then suggest an activation scheme in which Ca^{2+} acts both as extracellular regulator and intracellular messenger. This suggestion was compatible with the action of even some membrane-impermeant divalent or trivalent ions that similarly triggered cytosolic $[Ca^{2+}]$ changes: this would remain compatible with their interaction with a surface membrane CaR [Malgaroli et al., 1989; Zaidi et al., 1991; Zaidi et al., 1992a; Shankar et al., 1992a,b]. Thus, applications of the divalent cation Ni^{2+} as surrogate extracellular trigger elicited rapid, concentration-dependent, cytosolic $[Ca^{2+}]$ elevations. These showed use-dependence inactivation, persisted despite extracellular $[Ca^{2+}]$ deprivation, and a dependence on agonist $[Ni^{2+}]$ suggesting a unity Hill coefficient. They were prevented by prior depletion of intracellular Ca^{2+} stores by ionomycin and modified by extracellular levels of the divalent cations, Ca^{2+} and Mg^{2+}. They were potentiated by acidification from pH 7.8 to 4 suggesting possible linkages between Ca^{2+} sensing and extracellular acidification. The latter effect persisted in Ca^{2+}-free, EGTA-containing solutions, implicating actions on the release of intracellularly stored Ca^{2+} as opposed to its entry from the extracellular space [Adebanjo et al., 1994]. Finally, alterations of membrane voltage produced by altered extracellular $[K^+]$ in the presence of a valinomycin ionophore modified both the activation and inactivation kinetics of the $[Ca^{2+}]$ transients [Shankar et al., 1995a; Pazianas et al., 1993]. Findings of this kind were clearly compatible with regulation of cytosolic $[Ca^{2+}]$ through a integral surface membrane receptor for the divalent cation regulators Ca^{2+} and Mg^{2+} that was also sensitive to the surrogate agonist Ni^{2+}, whose occupancy activated and subsequently inactivated release of intracellularly stored Ca^{2+}.

5.3. Functional Consequences of CaR Activation

Such a CaR activation were accompanied by a specific causally related set of functional and morphometric events culminated in a reduction of bone resorptive activity over hours [Bax et al., 1993; Bax et al., 1992; Zaidi et al., 1992b; Shankar et al., 1993] and reduced acid phosphatase release, whose extent depended on agonist concentration. These effects followed a pronounced cell retraction (R effect) but preserved granule movement, cell migration, and

quantitative indicators of margin ruffling. Again, they could be reproduced following application of different alkaline earth or other metal cations in a common potency sequence: $La^{3+} > Cd^{2+} > Ni^{2+} > Ca^{2+} > Ba^{2+} = Sr^{2+} > Mg^{2+}$ [Zaidi et al., 1991; Shankar et al., 1992a], again consistent with actions at a single surface membrane CaR [Zaidi et al., 1991].

The CaR activation mechanism also appeared to interact with other regulatory processes involving more systemic mechanisms that influence osteoclast activity. Physiological, femtomolar, CT concentrations reduced the sensitivity of the CaR system to both Ca^{2+} and Ni^{2+}. Amylin, calcitonin gene-related peptide, cholera toxin and dibutyryl-cAMP, all believed to act through the osteoclast cAMP signaling system exerted similar effects [Zaidi et al., 1996]. Such cross coupling phenomena may also involve cytokine systems particularly those involving interleukin-6 (IL-6). Osteoclast surface membrane expresses IL-6 receptor, and IL-6 but not IL-11 reversed the inhibition of osteoclastic bone resorption induced by high extracellular Ca^{2+}. This effect was reversed by excess soluble IL-6 receptor. IL-6 also inhibited cytosolic $[Ca^{2+}]$ signals triggered by extracellular Ca^{2+} or Ni^{2+}. Conversely, elevated ambient $[Ca^{2+}]$ or their culture on a bony matrix increased osteoclastic IL-6 secretion as well as IL-6 receptor mRNA expression. Together such observations suggest that ambient $[Ca^{2+}]$ enhances secretion of IL-6 that in turn attenuates Ca^{2+} sensing and Ca^{2+} inhibition of bone resorption in an autocrine-paracrine loop that sustains osteoclastic activity despite local elevations in extracellular $[Ca^{2+}]$ generated by bone resorption. [Adebanjo et al 1998c].

Finally, both vitamin D-binding protein (DBP) and the macrophage-activating factor (DBP-MAF) left following removal of its sialic acid or galactose residue inhibit extracellular Ca^{2+} and cation sensing with the order of potency: native DBP = sialidase-treated DBP > beta-galactosidase-treated DBP [Adebanjo et al., 1998b].

5.4. Ca^{2+} Recovery in the Osteoclast

Mechanisms that then restore the basal cytosolic $[Ca^{2+}]$ levels remain unclear. Besides a Ca^{2+}-ATPase on the osteoclast dorsal surface relatively little is known of alternative or parallel methods for Ca^{2+} extrusion [Zaidi et al., 1993] although there is recent functional, evidence for a Na^+/Ca^{2+} exchanger that, in analogy to the regulation of cytoplasmic $[Ca^{2+}]$ in cardiac muscle could be linked to the proton extrusion that is a primary determinant of the rate and extent of bone resorption [Moonga et al., 2001].

In common with other cellular systems, refilling of Ca^{2+} stores following their release appears to depend upon a thapsigargin-sensitive Ca^{2+}-ATPase. Furthermore, such store depletion appears to induce a capacitative Ca^{2+} influx. Thus, the Ca^{2+}-ATPase inhibitor thapsigargin produced the expected elevation of cytosolic $[Ca^{2+}]$ in osteoclasts studied in Ca^{2+}-free extracellular solutions. Restoration of the extracellular $[Ca^{2+}]$ then produced a cytosolic $[Ca^{2+}]$ overshoot. Similar effects followed Ca^{2+} store depletion by ionomycin in cells bathed in EGTA-containing solutions when extracellular $[Ca^{2+}]$ was similarly restored. Both studies suggested capac-itative Ca^{2+} influx processes from the extracellular space by a cytosolic route [Shankar et al., 1994].

5.5. Functional Evidence for Ryanodine Receptor (RyR) Participation in Osteoclast Ca^{2+} Sensing

Increasing evidence implicates the ryanodine-receptor (RyR) in the transduction processes described here. Initial indications for this came from experiments that investigated the effect of perchlorate ions, known to facilitates skeletal muscle excitation-contraction coupling processes through acting on the RyR-Ca^{2+} release channel, on osteoclast function. Its intravenous infusion reduced plasma [Ca] in rats. Perchlorate reduced in vitro cortical bone resorption, induced transient cytosolic free $[Ca^{2+}]$ elevations, and marked and sustained cell

retraction in isolated cultured osteoclasts whilst conserving cell motility parameters or of supernatant concentrations of tartrate-resistant (osteoclastic) acid phosphatase [Moonga et al., 1991]. Conversely, the RyR-inhibitor dantrolene Na inhibits Ca^{2+}-induced cytosolic $[Ca^{2+}]$ elevations [Miyauchi et al., 1990]. Furthermore, ryanodine itself induced transient elevations of cytosolic $[Ca^{2+}]$ in fura 2-loaded osteoclasts to extents dependent upon surface membrane potential. Conversely the RyR agonist caffeine triggered releases of intracellularly stored Ca^{2+} through a bell-shaped concentration-response curve that varied with extracellular $[Ca^{2+}]$ whilst inhibiting Ni^{2+}-induced elevations in cytosolic $[Ca^{2+}]$ [Shankar et al., 1995b].

