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PROSPECTS

Molecular Mechanisms of Bone Resorption

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Abstract This review focuses on osteoclast ontogeny and function, emphasizing three aspects. We describe how a combination of laboratory models available to study the cell plus examination of the osteopetroses, a family of sclerotic diseases of the skeleton, have yielded major insights into osteoclast ontogeny and function. We proceed to describe the cell and molecular machinery enabling osteoclasts to resorb bone. The final, and most speculative, aspect of the review addresses possible mechanisms by which the osteoclast assumes its characteristic morphology, that of a polarized cell on bone. Since little direct information has been forthcoming as to how the osteoclast polarizes, we draw on other polarized cells. In particular, we examine the role of microtubules and members of the small GTPase family, the latter mediating polarized targeting of intracellular vesicles. In the case of the osteoclast, such vesicles probably represent the origin of the highly convoluted ruffled membrane, the cell's characteristic bone resorptive organ.

Key words: osteoclastogenesis, bone resorption, integrins, polarization, rab proteins, microtubules

The osteoclast is a physiological polykaryon and a member of the monocyte/macrophage family [Udagawa et al., 1990; Suda et al., 1992]. While it is the principal, if not exclusive, resorptive cell of bone, the mechanisms by which the osteoclast degrades skeletal matrix have begun to clarify only recently. The purpose of this review is to summarize current knowledge concerning the ontogeny and mode of action of the osteoclast. In the first section we examine the cellular lineage of the osteoclast, with emphasis on the signals which control production of these polykaryons. A second subject concerns the mechanisms by which osteoclasts resorb bone, again emphasizing the process at a molecular level. In the final section we discuss the possible pathways by which the osteoclast assumes its characteristic morphology, that of a polarized cell on bone. In this latter section, since there is little direct evidence relating to the osteoclast, we have drawn on recent findings as they apply to other polarized cells. In particular, we examine the role of both microtubules and members of the small GTPase family, the latter known to mediate polarized targeting of intracellular vesicles. In the case of the osteoclast, such vesicles probably represent the origin of the highly convoluted ruffled membrane, the characteristic feature of a polarized, fully functional bone resorbing cell.

New insights into osteoclast physiology derive largely from two sources. First, the disease osteopetrosis has provided a wealth of information regarding critical events in osteoclastogenesis and skeletal degradation. This rare family of disorders is characterized by failure of osteoclasts to resorb mineralized tissue, which therefore progressively accumulates within the skeleton. Thus, the skeletons of osteopetrotic animals and patients are sclerotic, with loss of distinction between cortex and trabeculum.

The osteopetroses fall into two general categories. The first is characterized by a paucity of osteoclasts. Because accessory cells, such as osteoblasts [Burger et al., 1984] or stromal cells [Udagawa et al., 1990], produce humoral [Tanaka et al., 1993a] and membrane-residing [Takahashi et al., 1988] factors critical to osteoclastogenesis, the molecular defect in osteoclast-deficient osteopetrosis may lie not in the osteoclast precursor per se but in cells providing factors necessary to promote its differentiation. Such a lesion exists in the op/op osteopetrotic mouse [Wiktor-Jedrzejczak et al., 1982]. This animal bears a homozygous mutation in the

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M-CSF (CSF-1) gene [Yoshida et al., 1990] whose product, macrophage specific growth factor, is necessary for osteoclastogenesis. As expected, administration of M-CSF to the op/op mouse cures its disease [Felix et al., 1990; Kodama et al., 1991].

In contrast to the osteoclast-deficient forms of osteopetrosis, those in which the cell is abundant are due not to abnormalities of osteoclast precursor differentiation but to failure of the *cell* to express an essential component of the resorptive apparatus. Osteoclast-abundant osteopetrosis is probably the most common form of the human disease [Teitelbaum et al., 1981] and is also seen in rodents such as the c-src −/− mouse [Soriano et al., 1991], a topic discussed later in this review. Predictably, this group of osteopetroses is curable by transplantation of normal osteoclast precursors derived from marrow, liver, or spleen [Coccia et al., 1980; Walker, 1975].

