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INTEGRIN-MEDIATED SIGNALING IN THE REGULATION OF OSTEOCLAST ADHESION AND ACTIVATION

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1. ABSTRACT

Integrins are heterodimeric membrane receptors that mediate cell-extracellular matrix (ECM), and cell-cell interactions. Integrins provide a physical link between the ECM and the cell cytoskeleton, and transduce signals which lead to elevation of cytosolic pH and calcium levels, changes in phospholipid metabolism and ultimately regulate gene expression. Osteoclast bone resorption is a complicated multistep process, that starts with matrix recognition, osteoclast attachment, polarization and formation of the sealing zone on the bone, followed by the directional secretion of acids and lysosomal enzymes to the resorbing surface. Osteoclasts exhibit high expression of the alpha v beta 3 integrin, which binds to a variety of RGD-containing proteins including vitronectin, osteopontin and bone sialoprotein. RGD-containing peptides, RGD-mimetics and blocking antibodies to alpha v beta 3 integrins were shown to inhibit bone resorption *in vitro* and *in vivo*, suggesting that this integrin plays an important role in regulating osteoclast activity. Furthermore, RGD-containing peptides and proteins modulate osteoclastic cytosolic calcium levels. Phosphatidyl inositol 3-kinase and c-Src were co-immunoprecipitated with alpha v beta 3 integrins in these cells. In addition, c-Cbl was found to be a substrate of c-Src in osteoclasts. More recently, ligand-engagement or clustering of alpha v beta 3 integrins in osteoclasts induced tyrosine phosphorylation of PYK2, a member of the focal adhesion kinase family, and of p130cas, a substrate of v-Src and v-Crk. Both PYK2 and p130cas were also found in the sealing zone of actively resorbing osteoclasts. How these signaling molecules interact with each other in mediating the alpha v beta 3 rate limiting effect on bone resorption is not well understood. They emerged however as key players in linking the adhesion of osteoclasts to the bone matrix, to cytoskeletal organization, and to the polarization and activation of these cells for bone resorption.

2. INTRODUCTION

Cell adhesion is a complex process mediated by several classes of cell adhesion molecules (CAMs) including integrins, cadherins and selectins. Development and maintenance of specialized tissues requires tightly regulated cell adhesion. Abnormal regulation of adhesion is responsible for or associated with a variety of diseases. Recent insight into the regulation of adhesion has come from observations that many adhesion molecules simultaneously mediate both adhesion and signal transduction (1-4). Here, we shall first briefly review the integrin-dependent signaling pathways in general, and then we discuss the role of integrins in osteoclast function and their signal transduction pathways in these cells.

3. INTEGRINS: AN OVERVIEW

Integrins mediate cell adhesion to the extracellular matrix (ECM) and specific classes of integrins also mediate cell-cell interactions (4, 5). The name of these receptors was coined by R.O. Hynes in 1986 to emphasize their role in integrating the intracellular cytoskeleton with the external enviroment. Integrin-mediated adhesion and signaling regulate a variety of cell functions including embryonic development, hemostasis, leukocyte homing and activation, bone resorption, clot retraction, the response of cells to mechanical stress, programmed cell death, tumor cell growth and metastases (6-11).

Integrins are heterodimeric membrane glycoproteins, composed of an alpha- and a beta-subunit. At present, more than 20 different members of the integrin family have been identified, produced by various combinations of the 16 alpha- and 8 beta-subunits. Each integrin subunit has a large extracellular domain, a single membrane spanning domain and usually a short cytoplasmic domain (40-60 amino acids). Association of

alpha- and beta-subunits define distinct, although largely overlapping, ligand binding specificity (4). Integrin binding to extracellular matrices can be classified as either RGD-dependent (such as fibronectin, vitronectin and fibrinogen) or RGD-independent (such as collagen and laminin). In addition, some integrins can bind to counterreceptors (such as intracellular adhesion molecules, ICAMs) on adjacent cells, leading to homotypic and heterotypic cell-cell interaction. Despite the high degree of apparent redundancy, most integrins seem to have specific biological functions, particularly during development (12), raising the possibility of signaling differences between integrins.

In addition to ligand occupancy, integrin receptor clustering is critical for the initiation of intracellular responses (13). Engagement of integrins usually leads to the formation of focal adhesions, where these receptors are linked to intracellular cytoskeletal complexes and bundles of actin filaments (14, 15). In addition to cell adhesion, the assembly of these receptors also modulates cell shape changes and participates in cell spreading and motility. It has long been recognized that the short cytoplasmic domains of the alpha and beta integrin subunits do not have intrinsic enzymatic activities, but can interact with a variety of cytoplasmic proteins, including cytoskeletal and signaling molecules (16). The alpha cytoplasmic domains are highly diverse, whereas the beta cytoplasmic domains are somewhat conserved (17). Analyses of chimeric molecules and mutations of the integrin subunits have indicated that the beta cytoplasmic domains are necessary and sufficient for integrin-dependent signaling. This region is also responsible for targeting the receptors to focal adhesions, while the alpha cytoplasmic domains play a role in regulating the specificity for the interaction with ligands (17-19).