These physiological findings prompted labeling and fluorescence studies that went on more directly to implicate a RyR, or at the very least a RyR-like molecule, that uniquely existed in the cell surface as opposed to the microsomal membrane of the osteoclast, in the process by which changes in extracellular $[Ca^{2+}]$ become transduced into elevations of cytosolic $[Ca^{2+}]$. Certainly extracellular applications of the cell-impermeant RyR modulators ruthenium red and adenosine $3',5'$-cyclic diphosphate ribose (cADPr) both triggered elevations in cytosolic $[Ca^{2+}]$ that were sensitive to manipulations of the surface membrane voltage. Both modulators additionally attenuated cytosolic $[Ca^{2+}]$ responses to external Ni^{2+} applications [Adebanjo et al., 1996]. This correlated well with binding and microscopy studies that demonstrated that $[^{3}H]$-ryanodine specifically bound to freshly isolated rat osteoclasts but was then displaced by ryanodine itself, the CaR agonist Ni^{2+} and the RyR antagonist ruthenium red. Labelled ryanodine inhibited but antisera raised to an epitope located within the channel-forming domain of the type II RyR potentiated Ni^{2+}-induced cytosolic Ca^{2+} elevations. Serial confocal sections and immunogold scanning electron microscopy localized a staining to the plasma membrane staining by antiserum directed to a putatively intracellular RyR epitope only staining fixed, permeabilized cells in a distinctive cytoplasmic pattern [Zaidi et al., 1995].

Additionally, possible roles for cADPr in regulation of a surface RyR were suggested by demonstrations of cytosolic mRNA for the multifunctional ADP-ribosyl cyclase, CD38 which catalyzes NAD^{+} cyclization to cADPr, known in turn to gate Ca^{2+} release through microsomal membrane-resident ryanodine receptors (RyRs). Both confocal microscopy and Western blotting then localized the CD38 protein to the plasma membrane [Sun et al., 1999].

5.6. Is Ca^{2+} Sensing Coupled to Changes in Cytosolic $[Ca^{2+}]$?

A possible involvement of a unique cell surface-situated RyR by which extracellular $[Ca^{2+}]$ is transduced into changes in intracellularly stored $[Ca^{2+}]$ nevertheless leaves a number of mechanistic questions unresolved. It remains uncertain as to whether the RyR-2 also itself functions as the Ca^{2+} sensor, conceivably through its intraluminal low-affinity Ca^{2+} binding site [Anderson et al., 1989] or is coupled to a distinct intramembrane entity of the conventional 7-pass G-protein coupled types described elsewhere [Kameda et al., 1998]. The mechanism by which such a surface event would induce a release of intracellularly stored Ca^{2+} remains unclear. Finally, the role of such a unique surface membrane site in the osteoclast for the RyR in longer term osteoclast regulation certainly merits further exploration [Adebanjo et al., 1999; Gerasimenko et al., 1995; Santella & Carafoli, 1997].

6. FINAL THOUGHTS

Calcium is a unique cation in living systems because of it dominant role in intra-cellular signaling. Therefore bone cells which must handle massive amounts of this mineral take special care in its regulation. Sustained elevation of intracellular calcium leads to cell death that is not effectively opposed by the usual regulators of apoptosis. We have undertaken to present what is known about how bone cells deal with calcium. However much remains to be learned and the acquisition of this knowledge will inform our treatment of many important medical conditions.

REFERENCES

Accardi A, Walden M, Nguitragool W, Jayaram H, Williams C, Miller C. Separate Ion Pathways in a Cl$^-$/H$^+$ Exchanger. J Gen Physiol 2005;126:263–570. [PubMed: 16129773]

Abu-Amer Y, Ross FP, Schlesinger P, Tondravi MM, Teitelbaum SL. Substrate recognition by osteoclast precursors induces {C}-src/microtubule association. J. Cell Biol 1997;137:247–258. [PubMed: 9105052]

Adebanjo OA, Biswas G, Moonga BS, Anandatheerthavarada HK, Sun L, Bevis PJ, Sodam BR, Lai FA, Avadhani NG, Zaidi M. Novel biochemical and functional insights into nuclear Ca^{2+} transport through IP3Rs and RyRs in osteoblasts. Am J Physiol Renal Physiol 2000;278:F784–F791. [PubMed: 10807590]

Adebanjo OA, Anandathreethavarada HK, Koval AP, Moonga BS, Biswas G, Sun L, Sodam BR, Bevis PJR, Huang CL-H, Epstein S, Lai FA, Avadhani NG, Zaidi M. A new function for CD38/ADP-ribosyl cyclase in nuclear Ca^{2+} homeostasis. Nature Cell Biology 1999;7:409–414.

Adebanjo OA, Igietseme J, Huang CL-H, Zaidi M. The effect of extracellularly applied divalent cations on cytosolic Ca^{2+} in murine leydig cells: evidence for a Ca^{2+}-sensing receptor. Journal of Physiology 1998a;513:399–410. [PubMed: 9806991]

Adebanjo OA, Moonga BS, Haddad JG, Huang CL-H, Zaidi M. A possible new role for vitamin D-binding protein in osteoclast control. Inhibition of Ca^{2+} sensing at low physiological concentrations. Biochem Biophys Res Commun 1998b;249:668–671. [PubMed: 9731194]

Adebanjo OA, Moonga BS, Yamate T, Sun L, Minkin C, Abe E, Zaidi M. Mode of action of interleukin-6 on mature osteoclasts. Novel interactions with extracellular Ca^{2+} sensing in the regulation of osteoclastic bone resorption. J Cell Biol 1998c;142:1347–1356. [PubMed: 9732294]

Adebanjo OA, Shankar VS, Pazianas M, Simon BJ, Lai FA, Huang CL-H, Zaidi M. Extracellularly applied ruthenium red and cADP ribose elevate cytosolic Ca^{2+} in isolated rat osteoclasts. Am J Physiol 1996;270:F469–F475. [PubMed: 8780250]

Adebanjo OA, Shankar VS, Pazianas M, Zaidi A, Huang CL-H, Zaidi M. Modulation of the osteo-clast Ca^{2+} receptor by extracellular protons. Possible linkage between Ca^{2+} sensing and extracellular acidification. Biochem Biophys Res Commun 1994;194:742–747. [PubMed: 8135818]

Akisaka T, Yoshida H, Suzuki R. The ruffled border and attachment regions of the apposing membrane of resorbing osteoclasts as visualized from the cytoplasmic face of the membrane. J Electron Microsc (Tokyo) 2006;55:53–61. [PubMed: 16775216]

Anderson K, Lai F, Liu QY, Rousseau E, Erickson HP, Meissner G. Structural and functional characterization of the purified cardiac ryanodine receptor-Ca^{2+} release channel complex. J Biol Chem 1989;264:1329–1335. [PubMed: 2463249]

Armour KE, Armour KJ, Gallagher ME, Godecke A, Helfrich MH, Reid DM, Ralston SH. Defective bone formation and anabolic response to estrogen in mice with targeted disruption of eNOS. Endocrinology 2001;142:760–766. [PubMed: 11159848]

Ashley RH. Challenging accepted ion channel biology: p64 and the CLIC family of putative intracellular anion channel proteins. Mol Membr Biol 2003;20:1–11. [PubMed: 12745921]

Balmain N, Berdal A, Hotton D, Cuisinier-Gleizes P, Mathieu H. Calbindin-D9K immunolo- calization and vitamin D-dependence in the bone of growing and adult rats. Histochemistry 1989;92:359–365. [PubMed: 2511166]

Bax BE, Shankar VS, Bax CMR, Alam ASMT, Zara SJ, Pazianas M, Huang CL-H, Zaidi M. Functional consequences of the interaction of Ni^{2+} with the osteoclast Ca^{2+} receptor. Exper Physiol 1993;78:517–529. [PubMed: 8398105]

Bax CM, Shankar VS, Moonga BS, Huang CL-H, Zaidi M. Is the osteoclast calcium "receptor" a receptor-operated calcium channel? Biochem Biophys Res Commun 1992;183:619–625. [PubMed: 1312836]

Bekker PJ, Gay CV. Characterization of a Ca^{2+}-ATPase in osteoclast plasma membrane. J Bone Miner Res 1990;5:557–567. [PubMed: 2143347]

Bennett BD, Alvarez U, Hruska KA. Receptor-operated osteoclast calcium sensing. Endocrinology 2001;142:1968–1974. [PubMed: 11316762]

Bergh JJ, Shao Y, Puente E, Duncan RL, Farach-Carson MC. Osteoblast Ca^{2+} permeability and voltage-sensitive Ca^{2+} channel expression is temporally regulated by 1,25-dihydroxyvitamin D3. Am J Physiol 2006;290:C822–C831.