The second event fostering major insights into osteoclast biology was the development of techniques whereby osteoclasts can be isolated or generated and maintained in culture. Such cells have been derived from chickens [Zamboni-Zallone et al., 1982], rodents [Arnett and Dempster, 1987], and man [Chambers et al., 1985], and each model has its advantages and disadvantages. Large numbers of avian osteoclasts can be isolated or produced by culture of uniform bone marrow–derived monocytic precursors [Alvarez et al., 1991]. Because of purity and abundance, these polykaryons are suitable for biochemical and/or cell biological experiments. In particular, the precursors respond to osteoclastogenic steroid hormones, which play a role in their differentiation [Suda et al., 1992]. On the other hand, bona fide avian osteoclasts and those derived in culture fail to express the calcitonin receptor [Arnett and Dempster, 1987], a hallmark of their mammalian counterpart [Nicholson et al., 1986; Hattersly and Chambers, 1989]. Furthermore, chicken hematopoietic growth factors are largely unavailable, thereby limiting experiments aimed at delineating cytokine-mediated mechanisms regulating osteoclastogenesis.

In contrast to the chicken, accessibility of recombinant murine hematopoietic cytokines facilitates performance, in the mouse, of critical experiments exploring the role of these molecules in osteoclastogenesis. Furthermore, gene targeting technology allows for direct, in vivo testing of hypotheses derived from in vitro experimentation. On the other hand, only limited numbers of mouse osteoclasts can be isolated or generated [Udagawa et al., 1990]. Moreover, murine osteoclast generation requires coculture of macrophages with either osteoblasts [Burger et al., 1984] or stromal cells [Udagawa et al., 1990], thus precluding a high degree of osteoclast purity. This fact, and the relatively small number of cells generated, makes biochemical experimentation more difficult than with the avian counterpart.

Finally, human osteoclasts can be isolated from giant cell tumors of bone (osteoclastomas), and important information has been forthcoming from these cells [Chambers et al., 1985; Ohsaki et al., 1992]. Such material is, however, unavailable to most laboratories, and, as yet, human polykaryons capable of osteoclastic bone resorption have not yet been generated in vitro.

Using these tools to unravel the resorptive process has led to a reasonable model by which the osteoclast degrades bone. Reflecting the defects in osteopetrosis, physiological resorption may be regulated by differentiation of osteoclast precursor cells or by governing the activity of mature polykaryons. In fact, stimulation of bone resorption appears to be exerted largely through regulation of osteoclast precursor differentiation. A number of agents are known to modulate differentiation of osteoclast precursors. These belong to the cytokine family of secreted factors and include a variety of interleukins as well as tumor necrosis factor (TNF) or lymphotoxin [Mundy, 1992]. Recent studies have clarified the mechanisms by which cytokine-mediated increases in osteoclastic bone resorption occur. IL-1, IL-6, IL-11, and TNF stimulate bone resorption indirectly by increasing proliferation and differentiation of osteoclast precursors [Roodman, 1992, Lerner and Ohlin, 1993]. These molecules crossregulate their production, as demonstrated by the fact that TNF amplifies IL-1 and parathyroid hormone-induced secretion of IL-6 [Passeri et al., 1994]. Recent publication suggests that IL-1 and TNF may act on early steps in the differentiation pathway, while IL-6 may be active later [Kitazawa et al., 1994].

IL-6 and IL-11 belong to a subfamily of cytokines in which signalling is mediated by receptors sharing a common subunit, gp130. Specificity is achieved by binding of each protein to separate subunits, which associate with gp130 to form the active signalling complex [Yin et al., 1993]. In the case of IL-6, an 80 kDa, soluble

form of the IL-6 binding subunit stimulates formation of osteoclasts in vitro [Tamura et al., 1993]. Thus, increased osteoclastogenesis by IL-6 and IL-11 may arise from a common intracellular signal.

