Molecular mechanisms of bone resorption

An update

Roland Baron

Departments of Orthopedics and Cell Biology, Yale University School of Medicine, 333 Cedar Street, P.O. Box 208044, New Haven, CT 06510, USA. Tel +1-203 785 4150. Fax +1-203 785 2744.

The osteoclast is morphologically and functionally polarized (Figure 1), with a pole facing the bone matrix, where attachment occurs and towards which most of the secretion is targeted (the apical pole), and a pole facing the soft tissues in the local microenvironment (bone marrow or periosteum) and which provides mostly, but not exclusively, regulatory functions (the basolateral pole).

Attachment apparatus

The most striking and unique feature of the osteoclast actin cytoskeleton is at the site of cell contact with the substratum, where there is a prominent peripheral ring of F-actin which contains actin filaments oriented parallel to the plane of the underlying substrate around the cell periphery as well as numerous punctate structures where the actin filaments are organized in bundles perpendicular to the plane of the substratum, the podosomes. When osteoclasts are cultured on bone, the ring circumscribes the area of active bone resorption.

The punctate actin structures in the peripheral band, termed "podosomes" (Marchisio et al. 1984; Teti et al. 1991), apparently occur only in cells of monocytic origin (osteoclasts and monocytes) and in cells which have been transformed by the *src, fps* and *abl* oncogenes (Marchisio et al. 1987). In addition to the bundles of actin filaments, the podosomes contain a number of other proteins which have been reported to occur at sites of cell-substratum or cell-cell interaction (see Teti et al. 1991 for review). These include fimbrin, α-actinin and gelsolin, which are closely associated with the actin filaments in the core of the podosome, as well as vinculin and talin, which appear to form rosette structures surrounding the podosome cores (Marchisio et al. 1984, Teti et al. 1991). Furthermore, several tyrosine kinases and/or their substrates have also been localized to focal adhesions and to the sealing zone in osteoclasts.

Recent observations by Ali et al. (1984), Lakkakorpi et al. (1989; Lakkakorpi and Vaananen 1991) and Kanehisa et al. (Kanehisa and Heersche

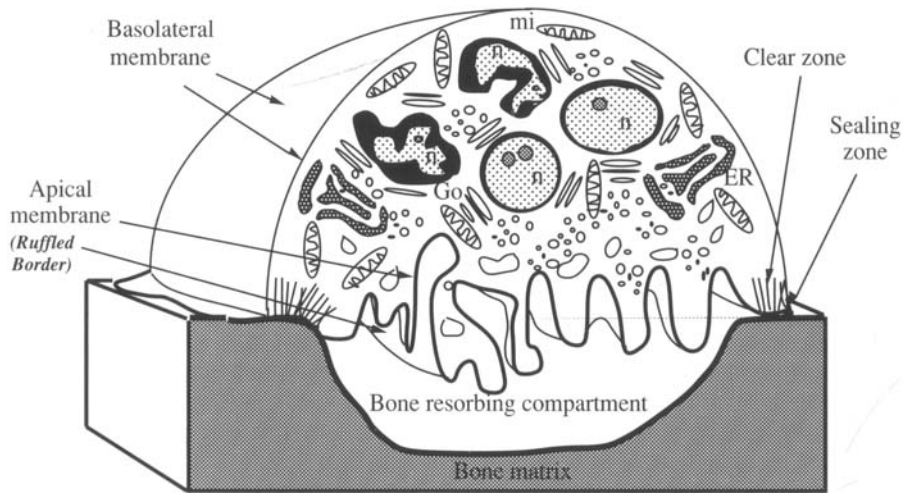

Figure 1. Functional organization and polarity of the osteoclast; n= nuclei; Go= Golgi complexes; mi= mitochondria; ER= endoplasmic reticulum.