Berry KL, Bulow HE, Hall DH, Hobert O. A C. elegans CLIC-like Protein Required for Intracellular Tube Formation and Maintenance. Science 2003;302:2134–2137. [PubMed: 14684823]

Berryman M, Bruno J, Price J, Edwards JC. CLIC-5A functions as a chloride channel in vitro and associates with the cortical actin cytoskeleton in vitro and in vivo. J Biol Chem 2004;279:34794–34801. [PubMed: 15184393]

Bikle DD, Halloran BP. The response of bone to unloading. J Bone Miner Metab 1999;17:233–244. [PubMed: 10575587]

Blair HC, Borysenko CW, Villa A, Schlesinger PH, Kalla SE, Yaroslavskiy BB, Garcia-Palacios V, Oakley JI, Orchard PJ. In vitro differentiation of CD14 cells from osteopetrotic subjects: contrasting phenotypes with TCIRG1, CLCN7, and attachment defects. J Bone Miner Res 2004;19:1329–1338. [PubMed: 15231021]

Blair HC, Zaidi M, Schlesinger PH. Mechanisms Balancing Skeletal Matrix Synthesis and Degradation. Biochem J 2002;364:329–341. [PubMed: 12023876]

Blair HC, Schlesinger PH. Purification of a stilbene sensitive chloride channel and reconstitution of chloride conductivity into phsopholipid vesicles. Biochem Biophys Res Commun 1990;171:920–925. [PubMed: 1699531]

Blair HC, Teitelbaum SL, Ghiselli R, Gluck S. Osteoclastic bone resorption by a polarized vacuolar proton pump. Science 1989;245:855–857. [PubMed: 2528207]

Blair HC, Teitelbaum SL, Tan HL, Koziol CM, Schlesinger PH. Passive chloride permeability charge coupled to H^+-ATPase of avian osteoclast ruffled membrane. Am J Physiol 1991;260:C1315–C1324. [PubMed: 1829326]

Boutahar N, Guignandon A, Vico L, Lafage-Proust MH. Mechanical strain on osteoblasts activates autophosphorylation of focal adhesion kinase and proline-rich tyrosine kinase-2 tyrosine sites involved in ERK activation. J Biol Chem 2004;279:30588–30599. [PubMed: 15096502]

Bowler WB, Littlewood-Evans A, Bilbe G, Gallagher JA, Dixon CJ. P2Y2 receptors are expressed by human osteoclasts of giant cell tumor but do not mediate ATP-induced bone resorption. Bone 1998;22:195–200. [PubMed: 9514211]

Boyer PD. The ATP synthase-a splendid molecular machine. Annu Rev Biochem 1997;66:717–749. [PubMed: 9242922]

Bradford PG, Maglich JM, Kirkwood KL. IL-1β increases type 1 inositol trisphosphate receptor expression and IL-6 secretory capacity in osteoblastic cell cultures. Mol Cell Biol Res Commun 2000;3:73–75. [PubMed: 10775502]

Brandt S, Jentsch TJ. ClC-6 and ClC-7 are two novel broadly expressed members of the CLC chloride channel family. FEBS Lett 1995;377:15–20. [PubMed: 8543009]

Brown EM. Extracellular Ca^{2+} sensing, regulation of parathyroid cell function, and role of Ca^{2+} and other ions as extracellular (first) messengers. Physiol Rev 1991;71:371–411. [PubMed: 2006218]

Brown EM, Gamba G, Riccardi ID, Lombardi M, Butters R, Kifor O, Sun A, Hediger MA, Lytton J, Hebert SC. Cloning and characterization of an extracellular calciumsensingreceptor from bovine parathyroid. Nature 1993;366:575–579. [PubMed: 8255296]

Bugel S. Vitamin k and bone health. Proc. Nutri.Soc 2005;62:839–843.

Bushinsky D. Acid-base imbalance and the skeleton. Eur J Nutr 2001;40:238–244. [PubMed: 11842949]

Carano A, Schlesinger PH, Athanasou NA, Teitelbaum SL, Blair HC. Acid and base effects on avian osteoclast activity. Am. J. Physiol 1993;264:C694–C701. [PubMed: 8460672]

Carn G, Koller DL, Peacock M, Hui SL, Evans WE, Conneally PM, Johnston CC Jr, Foroud T, Econs MJ. Sibling pair linkage and association studies between peak bone mineral density and the gene locus for the osteoclast-specific subunit of the vacuolar proton pumpon chromosome 11p12-13. J Clin Endocrinol Metab 2002;87:3819–3824. [PubMed: 12161516]

Chen X, Macica CM, Ng KW, Broadus AE. Stretch-induced PTH-related protein gene expression in osteoblasts. J Bone Miner Res 2005;20:1454–1461. [PubMed: 16007342]

Civitelli R, Beyer EC, Warlow PM, Robertson AJ, Geist ST, Steinberg TH. Connexin43 mediates direct intercellular communication in human osteoblastic cell networks. J. Clin. Invest 1993;91:1888–1896. [PubMed: 8387535]

Cleiren E, Benichou O, Van Hul E, Gram J, Bollerslev J, Singer FR, Beaverson K, Aledo A, Whyte MP, Yoneyama T, deVernejoul MC, Van Hul W. Albers-Schonberg disease (autosomal dominant osteopetrosis, type II) results from mutations in the ClCN7 chloride channel gene. Hum Mol Genet 2001;10:2861–2867. [PubMed: 11741829]

Cretin A. Contribution histochimique a etude de la construction et de la destruction osseuse. La Presse Medicale 1951;59:1240–1242. [PubMed: 14875726]

Danciu TE, Adam RM, Naruse K, Freeman MR, Hauschka PV. Calcium regulates the PI3K–Akt pathway in stretched osteoblasts. FEBS Lett 2003;536:193–197. [PubMed: 12586362]

Datta HK, MacIntyre I, Zaidi M. The effect of extracellular calcium elevation on morphology and function of isolated rat osteoclasts. Bioscience Reports 1989;9:747–751. [PubMed: 2692724]

Datta HK, MacIntyre I, Zaidi M. Intracellular calcium in the control of osteoclast function. I. Voltage-insensitivity and lack of effects of nifedipine, BAYK8644 and diltiazem. Biochem Biophys Res Commun 1990;167:183–188. [PubMed: 1690001]

Davidson RM. Membrane stretch activates a high-conductance K+ channel in G292 osteoblastic-like cells. J Membr Biol 1993;131:81–92. [PubMed: 8433353]

Davies GHE, Madesh M. Calcium signaling and apoptosis. Biochem Biophys Res Commun 2004;304:445–454. [PubMed: 12729578]

Deakins M, Burt RL. The deposition of calcium, phosphorus, and carbon dioxide in calcifying dental enamel. J Biol Chem 2002;156:77.