Steroid hormones, particularly 1,25 dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) [Udagawa et al., 1990; Perkins and Teitelbaum, 1991] and probably retinoic acid [Hough et al., 1988], are also critical for the maturation of precursor cells. Thus, it is not surprising that vitamin D receptors are present in osteoclast precursors and lost upon formation of the terminally differentiated polykaryon [Merke et al., 1986]. Additionally, $1,25(OH)_2D_3$ upregulates the estrogen receptor in human bone marrow stromal cells [Bellido et al., 1993]. The ability of specific inhibitors of IL-1 and TNF to reverse the consequences of estrogen withdrawal [Kitazawa et al., 1994] indicates that at least part of the effects of this steroid on osteoclast function are mediated via these cytokines.

Finally, mature osteoclasts contain estrogen receptors [Oursler et al., 1991], and treatment with the sex steroid stimulates lysosomal enzyme secretion [Oursler et al., 1994]. Given the above, and the recent demonstration that estrogen regulates expression of IL-1 and IL-6 in vivo [Jilka et al., 1992; Kimble et al., 1994; Pacifici et al., 1991; Ralston, 1994] and in vitro [Pioli et al., 1992; Girasole et al., 1992; Passeri et al., 1993], steroid hormones probably directly and indirectly modulate osteoclast formation.

Osteoclast precursor differentiation is characterized by acquisition of matrix adherence, a step apparently essential for physiological multinucleation. The entire repertoire of molecules responsible for osteoclast-bone recognition is probably not yet known, but the integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ appear to be important. For example an anti-$\alpha_v\beta_3$ antibody blunts the bone binding and resorbing capacity of osteoclasts [Chambers et al., 1986; Ross et al., 1993]. Furthermore, expression of this integrin heterodimer by osteoclast precursors is enhanced by the resorptive steroids $1,25(OH)_2D_3$ and retinoic acid, an event mediated by direct transactivation of the β_3 gene [Mimura et al., 1994; Chiba et al., 1993].

While $\alpha_v\beta_3$ function is pivotal to the resorptive process, it may not be the molecule which anchors the osteoclast directly to bone. For example, there is controversy [Teti et al., 1991; Lakkakorpi et al., 1991] as to whether the heterodimer localizes to the attachment region of the cell (the "sealing zone"), and we find the ability of avian osteoclast precursors to bind matrix precedes appearance of $\alpha_v\beta_3$. The closely related integrin $\alpha_v\beta_5$, on the other hand, is expressed in the earliest identifiable adherent avian osteoclast precursors [Sago et al., 1993]. This heterodimer, which, like $\alpha_v\beta_3$, recognizes the bone matrix protein osteopontin [J. Smith, personal communication], disappears as the cells differentiate under the influence of retinoic acid [Sago et al., 1993]. Thus, $\alpha_v\beta_5$ may be the initial homing receptor by which osteoclast precursors bind bone, only to be replaced by other molecules once matrix-associated differentiation is under way.

The principal steps by which osteoclasts, once differentiated and attached to bone, resorb matrix appear largely in hand. The initial event in this process, acidification of the isolated extracellular resorptive microenvironment, is mediated by a vacuolar H^+-ATPase in the ruffled membrane of the polarized cell. The structure and functional activity of this multienzyme complex is very similar, if not identical, to the analogous proton pump in the intercalated cell of the kidney [Blair et al., 1989; Mattsson et al., 1994]. The acidification step is critical, permitting not only mineral mobilization, but subsequent degradation of the organic phase of bone [Blair et al., 1986] by acidic proteases such as cathepsin B and G [Blair et al., 1993; Sasaki and Ueno-Matsuda, 1993].

One would expect, given the pivotal role extracellular acidification plays in osteoclastic bone resorption, to encounter osteopetrotic phenotypes with defects in proton transport. In fact, human osteopetrosis is associated with failure to express the osteoclast carbonic anhydrase isoenzyme [Sly et al., 1983], and recently a sclerotic disease akin to osteopetrosis was found in a patient whose osteoclast precursors lack the plasma membrane H^+-ATPase [Yamamoto et al., 1993].