1988, Kanehisa et al. 1990) provide us with a dynamic view of the attachment of the osteoclast to the bone matrix (Figure 2). In highly motile ("walking") osteoclasts, few podosomes are observed and seem to be confined to the irregularly shaped leading edge of the cell, or lamellipodium. Upon arrest and attachment, much more numerous podosomes are formed and are organized in a peripheral ring, as described in cells attached on glass. Rapidly thereafter, the seal is established: then the punctate "podosome" structures are either replaced by two concentric rings of vinculin and talin circumscribing a broad central zone of F-actin (Kanehisa et al. 1990, Lakkakorpi and Vaananen 1991) or reach such density that they cannot be individually observed when they arrange into two concentric rings. These observations therefore suggest a distinction in time and in specific cell-matrix interactions between the motile cell ("walking"), the cell recently arrested at a future resorbing site ("sitting"), forming a first ring-like structure of punctate attachment sites, and the resorbing cell, with a functionally tight sealing zone.

Transmembrane proteins of the integrin family of adhesion molecules, mediate cell-substratum interactions at the interface between the osteoclast and bone. Integrins are heterodimeric molecular assemblies of an α subunit and a ß subunit with specific, receptor-like, extracellular binding sites which recognize the Arg-Gly-Asp (RGD) sequence, a motif known to represent the core ligand for all members of the integrin family (Ruoslahti and Pierschbacher 1987). The amino acid sequence surrounding the RGD motif determines which integrin will recognize and bind a specific matrix protein (Ruoslahti and Pierschbacher 1987, Horton and Davies, 1989).

Osteoclasts express multiple integrins ($\alpha v\beta 3$, $\alpha 2\beta 1$ and $\alpha v\beta 1$; Nesbitt et al. 1993) some of which are involved in osteoclast adhesion to the bone matrix. Several RGD-containing matrix proteins have been identified (Teti et al. 1991). Of these, collagen-type I, osteopontin and bone sialoprotein II are the most likely candidates to fill the role of integrin-binding proteins in bone (Teti et al. 1991). Most interestingly, data is now accumulating that suggests that the osteoclast synthesizes and secretes both osteopontin and BSPII.

That the integrins play an important role in the osteoclast function and, despite the controversy regarding their presence at the sealing-zone membrane, most likely mediate the attachment of the osteoclast to the bone surface, is demonstrated by the fact that several RGD-containing proteins have been shown to inhibit bone resorption *in vitro* and *in vivo*.

The osteoclast secretes lysosomal enzymes as well as matrix-metalloproteinases in the bone resorbing compartment

The morphological polarity of the osteoclast is paralleled by a functional polarity, with the osteoclasts synthesizing and vectorially secreting several classes of enzymes towards the apical ruffled border (Baron et al. 1985. Baron et al. 1988). It is well established that these enzymes comprise acid phosphatases, aryl-sulfatases, beta-glucuronidase, beta-glycerophosphatase as well as several cysteine-proteinases (Baron et al. 1985. Baron et al. 1988. Vaes 1988), including cathepsins B, C, D, L and a novel cathepsin K, which are capable of fully digesting collagen at low pH. Recent evidence from several laboratories however suggests that osteoclasts also secrete metalloproteinases (collagenase type I and type IV, stromelysin) and that these are important in the process of bone resorption (4–7). Thus, it is the cooperative action of a set of enzymes with acidic and neutral optimal pH that leads to a complete degradation of the extracellular matrix at the resorbing site.

The apical ruffled-border membrane and the process of acidification

The apical membrane of the osteoclast, or ruffled-border, is directly involved in the molecular mechanisms of bone resorption. It is the target for the specific delivery of the newly synthetized secretory enzymes (Baron et al. 1985, 1988) and it is the site of proton extrusion for acidification of the bone resorbing compartment (Baron et al. 1985, Blair et al. 1989). The H^+ ATPase present in osteoclast membranes is of the Vacuolar-type, closely resembling the pumps present in lysosomes, coated vesicles or kidney membranes (Blair et al. 1989, Bekker and Gay 1990a, Vaananen et al. 1990).