Diewald L, Rupp J, Dreger M, Hucho F, Gillen C, Nawrath H. Activation by acidic pH of CLC-7 expressed in oocytes from Xenopus laevis. Biochem Biophys Res Commun 2002;291:421–424. [PubMed: 11846422]

Duncan R, Misler S. Voltage-activated and stretch-activated Ba2+ conducting channels in an osteoblast-like cell line (UMR 106). FEBS Lett 1989;251:17–21. [PubMed: 2473926]

Eberhardt AW, Yeager-Jones A, Blair HC. Regional trabecular bone matrix degeneration and osteocyte death in femora of glucocorticoid- treated rabbits. Endocrinology 2001;142:1333–1340. [PubMed: 11181552]

Edwards JC, Cohen C, Xu W, Schlesinger PH. c-Src Control of Chloride Channel Support for Osteoclast HCl Transport and Bone Resorption. J Biol Chem 2006;281:28011–28022. [PubMed: 16831863]

Elsegood CL, Zhuo Y, Wesolowski GA, Hamilton JA, Rodan GA, Duong LT. M-CSF induces the stable interaction of cFms with $\alpha V\beta 3$ integrin in osteoclasts. Int J Biochem Cell Biol 2006;38:1518–1529. [PubMed: 16600665]

Eu JP, Xu L, Stamler JS, Meissner G. Regulation of ryanodine receptors by reactive nitrogen species. Biochem Pharmacol 1999;57:1079–1084. [PubMed: 11230795]

Falsafi R, Tatakis DN, Hagel-Bradway S, Dziak R. Effects of inositol trisphosphate on calcium mobilization in bone cells. Calcif Tissue Int 1991;49:333–339. [PubMed: 1782574]

Finbow ME, Harrison MA. The vacuolar H^+-ATPase: a universal proton pump of eukaryotes. Biochem J 1997;324:697–712. [PubMed: 9210392]

Gama L, Baxendale-Cox LM, Breitwieser GE. Ca^{2+}-sensing receptors in intestinal epithelium. Am J Physiol 1997;273:C1168–C1175. [PubMed: 9357760]

Gay CV, Lloyd QP. Characterization of calcium efflux by osteoblasts derived from long bone periosteum. Comp Biochem Physiol A Physiol 1995;111:257–261. [PubMed: 7669110]

Gerasimenko OV, Gerasimenko JV, Tepikin AV, Petersen OH. ATP-dependent accumulation and inositol trisphosphate- or cyclic ADP-ribose-mediated release of Ca^{2+} from the nuclear envelope. Cell 1995;80:439–444. [PubMed: 7859285]

Gofa A, Davidson RM. NaF potentiates a K+-selective ion channel in G292 osteoblastic cells. J Membr Biol 1996;149:211–219. [PubMed: 8801353]

Golden LH, Insogna KL. The expanding role of PI3-kinase in bone. Bone 2004;34:3–12. [PubMed: 14751558]

Grabe M, Oster G. Regulation of Organelle Acidity. J. Gen. Physiol 2001;117:329–343. [PubMed: 11279253]

Gu Y, Preston MR, Magnay J, El Haj AJ, Publicover SJ. Hormonally-regulated expression of voltage-operated Ca^{2+} channels in osteocytic (MLO-Y4) cells. Biochem Biophys Res Commun 2001;282:536–542. [PubMed: 11401493]

Hattori T, Maehashi H, Miyazawa T, Naito M. Potentiation by stannous chloride of calcium entry into osteoblastic MC3T3-E1 cells through voltage-dependent L-type calcium channels. Cell Calcium 2001;30:67–72. [PubMed: 11396989]

Heiss NS, Poustka A. Genomic structure of a novel chloride channel gene, CLIC2, in Xq28. Genomics 1997;45:224–228. [PubMed: 9339381]

Henriksen Z, Hiken JF, Steinberg TH, Jorgensen NR. The predominant mechanism of intercellular calcium wave propagation changes during long-term culture of human osteoblast-like cells. Cell Calcium 2006;39:435–444. [PubMed: 16545868]

Hessle L, Johnson KA, Anderson HC, Narisawa S, Sali A, Goding JW, Terkeltaub R, Millan JL. Tissue-nonspecific alkaline phosphatase and plasma cell membrane glycoprotein-1 are central antagonistic regulators of bone mineralization. Proc Natl Acad Sci USA 2002;99:9445–9449. [PubMed: 12082181]

Hirota J, Baba M, Matsumoto M, Furuichi T, Takatsu K, Mikoshiba K. T-cell-receptor signalling in inositol 1,4,5-trisphosphate receptor (IP3R) type-1-deficient mice: is IP3R type 1 essential for T-cell-receptor signalling? Biochem J 1998;333:615–619. [PubMed: 9677320]

Ho AM, Johnson MD, Kingsley DM. Role of the mouse ank gene in control of tissue calcification and arthritis. Science 2000;289:265–270. [PubMed: 10894769]

Hoebertz A, Arnett TR, Burnstock G. Regulation of bone resorption and formation by purines and pyrimidines. TRENDS in Pharm. Sci 2003;24:290–297. [PubMed: 12823955]

Huang MS, Adebanjo OA, Awumey E, Biswas G, Koval A, Sodam BR, Sun L, Moonga BS, Epstein J, Goldstein S, Lai FA, Lipschitz D, Zaidi M. IP(3), IP(3) receptor, and cellular senescence. Am J Physiol 2000;278:F576–F584.

Hughes S, Magnay J, Foreman M, Publicover SJ, Dobson JP, El Haj AJ. Expression of the mechanosensitive $2PK^{+}$ channel TREK-1 in human osteoblasts. J Cell Physiol 2006;206:738–748. [PubMed: 16250016]

Hughes-Fulford M. Signal transduction and mechanical stress. Sci STKE 2004;2004:RE12. [PubMed: 15353762]

Hurle B, Ignatova E, Massironi SM, Mashimo T, Rios X, Thalmann I, Thalman R, Ornitz DM. Non-syndromic vestibular disorder with otoconial agenesis in Tilted/Mergulhador mice caused by mutations in otopetrin 1. Human Molecular Genetics 2003;12:777–789. [PubMed: 12651873]

Hurle B, Lane K, Kenney J, Tarantino LM, Bucan M, Brownstein BH, Ornitz M. Physical mapping of the mouse tilted locus identifies an association between human deafness loci dfna6/14 and vestibular system development. Genomics 2001;77:189–199. [PubMed: 11597144]

Iqbal J, Kumar K, Sun L, Zaidi M. Selective Upregulation of the ADPribosyl-cyclases CD38 and CD157 by TNF but not by RANK-L Reveals Differences in Downstream Signaling. Am J Physiol Renal Physiol 2006;291:F557–F566. [PubMed: 16705149]

Jentsch TJ, Poet M, Fuhrmann JC, A A, Zdebik AA. Physiological Functions of ClC Cl Channels Gleaned from Human Genetic Disease and Mouse Models. Ann Rev Physiol 2005;67:779–807. [PubMed: 15709978]

Jorgensen NR, Teilmann SC, Henriksen Z, Civitelli R, Sorensen OH, Steinberg TH. Activation of L-type calcium channels is required for gap junction-mediated intercellular calcium signaling in osteoblastic cells. J Biol Chem 2003;278:4082–4086. [PubMed: 12446698]

Kameda T, Mano H, Yamada Y, Takai H, Amizuka N, Kobori M, Izumi N, Kawashima H, Ozawa H, Ikeda K, Kameda A, Hakeda Y, Kumegawa M. Calcium-sensing receptor in mature osteoclasts, which are bone resorbing cells. Biochem Biophys Res Commun 1998;245:419–422. [PubMed: 9571166]