Having documented the osteoclast transports protons extracellularly by an electrogenic mechanism raised the issue of maintenance of intracellular pH. Turning to the paradigm of the renal intercalated cell, Teti et al. [1989] found that osteoclasts express, on their antiresorptive border, an energy-independent Cl^-/HCO_3^- exchanger similar to band 3 of the erythrocyte. Finally, electroneutrality is preserved by a plasma membrane Cl^- channel, charge coupled to the H^+-ATPase, resulting in secretion of HCl

into the resorptive microenvironment [Blair et al., 1991]. Although no evidence has been forthcoming which links abnormalities in chloride transport to impaired bone resorption, this remains a reasonable hypothesis.

While much is known about the mechanisms by which osteoclasts degrade bone, less is evident regarding regulation of these events. For example, while their activity is directly blunted by calcitonin [Arnett and Dempster, 1987] mature osteoclasts seem generally unresponsive to humoral agonists, such as $1,25(OH)_2D_3$, which target to their precursors [Udagawa et al., 1990; Merke et al., 1986].

Being a member of the monocyte/macrophage family, osteoclasts share many similarities with other polykaryons of this lineage, such as those elicited by foreign bodies [Quinn et al., 1991]. The distinguishing feature of the osteoclast in this regard is its polarization. In particular, the interface of the cell with bone is highly convoluted and, thus, known as its ruffled membrane. This structure, appearing only in bone-bound osteoclasts, is rich in H^+-ATPase and is the cell's resorptive organ [Blair et al., 1989]. Available evidence [Baron et al., 1988, 1990; Blair et al., 1988] suggests the osteoclast ruffled membrane forms by polarized insertion of H^+-ATPase–bearing vesicles into the osteoclast plasma membrane (Fig. 1). A major unsolved issue regarding osteoclast function pertains to the detailed mechanisms by which such vesicles target to the bone-residing surface of the cell, thereby permitting the initial step in skeletal degradation, namely acidification of the resorptive microenvironment. The fact that polarization follows attachment suggests that cell-matrix interactions produce a signal resulting in vesicular movement.

Insights gained into the mechanisms of protein transport in other systems [Zerial and Stenmark, 1993; Rothman and Orci, 1992; Bauerfeind and Huttner, 1993] offer suggestions as to how intraosteoclast vesicles target to and fuse with the bone-polarized plasma membrane. Movement of proteins from a cell's center to its surface involves generation of specialized vesicles, with subsequent targeting to and fusion with sequential membrane compartments [Zerial and Stenmark, 1993; Novick and Brennwald, 1993; Novick and Garrett, 1994] (Figs. 2, 3). Budding of a nascent vesicle requires formation, on its surface, of a multimer (a coating with nonclathrin proteins) which stimulates