There are however subtle differences in the properties and structure of mammalian V-ATPases (Nelson, 1991, Forgac 1989, Wang and Gluck 1990) and recent results from our laboratory indeed show that the osteoclast proton pumps, at least in chicken, differ in some respects from kidney vacuolar ATPases (Chatterjee et al. 1992). Briefly, proton transport by osteoclast-derived vesicles is more sensitive to inhibition by vanadate, nitrate and Tiludronate than similar preparations derived from the kidney in the same animals. Furthermore, cloning of the catalytic domain of the enzyme showed that the B subunit is of the ubiquitous type, different from the one expressed in kidney and that there are two isoforms of the catalytic subunit A, although their exact distribution in cells and tissues is not clearly elucidated.

The osteoclast proton pump may therefore have a

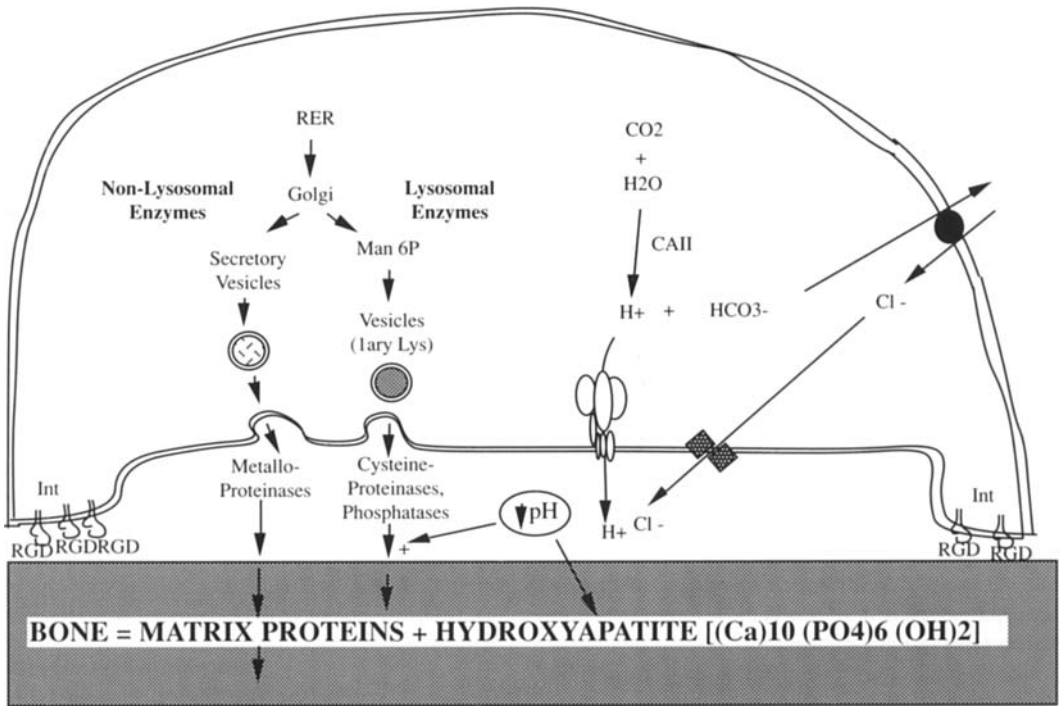

Figure 2. Schematic representation of the relationship of enzyme secretion, attachment and acidification in the mechanisms of bone resorption. Sealing at the periphery of the resorbing compartment is assured by binding of integrin receptors to RGD-containing extracellular proteins; cysteine-proteinases and metalloproteases are secreted into this sealed-off compartment, possibly transported in two separate secretory vesicles; acidification is ensured by vacuolar-like proton ATPase in the ruffled-border membrane; the protons are generated intracellularly by carbonic anhydrase and the by-product of the reaction, bicarbonate, is secreted basolaterally in exchange for chloride by a band 3-like anion exchanger; the chloride is then transported into the resorbing compartment by chloride channels, thereby avoiding hyperpolarization of the osteoclast's membrane that would result from proton transport. The low pH favors the action of lysosomal enzymes and dissolves the hydroxyapatite, and the presence of metalloproteases broadens the pH range at which resorption can occur.

unique pharmacology and the assembly of specific isoforms of two subunits in the catalytic portion of the enzyme may confer specificities to this ATPase.