Kamioka H, Sugawara Y, Murshid SA, Ishihara Y, Honjo T, Takano-Yamamoto T. Fluid shear stress induces less calcium response in a single primary osteocyte than in a single osteoblast: implication of different focal adhesion formation. J Bone Miner Res 2006;21:1012–1021. [PubMed: 16813522]

Kasper D, Planells-Cases R, Fuhrmann JC, Scheel O, Zeitz O, Ruether K, Schmitt A, Poe M, Steinfeld R, Schweizer M, Kornak U, Jentsch TJ. Loss of the chloridechannel ClC-7 leads to lysosomal storage disease and neurodegeneration. EMBO J 2005;24:1079–1091. [PubMed: 15706348]

Kirkwood KL, Homick K, Dragon MB, Bradford PG. Cloning and characterization of the type I inositol 1,4,5-trisphosphate receptor gene promoter. Regulation by 17β-estradiol in osteoblasts. J Biol Chem 1997;272:22425–22431. [PubMed: 9278393]

Kizer N, Guo XL, Hruska K. Reconstitution of stretch-activatedcation channels by expression of the alpha-subunit of the epithelial sodium channel cloned from osteoblasts. Proc Natl Acad Sci USA 1997;94:1013–1018. [PubMed: 9023374]

Kornak U, Kasper D, Bosl MR, Kaiser E, Schweizer M, Schulz A, Friedrich W, Delling G, Jentsch TJ. Loss of the ClC-7 chloride channel leads to osteopetrosis in mice and man. Cell 2001;104:205–215. [PubMed: 11207362]

Kos CH, Karaplis AC, Peng JB, Hediger MA, Goltzman D, Mohammad KS, Guise TA, Pollak MR. The calcium-sensing receptor is required for normal calcium homeostasis independent of parathyroid hormone. J Clin Invest 2003;111:1021–1028. [PubMed: 12671051]

Lakkakorpi PT, Lehenkari PP, Rautiala TJ, Vaananen HK. Different calcium sensitivity in osteoclasts on glass and on bone and maintenance of cytoskeletal structures on bone in the presence of high extracellular calcium. J Cell Physiol 1996;168:668–677. [PubMed: 8816921]

Lee HS, Millward-Sadler SJ, Wright MO, Nuki G, Salter DM. Integrin and mechanosensitive ion channel-dependent tyrosine phosphorylation of focal adhesion proteins and beta-catenin in human articular chondrocytes after mechanical stimulation. J Bone Miner Res 2000;15:1501–1509. [PubMed: 10934648]

Li W, Duncan RL, Karin NJ, Farach-Carson MC. 1,25 $(OH)_2D3$ enhances PTH-induced Ca^{2+} transients in preosteoblasts by activating L-type Ca^{2+} channels. Am J Physiol 1997;273:E599–E605. [PubMed: 9316451]

Li YP, Chen W, Liang Y, Li E, Stashenko P. Atp6i–deficient mice exhibit severe osteopetrosis due to loss of osteoclast-mediated extracellular acidification. Nat Genet 1999;23:447–451. [PubMed: 10581033]

Lotz M, Rosen F, McCabe G, Quach J, Blanco F, Dudler J, Solan J, Goding J, Seegmiller JE, Terkeltaub R. Interleukin 1 beta suppresses transforming growth factor-induced inorganic pyrophosphate (PPi) production and expression of the PPi-generating enzyme PC-1 in human chondrocytes. Proc Natl Acad Sci USA 1995;92:10364–10368. [PubMed: 7479785]

Lu Z, Jiang YP, Ballou LM, Cohen IS, Lin RZ. Gα q inhibits cardiac L-type Ca^{2+} channels through phosphatidylinositol 3-kinase. J Biol Chem 2005 2005;280:40347–40354.

Lundgren S, Hjalm G, Hellman P, Ek B, Juhlin C, Rastad J, Klareskog L, Akerstrom G, Rask L. A protein involved in calcium sensing of the human parathyroid and placental cytotrophoblast cells belongs to the LDL-receptor protein superfamily. Exper Cell Res 1994;212:344–350. [PubMed: 8187828]

MacKenna DA, Dolfi F, Vuori K, Ruoslahti E. Extracellular signal-regulated kinase and c-Jun NH2-terminal kinase activation by mechanical stretch is integrin-dependent and matrix-specific in rat cardiac fibroblasts. J Clin Invest 1998;101:301–310. [PubMed: 9435301]

Malgaroli A, Meldolesi J, Zallone AZ, Teti A. Control of cytosolic free calcium in rat and chicken osteoclasts. The role of extracellular calcium and calcitonin. J Biol Chem 1989;264:14342–14347. [PubMed: 2547794]

Marcantoni A, Levi RC, Gallo MP, Hirsch E, Alloatti G. Phosphoinositide 3-kinaseγ controls L-type calcium current through its positive modulation of type-3 phosphodiesterase. J Cell Physiol 2006;206:329–336. [PubMed: 16110482]

Mattsson JP, Li X, Peng S-B, Nilsson F, Adersen P, Lundberg LG, Stone DK, Keeling DJ. Properties of three isoforms of the 116-kDa Subunit of vacuolar H^+-ATPase from a single vertebrate species. Cloning, gene expression and protein characterization of functionally distinct isoforms in *Gallus domesticus*. Euro J Bioch 2000;267:4115–4126.

Miyauchi A, Alvarez J, Greenfield EM, Teti A, Grano M, Colucci S, Zambonin-Zallone A, Ross FP, Teitelbaum SL, Cheresh D. Recognition of osteopontin and related peptides by an alpha v beta 3 integrin stimulates immediate cell signals in osteoclasts. J Biol Chem 1991;266:20369–20374. [PubMed: 1939092]

Miyauchi A, Hruska KA, Greenfield EM, Duncan R, Alvarez J, Barattolo R, Colucci S, Zambonin-Zallone A, Teitelbaum SL, Teti A. Osteoclast cytosolic calcium, regulated by voltage-gated calcium channels and extracellular calcium, controls podosome assembly and bone resorption. J Cell Biol 1990;111:2543–2552. [PubMed: 1703539]

Moonga BS, Li S, Iqbal J, Davidson R, Shankar VS, Bevis PJ, Inzerillo A, Abe E, Huang CL, Zaidi M. Ca^{2+} influx through the osteoclastic plasma membrane ryanodine receptor. Am J Physiol Renal Physiol 2002;282:F921–F932. [PubMed: 11934703]

Moonga BS, Datta HK, Bevis PJR, Huang C-H, MacIntyre I, Zaidi M. Correlates of osteoclast function in the presence of perchlorate ions in the rat. Exper Physiol 1991;76:923–933. [PubMed: 1662966]

Moonga BS, Davidson R, Sun L, Adebanjo OA, Moser J, Abedin M, Zaidi N, Huang CL-H, Zaidi M. Identification and characterization of a sodium/calcium exchanger, NCX-1, in osteoclasts and its role in bone resorption. Biochem Biophys Res Commun 2001;283:770–775. [PubMed: 11350050]

Moonga BS, Moss DW, Patchell A, Zaidi M. Intracellular regulation of enzyme secretion from rat osteoclasts and evidence for a functional role in bone resorption. J Physiol 1990;429:29–45. [PubMed: 2277349]

Morrison MS, Turin L, King BF, Burnstock G, Arnett TR. ATP is a potent stimulator of the activation and formation of rodent osteoclasts. J Physiol 1998;511:495–500. [PubMed: 9706025]

Nesbitt SA, Horton MA. Trafficking of matrix collagens through bone-resorbing osteoclasts. Science 1997;27:266–269. [PubMed: 9092478]

Neuman, WF.; Neuman, MW. The chemical dynamics of bone mineral. Chicago: University of Chicago Press; 1958.