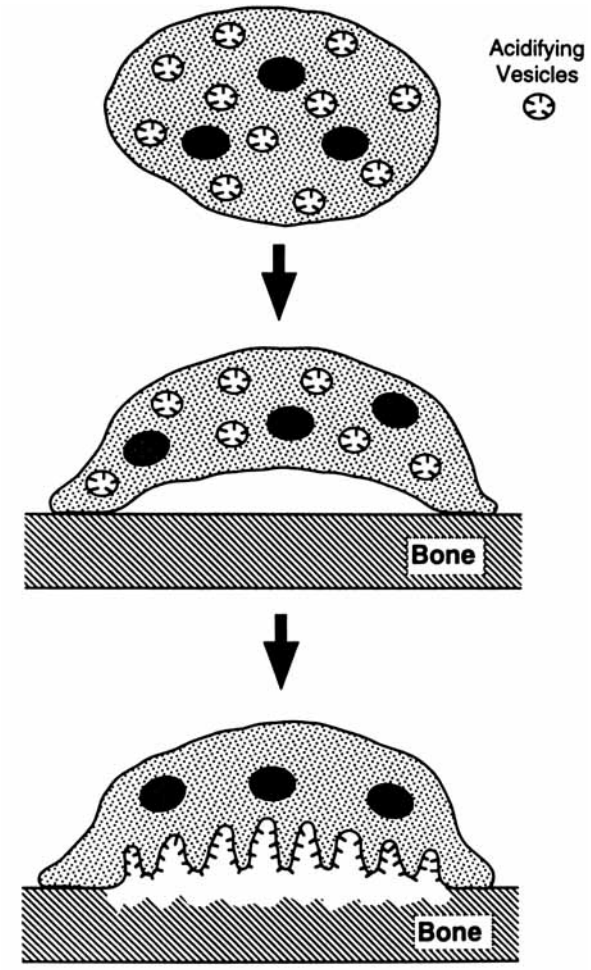

Fig. 1. Model for generation of the osteoclast ruffled membrane. A nonadherent, nonpolarized osteoclast binds to bone leading to the generation of an apical (resorptive) surface. Adapting the paradigm of epithelial cells [Fath et al., 1993; Rizzolo and Joshi, 1993], microtubules in polarized osteoclasts would be expected to orient their + ends toward the cell center and − poles facing the plasma membrane. If such is the case, the retrograde (+ to −) microtubule motor dynein will aid movement of osteoclast vesicles destined for the plasma membrane along the microtubular network. Vesicles associating with osteoclast microtubules may derive directly from the transgolgi system and thus accommodate newly synthesized proteins [Fath et al., 1993]. Alternatively, acidifying structures containing critical components of the ruffled membrane, such as its proton pump [Baron et al., 1988], may reside in the cytoplasm prior to microtubule association. Extensive fusion of vesicles with the apical membrane leads to formation of the characteristic ruffled appearance. The central issue in osteoclast polarization, namely the signalling pathways leading to vesicle movement, remains undefined.

vesicle formation and movement from one membrane surface to the next [Rothman and Orci, 1992; Novick and Garett, 1994]. Energy for these events is derived from hydrolysis of ATP and GTP [Ferro-Novick and Novick, 1993], uti-

lizing members of several families of nucleotide-binding proteins involved in vesicle targeting [Zerial and Stenmark, 1993; Novick and Brennwald, 1993; Novick and Garrett, 1994; von Mollard et al., 1994a; Zahraoui et al., 1994].

Antero (− to +) and retrograde (+ to −) vesicle movement occurs by association with microtubules [Raff 1994; Mellman et al., 1993]. The molecular basis of these events involves interactions between the directional motors dynein and kinesin with microtubular proteins on the one hand [Collins 1994; Scholey and Vale, 1994] and receptors on the vesicular membrane on the other [Fath et al., 1993; Walker and Sheetz, 1993]. Microtubules maintain composition, organization, and position in the cytoplasm of many membrane-bound organelles or specialized compartments. They also move materials packaged into vesicles from one compartment to another [Kelly 1990; Bauerfeind and Huttner, 1993; Wordeman and Mitchison, 1994]. In the context of this review, relevant examples include transport from the endoplasmic reticulum to the plasma membrane [van den Bosch et al., 1990; Gilbert et al., 1991; Saucan and Palade, 1992].

The role of the microtubules in polarized vesicular transport has been documented primarily for epithelial and neuronal cells [Rodriguez and Powell, 1992; Brown and Sabolic, 1993; Elferink and Scheller, 1993; Fath et al., 1993] and hepatocytes [Saucan and Palade, 1994]. As targeted vesicular transport is necessary for bone resorption [Baron et al., 1988; Blair et al., 1989], it is likely that microtubules play a role in the polarization of osteoclasts. For example, that administration of microtubule-dissolving drugs in vivo blunts bone resorption [Ohya and Ogura, 1993] may result from alterations in osteoclast cytoskeleton. Likewise, osteoclast microtubular structure is altered following treatment with calcitonin in vitro [Warshafsky et al., 1985].