In parallel with proton pumping, the plasma membrane of the osteoclast in the apical ruffled border domain also expresses chloride channels, measured as a chloride conductance, and the extrusion of this anion is absolutely required for the acidification process.

Basolateral ion transport systems are tightly coupled to the process of apical acidification

The apparently simple process of pumping protons across the ruffled border membrane to acidify the resorption lacuna imposes, in fact, a complex and demanding set of ionic requirements to the cell, essentially designed to maintain the electrochemical balance of the osteoclast during bone resorption. This ionic balancing act requires the coordinated activity of electrogenic ion pumps, ion channels and electroneutral ion exchangers in order to maintain the cytoplasmic pH and the transmembrane electrical potential within narrow physiological ranges.

Both an acid extruder (the Na^+/H^+ exchanger) and an acid-loader (i.e. base-extruder, the HCO_3^-/Cl^- exchanger) are present in the osteoclast (Teti et al. 1989, Ravesloot et al. 1995). The Na^+/H^+ exchanger is driven by a Na^+ gradient established across the basolateral membrane by the activity of the sodium pumps (Baron et al. 1986).

The hyperpolarization resulting from the activities of the H^+ pump and the Na^+/K^+ ATPase, to which the activity of the Ca^{2+} ATPase also contributes (Bekker and Gay 1990b), is alleviated by the activity of voltage-sensitive ion channels, which transport positively charged ions into the cell (K channels) and/or negatively charged ions out of the cell (Cl channels), thus discharging the electrical potential (Ravesloot et al. 1989b, Ravesloot et al. 1989a, Sims and Dixon 1989, Sims et al. 1991, Schoppa et al. 1990, Blair et al. 1991). Studies performed by our laboratory, mostly in kidney cells, suggest that the main mode of action of calcitonin may be via the regulation of several of these basolateral transporters.

The proto-oncogene c-src plays a critical functional role while the proto-oncogene c-fos is required for the differentiation of osteoclasts

The discovery that deletion of the genes encoding either *c-src* or *c-fos* lead to osteopetrosis, directly and unequivocally demonstrated that these proteins, one a cytoplasmic tyrosine kinase and the other a nuclear transcription factor, play critical and unique roles in bone resorption (Soriano et al. 1991, Grigoriadis et al. 1994).

The c-fos deletion has been demonstrated to result in the absence of osteoclast formation from hematopoietic precursors while macrophages differentiate normally and are even markedly increased (Grigoriadis et al. 1994), thereby demonstrating that c-fos is required for the branching from monocytes to osteoclasts but not to macrophages.

In contrast, c-src is playing its critical role within the osteoclast itself, i.e. the osteopetrotic phenotype in the c-src mutants is cell autonomous (Lowe et al. 1993). C-Src is expressed at high levels in osteoclasts, where it is associated with both the plasma membrane and intracellular organelles, but not in other bone cells (Horne et al. 1992).

Recent studies in our laboratory have indicated that c-src is involved in several critical signaling pathways in the osteoclast, downstream of integrin and CSF-1 receptors. Integrin binding leads to a rapid and transient wave of tyrosine phosphorylation in isolated osteoclasts. c-src-kinase gets phosphorylated in osteoclast-like cells. Such responses are blunted in src-deficient cells. In contrast, calcium signaling from integrins is not affected. Furthermore, src substrates are found in association with several intracellular vesicles in the osteoclast as well as with both the sealing zone and the ruffled border membrane. c-src may therefore play several roles in osteoclast function, one or more of which cannot be compensated when the gene for c-src is deleted.