Nishi T, Forgac M. The vacuolar H^+-ATPases - Natures most versitile proton pumps. Nature Rev 2002;3:94–102.

Nishiya Y, Kosaka N, Uchii M, Sugimoto S. A potent 1,4-dihydropyridine L-type calcium channel blocker, benidipine, promotes osteoblast differentiation. Calcif Tissue Int 2002;70:30–39. [PubMed: 11907705]

Nomura S, Takano-Yamamoto T. Molecular events caused by mechanical stress in bone. Matrix Biol 2000;19:91–96. [PubMed: 10842092]

Nurnberg P, Thiele H, Chandler D, Hohne W, Cunningham ML, Ritter H, Leschik G, Uhlmann K, Mischung C, Harrop K, Goldblatt J, Borochowitz ZU, Kotzot D, Westermann F, Mundlos S, Braun HS, Laing N, Tinschert S. Heterozygous mutations in ANKH, the human ortholog of the mouse progressive ankylosis gene, result in craniometaphyseal dysplasia. Nat Genet 2001;28:37–41. [PubMed: 11326272]

Pazianas M, Adebanjo OA, Shankar VS, James SY, Colston KW, Maxwell JD, Zaidi M. Extracellular cation sensing by the enterocyte. Prediction of a novel divalent cation receptor. Biochem Biophys Res Comm 1995;210:948–953. [PubMed: 7763267]

Pazianas M, Zaidi M, Huang CL-H, Moonga BS, Shankar VS. Voltage-sensitivity of the osteoclast calcium receptor. Biochem Biophys Res Commun 1993;192:1100–1105. [PubMed: 8507183]

Pendleton A, Johnson MD, Hughes A, Gurley KA, Ho AM, Doherty M, Dixey J, Gillet P, Loeuille D, McGrath R, Reginato A, Shiang R, Wright G, Netter P, Williams C, Kingsley DM. Mutations in ANKH cause chondrocalcinosis. Am J Hum Genet 2002;71:933–940. [PubMed: 12297987]

Pi M, Faber P, Ekema G, Jackson PD, Ting A, Wang N, Fontilla-Poole M, Mays RW, Brunden KR, Harrington JJ, Quarles LD. Identification of a novel extracellular cation-sensing G-protein-coupled receptor. J Biol Chem 2005;280:40201–40209. [PubMed: 16199532]

Picollo A, Pusch M. Chloride/proton antiporter activity of mammalian CLC proteins ClC-4 and ClC-5. Nature 2005;436:420–423. [PubMed: 16034421]

Piper RC, Luzio JP. CUPpling calcium to lysosomal biogenesis. TRENDS in Cell Biol 2004;14:471–473. [PubMed: 15350973]

Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143–147. [PubMed: 10102814]

Quinn SJ, Ye CP, Diaz R, Kifor O, Bai M, Vassilev P, Brown E. The Ca^{2+}-sensing receptor: a target for polyamines. Am J Physiol 1997;273:C1315–C1323. [PubMed: 9357776]

Radding W, Jordan SE, Hester RB, Blair HC. Intracellular calcium puffs in osteoclasts. Exp Cell Res 1999;253:689–696. [PubMed: 10585292]

Reichenberger E, Tiziani V, Watanabe S, Park L, Ueki Y, Santanna C, Baur ST, Shiang R, Grange DK, Beighton P, Gardner J, Hamersma H, Sellars S, Ramesar R, Lidral AC, Sommer A, Raposo do Amaral CM, Gorlin RJ, Mulliken JB, Olsen BR. Autosomal dominant craniometaphyseal dysplasia is caused by mutations in the transmembrane protein ANK. Am J Hum Genet 2001;68:1321–1326. [PubMed: 11326338]

Renkema KY, Nijenhuis T, van der Eerden BC, van der Kemp AW, Weinans H, van Leeuwen JP, Bindels RJ, Hoenderop JG. Hypervitaminosis D mediates compensatory Ca^{2+} hyperabsorption in TRPV5 knockout mice. J Am Soc Nephrol 2005;16:3188–3195. [PubMed: 16148038]

Rezzonico R, Cayatte C, Bourget-Ponzio I, Romey G, Belhacene N, Loubat A, Rocchi S, Van Obberghen E, Girault JA, Rossi B, Schmid-Antomarchi H. Focal adhesion kinase pp125FAK interacts with the large conductance calcium-activated hSlo potassium channel in human osteoblasts: potential role in mechanotransduction. J Bone Miner Res 2003;18:1863–1871. [PubMed: 14584897]

Riccardi D, Park J, Lee WS, Gamba G, Brown EM, Hebert SC. Cloning and functional expression of a rat kidney extracellular calcium/polyvalent cation-sensing receptor. Proc Natl Acad Sci USA 1995;92:131–135. [PubMed: 7816802]

Riddle RC, Taylor AF, Genetos DC, Donahue HJ. MAP kinase and calcium signaling mediate fluid flow-induced human mesenchymal stem cell proliferation. Am J Physiol Cell Physiol 2006;290:C776–C784. [PubMed: 16267109]

Romanello M, Padoan M, Franco L, Veronesi V, Moro L, D'Andrea P. Extracellular NAD^+ induces calcium signaling and apoptosis in human osteoblastic cells. Biochem Biophys Res Commun 2001;285:1226–1231. [PubMed: 11478787]

Ruwhof C, van der Laarse A. Mechanical stress-induced cardiac hypertrophy: mechanisms and signal transduction pathways. Cardiovasc Res 2000;47:23–37. [PubMed: 10869527]

Ryder KD, Duncan RL. Parathyroid hormone enhances fluid shear-induced $[Ca^{2+}]i$ signaling in osteoblastic cells through activation of mechanosensitive and voltage-sensitive Ca^{2+} channels. J Bone Miner Res 2001;16:240–248. [PubMed: 11204424]

Sadoshima J, Takahashi T, Jahn L, Izumo S. Roles of mechano-sensitive ion channels, cytoskeleton, and contractile activity in stretch-induced immediate-early gene expression and hypertrophy of cardiac myocytes. Proc Natl Acad Sci USA 1992;89:9905–9909. [PubMed: 1384064]

Salo J, Lehenkari P, Metsikko K, Vanananen HK. Removal of osteoclast bone resorption products by transcytosis. Science 1997;276:270–273. [PubMed: 9092479]

Santella L, Carafoli E. Calcium signaling in the cell nucleus. FASEB J 1997;11:1091–2109. [PubMed: 9367344]

Scheel O, Zdebik AA, Lourdel S, Jentsch TJ. Voltage-dependent electrogenic chloride/proton exchange by endosomal CLC proteins. Nature 2005;436:424–427. [PubMed: 16034422]

Schlesinger PH, Blair HC, Teitelbaum SL, Edwards JC. Characterization of the osteoclast ruffled border chloride channel and its role in bone resorption. J Biol Chem 1997;272:18636–18643. [PubMed: 9228032]

Schlesinger PH, Mattsson JP, Blair HC. Osteoclastic Acid Transport: Mechanism and Implications for Physiology and Pharmacological Regulation. Mineral Eletrolyte Metab 1994;20:31–39.