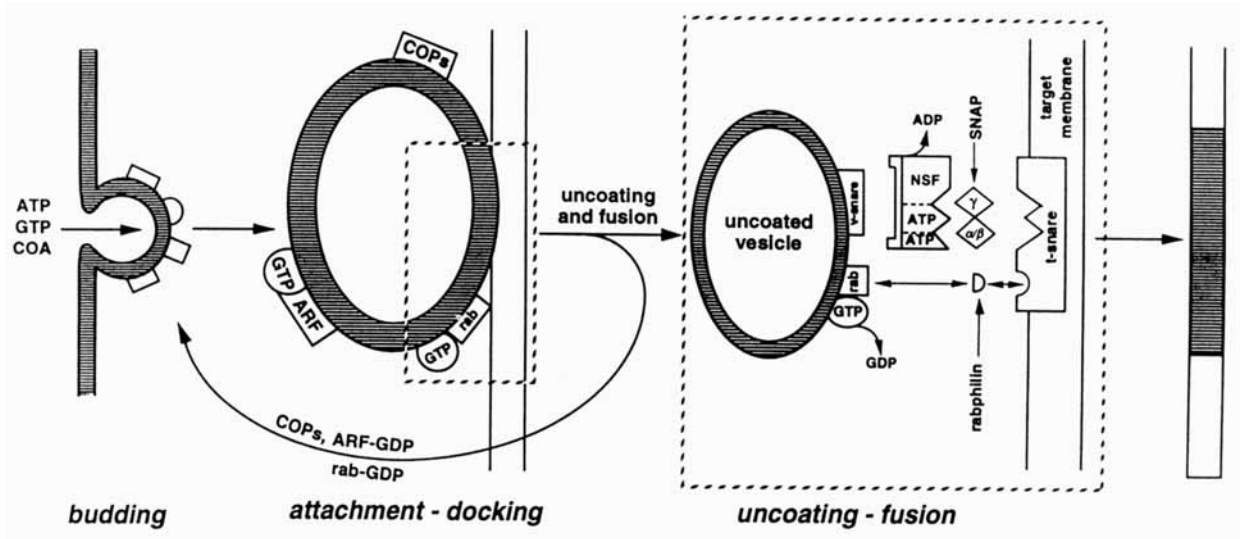

Fig. 2. Mechanism for targeting vesicles from one membrane to another. Budding of a vesicle from a donor membrane requires expenditure of energy in the form of both GTP and ATP. The budding process is initiated by association of individual members of several groups of proteins, including the small GTP-binding family (rabs) [reviewed in von Mollard et al., 1994a] the ADP ribosylation factor family (ARF), and a complex of coatamer proteins (COPs) [Takizawa and Malhotra, 1993]. COPs are structurally related to but functionally separate from clathrins. The macromolecular structure so formed is capable of migrating to and fusing with an acceptor membrane. N-ethylmaleimide sensitive factor (NSF), a soluble molecule which contains, at its amino terminus, two binding sites for ATP, mediates docking of the vesicle to the acceptor membrane. Soluble proteins (SNAPs) which activate NSF bind to receptors (SNAREs) on vesicles and acceptor membranes [Takizawa and Malhotra, 1993], thereby aiding vesicle-membrane attachment. Individual members of the rab family dictate trafficking of vesicles to specific membranes [reviewed in von Mollard et al., 1994b]. In the case of plasma membrane targeting, to date rab8, rab13, and several proteins homologous to rab3 have been shown to play a role in this process [Holz et al., 1994; Huber et al., 1993; Jena et al., 1994; von Mollard et al., 1994a; Weber et al., 1994]. Budding requires that a given rab associates with a nascent vesicle as it exits the donor membrane compartment. At this time, binding of ATP to NSF triggers assembly of a fusion complex which includes SNAPs and a vesicular SNARE (vSNARE). The entire complex recognizes a SNARE on the acceptor (target) membrane (tSNARE), prompting hydrolysis of NSF-associated ATP, with consequent vesicle-membrane fusion. The ADP-bound form of NSF dissociates from the vesicle, resulting in detachment of the other proteins mediating targeting/fusion. All facilitatory proteins recycle to the donor membrane, where they are used in another round of vesicle transport.