Acknowledgements

Supported by grants NIH #DE-04724, AR41339 and AR42927.

References

Ali N N, Boyde A, Jones S J. Motility and resorption: osteoclastic activity in vitro. Anat Embryol 1984; 170: 51–6.

Baron R, Neff L, Louvard D, Courtoy P J. Cell-mediated extracellular acidification and bone resorption: Evidence for a low pH in resorbing lacunae and localization of a 100 kD lysosomal membrane protein at the osteoclast ruffled border. J Cell Biol 1985; 101: 2210–22.

Baron R, Neff L, Roy C, Boisvert A, Caplan M. Evidence for a high and specific concentration of (Na^+, K^+)-ATPase in the plasma membrane of the osteoclast. Cell 1986; 46: 311–20.

Baron R, Neff L, Brown W, Courtoy P J, Louvard D, Farquhar M G. Polarized secretion of lysosomal enzymes: Co-distribution of cation-independent mannose-6-phosphate receptors and lysosomal enzymes along the osteoclast exocytic pathway. J Cell Biol 1988; 106: 1863–72.

Bekker P J, Gay C V. Biochemical characterization of an electrogenic vacuolar proton pump in purified chicken osteoclast plasma membrane vesicles. J Bone Miner Res 1990a; 5: 569–79.

Bekker P J, Gay C V. Characterization of a calcium ATPase in osteoclast plasma membrane. J Bone Miner Res 1990b; 5: 557–67.

Blair H C, Teitelbaum S L, Ghiselli R, Gluck S. Osteoclastic bone resorption by a polarized vacuolar proton pump. Science 1989; 245: 855–7.

Blair H C, Teitelbaum S L, Tan H L, Koziol C M, Schlesinger P H. Passive chloride permeability charge coupled to H^+-ATPase of avian osteoclast ruffled membrane. Am J Physiol 1991; 260: C1315–24.

Chatterjee D, Neff L, Chakraborty M, Leit M, Jamsa-Kellokumpu S, Fuchs R, Baron R. Sensitivity to vanadate and isoforms of subunits A and B distinguish the osteoclast proton-pump from other vacuolar H^+-ATPases. Proc Natl Acad Sci USA 1992; 89: 6257–61.

Forgac M. Structure and function of vacuolar class of ATP-driven proton pumps. Physiol Rev 1989; 69: 765–96.

Grigoriadis A, Wang Z, Cecchini M, Hofstetter W, Felix R, Fleisch H, Wagner E. c-Fos: A key regulator of osteocalst-macrophage lineage determination and bone remodeling. Science 1994; 266: 443–8.

Horne W C, Neff L, Chatterjee D, Lomri A, Levy J B, Baron R. Osteoclasts express high levels of pp60 *c-src* in association with intracellular membranes. J Cell Biol 1992; 119: 1003–13.

Horton M A, Davies J. Perspectives: Adhesion receptors in bone. J Bone Miner Res 1989; 4: 803–7.

Kanehisa J, Heersche J N M. Osteoclastic bone resorption: In vitro analysis of the rate of resorption and migration of individual osteoclasts. Bone 1988; 9: 73–9.

Kanehisa J, Yamanaka T, Doi S, Turksen K, Heersche J N M, Aubin J E, Takeuchi H. A band of F-actin containing podosomes is involved in bone resorption by osteoclasts. Bone 1990; 11: 287–93.

Lakkakorpi P, Tuukkanen J, Hentunen T, Jarvelin K, Vaananen K. Organization of osteoclast microfilaments during the attachment to bone surface in vitro. J Bone Miner Res 1989; 4: 817–25.

Lakkakorpi P T, Vaananen H K. Kinetics of the osteoclast cytoskeleton during the resorption cycle in vitro. J Bone Miner Res 1991; 6: 817–26.