Seisenberger C, Specht V, Welling A, Platzer J, Pfeifer A, Kuhbandner S, Striessnig J, Klugbauer N, Feil R, Hofmann F. Functional embryonic cardiomyocytes after disruption of the L-type alpha1C (Cav1.2) calcium channel gene in the mouse. J. Biol. Chem 2000;275:39193–39199. [PubMed: 10973973]

Shankar VS, Alam AS, Bax CM, Bax BE, Pazianas M, Huang CL-H, Zaidi M. Activation and inactivation of the osteoclast Ca^{2+} receptor by the trivalent cation, La^{3+} Biochem Biophys Res Commun 1992a; 187:907–912. [PubMed: 1530645]

Shankar VS, Bax CMR, Bax BE, Alam ASMT, Simon B, Pazianas M, Moonga BS, Huang CL-H, Zaidi M. Activation of the Ca^{2+} receptor on the osteoclast by Ni^{2+} elicits cytosolic Ca^{2+} signals: Evidence for receptor activation and inactivation, intracellular Ca^{2+} redistribution and divalent cation modulation. J Cell Physiol 1993;155:120–129. [PubMed: 8385675]

Shankar VS, Bax CM, Alam A, Bax BE, Huang CL-H, Zaidi M. The osteoclast Ca^{2+} receptor is highly sensitive to activation by transition metal cations. Biochem Biophys Res Commun 1992b;187:913–918. [PubMed: 1530646]

Shankar VS, Huang CL-H, Adebanjo OA, Pazianas M, Zaidi M. Calcium influx and release in isolated rat osteoclasts. Exper Physiol 1994;79:537–545. [PubMed: 7946282]

Shankar VS, Huang CL-H, Adebanjo OA, Simon BJ, Alam ASMT, Moonga BS, Pazianas M, Scott RH, Zaidi M. The effect of membrane potential on surface Ca^{2+} receptor activation in rat osteoclasts. J Cell Physiol 1995;162:1–8. [PubMed: 7814441]

Shankar VS, Pazianas M, Huang CL-H, Simon B, Adebanjo OA, Zaidi M. Caffeine modulates Ca^{2+} receptor activation in isolated rat osteoclasts and induces intracellular Ca^{2+} release. Am J Physiol 1995;268:F447–F454. [PubMed: 7900844]

Silver IA, Murrills RJ, Etherington DJ. Microelectrode studies on the acid microenvironment beneath adherent macrophages and osteoclasts. Exper Cell Res 1988;175:266–276. [PubMed: 3360056]

Soriano P, Montgomery C, Geske R, Bradley A. Targeted disruption of the c-src proto-oncogene leads to osteopetrosis in mice. Cell 1991;64:693–702. [PubMed: 1997203]

Splawski I, Timothy KW, Decher N, Kumar P, Sachse FB, Beggs AH, Sanguinetti MC, Keating MT. Severe arrhythmia disorder caused by cardiac L-type calcium channel mutations. Proc Natl Acad Sci USA 2005;102:8089–8096. [PubMed: 15863612]

Stains JP, Weber JA, Gay CV. Expression of Na+/Ca^{2+} exchanger isoforms (NCX1 and NCX3) and plasma membrane Ca^{2+}-ATPase during osteoblast differentiation. J Cell Biochem 2002;84:625–635. [PubMed: 11813267]

Sun L, Adebanjo OA, Koval A, Anandatheerthavarada HK, Iqbal J, Wu XY, Moonga BS, Wu XB, Biswas G, Bevis PJ, Kumegawa M, Epstein S, Huang CL, Avadhani NG, Abe E, Zaidi M. A novel mechanism for coupling cellular intermediary metabolism to cytosolic Ca^{2+} signaling via CD38/ADP-ribosyl cyclase, a putative intracellular NAD+ sensor. FASEB J 2002;16:302–314. [PubMed: 11874980]

Sun L, Iqbal J, Dolgilevich S, Yuen T, Wu XB, Moonga BS, Adebanjo OA, Bevis PJ, Lund F, Huang CL, Blair HC, Abe E, Zaidi M. Disordered osteoclast formation and function in a CD38 (ADP-ribosyl cyclase)-deficient mouse establishes an essential role for CD38 in bone resorption. FASEB J 2003;17:369–375. [PubMed: 12631576]

Sun L, Adebanjo OA, Moonga BS, Corisdeo S, Anandatheerthavarada HK, Biswas G, Arakawa T, Hakeda Y, Koval A, Sodam B, Bevis PJ, Moser AJ, Lai FA, Epstein S, Troen BR, Kumegawa M, Zaidi M. CD38/ADP-ribosyl cyclase: a new role in the regulation of osteoclastic bone resorption. J Cell Biology 1999;146:1161–1171.

Suzuki H, Ikeda K, Furukawa M, Takasaka T. P2 purinoceptor of the globular substance in the otoconial membrane of the guinea pig inner ear. Am J Physiol 1997;273:C1533–C1540. [PubMed: 9374638]

Tehrani S, Faccio R, Ross FP, Cooper JA. Cortactin has an essential and highly specific role in osteoclast actin assembly. Molec. Biol. Cell. 2006 epub 2006.

Teti A, Grano M, Colucci S, Argentino L, Barattolo R, Miyauchi A, Teitelbaum SL, Hruska KA, Zambonin Zallone A. Voltage dependent calcium channel expression in isolated osteoclasts. Boll Soc Ital Biol Sper 1989;65:1115–1118. [PubMed: 2560636]

Turnbull CI, Looi K, Mangum JE, Meyer M, Sayer RJ, Hubbard MJ. Calbindin independence of calcium transport in developing teeth contradicts the calcium ferry dogma. J Biol Chem 2004;279:55850–55854. [PubMed: 15494408]

van der Eerden BC, Hoenderop JG, de Vries TJ, Schoenmaker T, Buurman CJ, Uitterlinden AG, Pols HA, Bindels RJ, van Leeuwen JP. The epithelial Ca^{2+} channel TRPV5 is essential for proper osteoclastic bone resorption. Proc Natl Acad Sci USA 2005;102:17507–17512. [PubMed: 16291808]

Vaananen HK, Zhao H, Halleen JH. The cell biology of osteoclast function. J Cell Sci 2000;113:377–381. [PubMed: 10639325]

Watanabe S, Fukumoto S, Chang H, Takeuchi Y, Hasegawa Y, Okazaki R, Chikatsu N, Fujita T. Association between activating mutations of calcium-sensing receptor and Bartter's syndrome. Lancet 2002;360:692–694. [PubMed: 12241879]

Weber P. Vitamin K and bone health. Nutrition 2001;17:880–887. [PubMed: 11684396]

Whyte MP, Landt M, Ryan LM, Mulivor RA, Henthorn PS, Fedde KN, Mahuren JD, Coburn SP. Alkaline phosphatase: placental and tissue-nonspecific isoenzymes hydrolyze phosphoethanolamine, inorganic pyrophosphate, and pyridoxal 5'-phosphate. Substrate accumulation in carriers of hypophos- phatasia corrects during pregnancy. J Clin Invest 1995;95:1440–1445. [PubMed: 7706447]

Wiebe SH, Sims SM, Dixon SJ. Calcium signalling via multiple P2 purinoceptor subtypes in rat osteoclasts. Cell Physiol Biochem 1999;9:323–337. [PubMed: 10749998]

Williams CJ, Zhang Y, Timms A, Bonavita G, Caeiro F, Broxholme J, Cuthbertson J, Jones Y, Marchegiani R, Reginato A, Russell RG, Wordsworth BP, Carr AJ, Brown MA. Autosomal dominant familial calcium pyrophosphate dihydrate deposition disease is caused by mutation in the transmembrane protein ANKH. Am J Hum Genet 2002;71:985–991. [PubMed: 12297989]

Wiltink A, Van Duijn B, Weidema AF, De Vos A, van der Meer JM, Nijweide PJ, Ypey DL. Differential depolarization-activated calcium responses in fetal and neonatal rat osteoblast-like cells. Calcif Tissue Int 1994;54:278–283. [PubMed: 8062143]