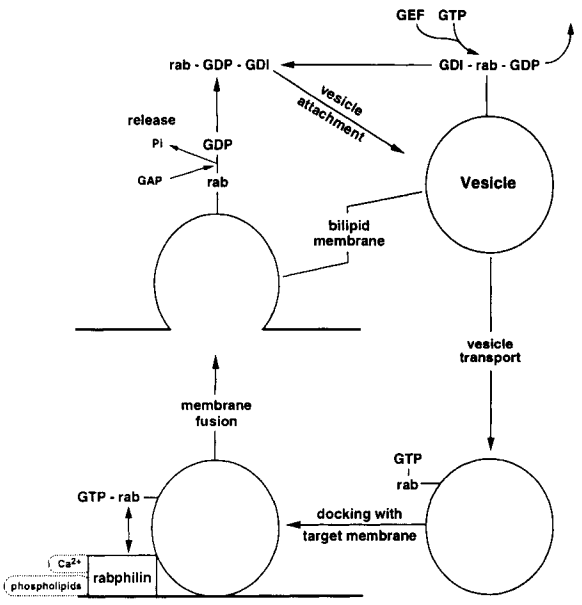

Fig. 3. Mechanism of rab-mediated vesicular targeting [for further details see Novick and Brennwald, 1993; von Mollard et al., 1994b]. A pool of inactive GDPrabs exists in solution bound to GDP dissociation inhibitor (GDI). A guanidine nucleotide exchange factor (GEF) on the donor membrane stimulates release of GDP, thereby facilitating membrane attachment of rab. GTP binding to membrane-bound rab triggers its interaction with vSNARE on the budding vesicle. Docking of the vesicle to an acceptor membrane is mediated by events involving vSNARE-NSF/SNAP and rab/rabphilin. Hydrolysis of NSF-bound ATP and rab-bound GTP results in vesicle-membrane fusion and release of rab, NSF, and COP proteins. Rab-GDP recycles to the inactive GDI-bound pool.

Polarization of the osteoclast, a prerequisite for resorption, requires it bind to the organic matrix of bone. Matrix-recognizing integrins localize in this cell to focal adhesions, subcellular complexes in close proximity to extracellular ligand [Marchisio et al., 1984]. It is within these structures that integrins associate with a number of intracellular proteins ultimately linking the heterodimers and cytoskeleton [Burridge and Fath, 1989; Sastry and Horwitz, 1993]. We [Ross et al., 1993] and others [Chambers et al., 1986; Fisher et al., 1993] have shown that the integrin $\alpha_v\beta_3$ is essential to the resorptive process. Moreover, this integrin, like other members of its family, transmits matrix-derived signals [Guan et al., 1991; Kornberg et al., 1991; Leavesley et al., 1993; Juliano and Haskill, 1993; Fox et al., 1993] and does so in the osteoclast. For example, interaction of $\alpha_v\beta_3$ with its bone matrix ligand, osteopontin, leads, in both avian [Miyauchi et al., 1991] and rodent osteoclasts [Zimolo et al., 1994], to immediate changes in intracellular calcium. In the case of avian cells the change in calcium arises via activation of a calmodulin-dependent plasma membrane Ca^{2+}-ATPase, probably protecting osteoclasts from the high ambient Ca^{2+} to which they are exposed. Occupancy of $\alpha_v\beta_3$ prompts osteoclasts to synthesize phosphatidylinositol triphosphate which binds, in turn, to gelsolin, thereby prompting cytoskeletal reorganization, an event likely to play a critical role in osteoclast polarization [Miyauchi et al., submitted].

With the observations that integrins transmit matrix-derived signals came the search for proteins distal to the heterodimer in the signalling pathway. The tyrosine kinases pp60 c-^{src} and focal adhesion kinase may be important in this regard as they physically associate with integrins [Rolnick et al., 1992; Hildebrand et al., 1993]. The report that liganding of $\alpha_v\beta_3$ on osteoclasts induces a wave of tyrosine phosphorylation [Neff et al., 1993] suggests these enzymes are activated by integrin occupancy.