Lowe C, Yoneda T, Boyce B F, Chen H, Mundy G R, Soriano P. Osteopetrosis in src-deficient mice is due to an autonomous defect of osteoclasts. Proc Natl Acad Sci USA 1993; 90: 4485–9.

Marchisio P C, Cirillo D, Naldini L, Primavera M V, Teti A, Zambonin-Zallone A. Cell-substratum interaction of cultured avian osteoclasts is mediated by specific adhesion structures. J Cell Biol 1984; 99: 1696–1705.

Marchisio P C, Cirillo D, Teti A, Zambonin-Zallone A, Tarone G. Rous sarcoma virus-transformed fibroblasts and cells of monocytic origin display a peculiar dot-like organization of cytoskeletal proteins involved in microfilament-membrane interactions. Exp Cell Res 1987; 169:, 202–14.

Nelson N. Structure and pharmacology of the proton-ATPases. Trends Pharmacol Sci 1991; 12: 71–5.

Nesbitt S, Nesbit A, Helfrich M, Horton M. Biochemical characterization of human osteoclast integrins: Osteoclasts express avB3, a2B1 and avB1 integrins. J Biol Chem 1993; 268: 16737–45.

Ravesloot J H, Ypey D L, Nijweide P J, Buisman H P, Vrijheid-Lammers T. Three voltage-activated K^+ conductances and an ATP-activated conductance in freshly isolated embrynic chick osteoclasts. Pflugers Arch 1989a; 414: S166–7.

Ravesloot J H, Ypey D L, Vrijheid-Lammers T, Nijweide P J. Voltage-activated K^+ conductances in freshly isolated embryonic chicken osteoclasts. Proc Natl Acad Sci USA 1989b; 86: 6821–5.

Ravesloot J H, Eisen T, Baron R, Boron W. Role of Na-H exchangers and vacuolar H^+ pumps in intracellular pH regulation in neonatal rat osteoclasts. J Gen Physiol 1995; 105: 177–208.

Ruoslahti E, Pierschbacher M D. New perspectives in cell adhesion: RGD and integrins. Science 1987; 238: 491–7.

Schoppa N E, Su Y, Baron R, Boulpaep E L. Identification of single ion channels in neonatal rat osteoclasts. J Bone Miner Res 1990; 5: S204.

Sims S M, Dixon S J. Inwardly rectifying K^+ current in osteoclasts. Am J Physiol 1989; 256: C1277–82.

Sims S M, Kelly M E M, Dixon S J. K^+ and Cl^- currents in freshly isolated rat osteoclasts. Pflugers Arch 1991; 419: 358–70.

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.

Teti A, Blair H C, Teitelbaum S L, Kahn A J, Koziol C, Konsek J, Zambonin-Zallone A, Schlesinger P H. Cytoplasmic pH regulation and chloride bicarbonate exchange in avian osteoclasts. J Clin Invest 1989; 83: 227–33.

Teti A, Marchisio P C, Zambonin Zallone A. Clear zone in osteoclast function: role of podosomes in regulation of bone-resorbing activity. Am J Physiol 1991; 261: C1–C7.

Vaananen H K, Karhukorpi E K, Sundquist K, Wallmark B, Roininen I, Hentunen T, Tuukkanen J, Lakkakorpi P. Evidence for the presence of a proton pump of the vacuolar H^+-ATPase type in the ruffled border of osteoclasts. J Cell Biol 1990 ; 111: 1305–11.

Vaes G. Cellular biology and biochemical mechanism of bone resorption. Clin Orthop 1988; 231: 239–71.

Wang Z-Q, Gluck S. Isolation and properties of bovine kidney brush border vacuolar H+-ATPase. A proton pump with enzymatic and structural differences from kidney microsomal H^+-ATPase. J Biol Chem 1990; 265: 21957–65.