Xia SL, Ferrier J. Propagation of a calcium pulse between osteoblastic cells. Biochem Biophys Res Commun 1992;186:1212–1219. [PubMed: 1510656]

Xia SL, Ferrier J. Localized calcium signaling in multinucleated osteoclasts. J Cell Physiol 1996;167:148–155. [PubMed: 8698832]

Xu M, Welling A, Paparisto S, Hofmann F, Klugbauer N. Enhanced expression of L-type Cav1.3 calcium channels in murine embryonic hearts from Cav1.2-deficient mice. J Biol Chem 2003;278:40837–40841. [PubMed: 12900400]

Yaroslavskiy BB, Zhang Y, Kalla SE, Garcia Palacios V, Sharrow AC, Li Y, Zaidi M, Wu C, Blair HC. NO-dependent osteoclast motility: reliance on cGMP-dependent protein kinase I and VASP. J Cell Science 2005;118:5479–5487. [PubMed: 16291726]

Young MF. Bone matrix proteins: their function, regulation, and relationship to osteoporosis. Osteoporos Int 2003;3(14 Suppl):S35–S42. [PubMed: 12730768]

Ypey DL, Weidema AF, Hold KM, Van der Laarse A, Ravesloot JH, Van Der Plas A, Nijweide PJ. Voltage, calcium, and stretch activated ionic channels and intracellular calcium in bone cells. J Bone Miner Res 1992;2(7 Suppl.):S377–S387. [PubMed: 1283043]

Zahanich I, Graf EM, Heubach JF, Hempel U, Boxberger S, Ravens U. Molecular and functional expression of voltage-operated calcium channels during osteogenic differentiation of human mesenchymal stem cells. J Bone Miner Res 2005;20:1637–1646. [PubMed: 16059635]

Zaidi M. "Calcium receptors" on eukaryotic cells with special reference to the osteoclast. Biosci Rep 1990;10:493–507. [PubMed: 1964812]

Zaidi M, Datta HK, Patchell A, Moonga B, MacIntyre I. 'Calcium-activated' intracellular calcium elevation: A novel mechanism of osteoclast regulation. Biochem Biophys Res Commun 1989;163:1461–1465. [PubMed: 2783143]

Zaidi M, Huang ASMT, Shankar VS, Pazianas M, Bax BE, Bax CMR, Moonga BS, Bevis PJR, Stevens CR, Blake DR, Huang CL-H. Cellular biology of bone resorption. Biological Reviews of the Cambridge Philosophical Society 1993;68:197–264. [PubMed: 8504194]

Zaidi M, Kerby J, Huang C-H, Alam ASMT, Rathod H, Chambers TJ, Moonga BS. Divalent cations mimic the inhibitory effects of extracellular ionized calcium on bone resorption by isolated rat osteoclasts: Further evidence for a "calcium receptor". J Cell Physiol 1991;149:422–427. [PubMed: 1660481]

Zaidi M, MacIntyre I, Datta H. Intracellular calcium in the control of osteoclast function. II. Paradoxical elevation of cytosolic free calcium by verapamil. Biochem Biophys Res Commun 1990;167:807–812. [PubMed: 2322253]

Zaidi M, Shankar VS, Adebanjo OA, Lai FA, Pazianas M, Sunavala G, Spielman AI, Rifkin BR. Regulation of extracellular calcium sensing in rat osteoclasts by femtomolar calcitonin concentrations. Am J Physiol 1996;271:F637–F644. [PubMed: 8853426]

Zaidi, M.; Shankar, VS.; Bax, CMR.; Bax, BE.; Alam, ASMT.; Banerji, B.; Bevis, PJR.; Gill, JS.; Moonga, BS.; Huang, CL-H. Characterization of the osteoclast calcium receptor. In: Cohn, DV.; Tashjian, AR., Jr, editors. Calcium Regulation and Bone metabolism. Amsterdam: Elsevier; 1992a. p. 170-174.

Zaidi M, Shankar VS, Towhidul Alam AS, Moonga BS, Pazianas M, Huang CL. Evidence that a ryanodine receptor triggers signal transduction in the osteoclast. Biochem Biophys Res Commun 1992b;188:1332–1336. [PubMed: 1445365]

Zaidi M, Shankar VS, Tunwell R, Adebanjo OA, Mackrill J, Pazianas M, O'Connell D, Simon BJ, Rifkin BR, Venkitaraman AR, Huang CL-H, Lai FA. A ryanodine receptor-like molecule expressed in the osteoclast plasma membrane functions in extracellular Ca^{2+} sensing. J Clin Invest 1995;96:1582–1590. [PubMed: 7657829]

Zuo J, Jiang J, Chen SH, Vergara S, Gong Y, Xue J, Huang H, Kaku M, Holliday LS. Actin binding activity of subunit B of vacuolar H^+-ATPase is involved in its targeting to ruffled membranes of osteoclasts. J Bone Miner Res 2006;21:714–721. [PubMed: 16734386]
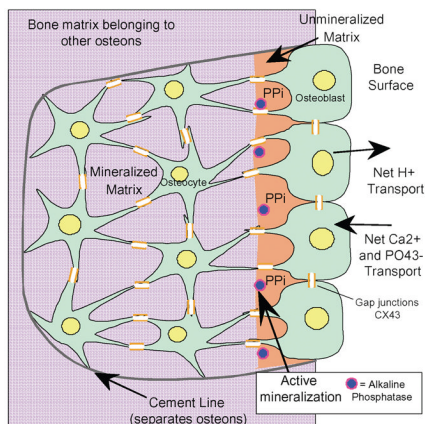
Figure 1.
Bulk calcium transport by the osteoblast. While chondrocytes are individual cells embedded in an acellular matrix, and calcify the matrix focally by producing high local concentrations of phosphate, osteoblasts are arrayed in a three-dimensional organized matrix that allows calcium to be deposited in an efficient site-directed mechanism. The osteoblasts are connected into sheets of cells at the surface of bone by gap junctions containing connexin-43. The osteoblasts secrete an organic matrix comprised mainly of type I collagen, which is oriented in layers alternately along the axis of stretch of the bone and orthogonal to this axis. There are also minor proteins, including the calcium binding low molecular weight protein osteocalcin, which facilitate mineral deposition. Mineral deposition is driven by alkaline phosphatase activity which degrades pyrophosphate. Pyrophosphate can be transported either by membrane transporters including ANKH, or may be produced locally by nucleoside pyrophosphatase (PC-1) activity. The high phosphate produced is balanced by calcium transport and by alkalinization of the mineralization site, which are required for continuing mineral deposition, but the specific transporters involved in these activities are unclear (See Colour Plate 28)
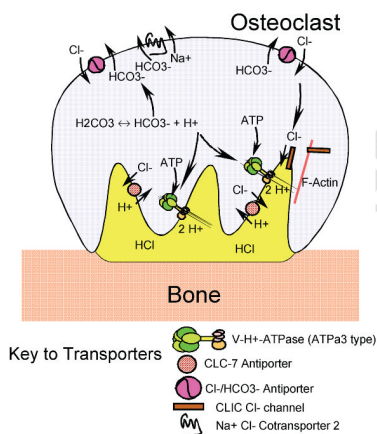
Figure 2.
Bulk calcium transport by the osteoclast. Net acid transport is driven by the vacuolar-type H$^+$-ATPase with a specialized large membrane subunit. Transport is balanced by chloride transport, probably involving both a chloride channel (CLIC-5) and a chloride bicarbonate antiporter (CLCN7). Supporting transport processes include chloride-bicarbonate exchange. Insertion of transporters is specific for subcellular locations and involves interaction of transporters with specific cytoskeletal components, including actin (See Colour Plate 29)