In 1991, Soriano et al. made the surprising observation that interruption of the c-src gene results in a form of osteopetrosis associated with abundant, yet dysfunctional, osteoclasts [Soriano et al., 1991]. The precise role c-src plays in osteoclast function is not yet understood. However, since pp60 c-^{src} is associated with both intracellular and plasma membranes in osteoclasts [Horne et al., 1992; Tanaka et al., 1992], and osteoclasts of c-src $-/-$ mice fail to form ruffled membranes [Soriano et al., 1991], the kinase may be critical to acidified vesicle polarization. This observation, and the fact that focal adhesion kinase, a pp60 c-^{src} substrate, is phosphorylated in vitro upon integrin occupancy [Burridge et al., 1992, Guan and Shalloway, 1992; Lipfert et al., 1992], prompted Suda and his colleagues to block focal adhesion kinase expression in osteoclasts. These experiments, performed with antisense technology, blunted osteoclastic bone resorption [Tanaka et al., 1993b]. Thus, nonreceptor tyrosine kinases, which impact on cytoskeletal function, appear central to activating osteoclasts, perhaps by inducing ruffled membrane formation.

Given that the integrin $\alpha_v\beta_3$ transmits matrix-derived signals, we propose that osteoclasts are activated upon binding of the heterodimer to its bone-residing ligands. Our finding that H^+-ATPase polarization occurs in osteoclasts only in contact with bone [unpublished observations] supports this hypothesis. The scenario which

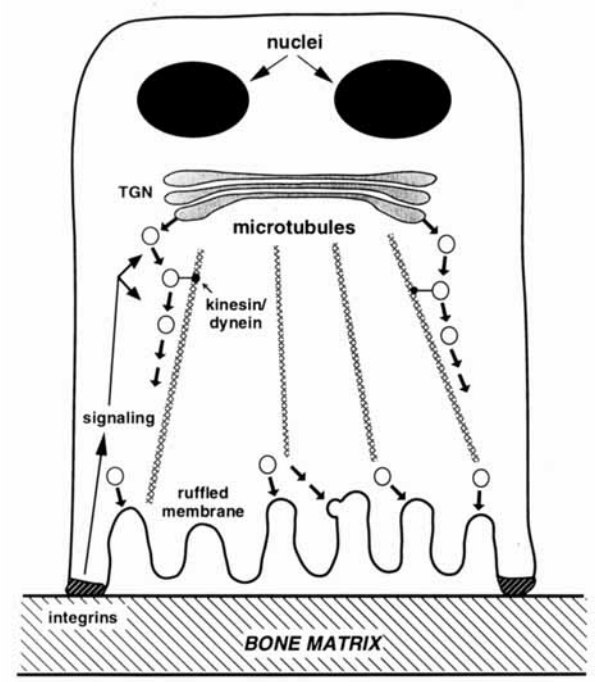

Fig. 4. Model of osteoclast polarization. In the nonattached state the osteoclast is unpolarized, with acidifying vesicles distributed throughout its cytoplasm. Once in contact with bone, matrix-derived signals, probably mediated via integrins such as $\alpha_v\beta_3$, prompt targeting, by trafficking along microtubules, of acidifying vesicles to the apical (resorptive) surface of the cell. Insertion of these vesicles into the bone-adjacent plasma membrane yields the characteristic, highly ruffled resorptive surface of the cell. The "spikes" within the vesicles represent the H^+-ATPase (proton pump).

follows would involve activation, via occupancy of $\alpha_v\beta_3$, of c-src and focal adhesion kinase, which, through protein phosphorylation, mobilize vesicles bearing H^+-ATPase complexes to the bone-adjacent plasma membrane. Insertion of these vesicles into the membrane would increase its complexity, forming the characteristic resorptive organ of the cell (summarized in Fig. 4).

Thus, the mystery of the osteoclast is beginning to unravel. It appears to be a cell whose differentiation is controlled by secreted and plasma membrane-residing factors. Osteoclast activation, on the other hand, may not be humorally responsive but governed by an intimate relationship with bone matrix which signals the cell to polarize and thus assume its characteristic phenotype. Many of these insights gained from studying the rare family of disorders osteopetrosis are likely to impact on preventing the endemic disease osteoporosis.

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