3.1. Proteins that associate with integrins

A number of molecules were shown to interact directly with the beta subunit cytoplasmic domains, using *in vitro* binding assays or yeast two-hybrid screens. These include cytoskeletal, regulatory and signal transduction proteins. The actin-binding proteins, alpha-actinin and talin, bind directly to the cytoplasmic domains of beta 1, beta 2 and beta 3, while the cytoskeletal protein filamin has recently been demonstrated to interact specifically with the beta 2 cytoplasmic domain (20-23). In addition to interaction with cytoskeletal molecules, the beta subunits can also bind to intracellular proteins that participate in the regulation of integrin function and integrin-mediated signal transduction. The interaction of the beta subunit cytoplasmic domains with a number of cytosolic molecules, such as a serine/threonine integrin-linked kinase (ILK), beta 3-endonexin and cytohesin-1 were recently identified in the yeast two-hybrid system (24-26). Focal adhesion kinase (FAK), has also been shown to bind *in vitro* to peptides derived from the cytoplasmic domains of beta 1, beta 2 and beta 3 integrin subunits (27). It has not been possible so far to demonstrate a direct association of FAK with integrins by other means such as co-immunoprecipitation, which indicates weak interaction between these proteins. However, the beta-integrin cytoplasmic domain is required for recruitment of FAK localization to focal adhesion plaques (28-30).

The primary sequence of the alpha subunit cytoplasmic domains is quite diverse with the exception of the highly conserved membrane proximal KXGFFKR sequence, which can interact directly with an intracellular calcium-binding protein, calreticulin (31). The interaction of calreticulin with alpha-subunits was suggested to play an important role in the regulation of integrin-affinity states (32). More recently, using yeast two-hybrid cloning another calcium binding protein, CIB, was found to interact with the cytoplasmic domain of the alpha IIb subunit (33).

There are many other intracellular proteins and membrane-associated proteins that were recently reported to interact with integrins. For example, regulatory proteins, such as insulin receptor substrate (IRS-1), were found to interact with the integrin upon growth factor stimulation. Others include the insulin receptor and platelet-derived growth factor (PDGF) receptor (34, 35). Integrin-associated protein (IAP or CD47) is a transmembrane protein that associates with the alpha v beta 3 and alpha IIb beta 3 integrins (36, 37). In addition, members of the transmembrane-4 (TM4SF) superfamily (CD9, D37, CD53, CD63, CD81 and CD82) interact with the extracellular domains of the alpha and beta integrin subunits (38-41). Reciprocal co-immunoprecipitations have established that alpha 3 beta 1, alpha 4 beta 1 and alpha 6 beta 1 integrins, but not other integrin receptors, associate with TM4SF proteins.

In addition to the direct interactions with integrin cytoplasmic domains discussed above, a number of cytoskeletal and signaling molecules have been shown to co-localize with integrins upon ligand binding and receptor clustering. The idea that integrins directly regulate the spatial arrangement of these molecules came from the initial observation that integrins form clusters at sites of tight cell-substrate adhesion contacts (focal adhesion contacts), which serve to anchor the cell to the extracellular matrix. Yamada and colleagues (16) have proposed a model of hierarchical association of cytoskeletal and signaling molecules based on experiments carried out with beads coated with ligands and anti-integrin antibodies, and the detection of colocalized intracellular proteins by immunofluorescence (13). This model suggests that integrin clustering triggers the accumulation of 20 signaling molecules, including FAK, and the cytoskeletal molecules alpha-actinin, talin, tensin, vinculin, paxillin and F-actin. Accumulation of some proteins appears to require tyrosine kinase activity (tensin) but others do not (FAK or vinculin). This model shows the complexity of integrin-cytosolic protein interactions, but does not yet account for the complex three-dimensional matrix composed of multiple ECM proteins and growth factors normally encountered by certain cells as part of their physiological function.

3.2. Integrin-mediated signal transduction

The signaling pathways activated by integrins have been identified through the analysis of biochemical events that are triggered by integrin engagement, and by the

indentification of proteins that associate with focal adhesion complexes. These signaling pathways control activation of both protein tyrosine kinases and members of the Rho family of small GTP-binding proteins (42).

Protein phosphorylation is one of the earliest events detected upon integrin stimulation. Increased tyrosine phosphorylation has been shown to be a common response to integrin engagement in many cell types including platelets, fibroblasts, carcinoma cells, and leukocytes (2, 43). Adhesion of fibroblasts to ECM proteins or antibody-mediated clustering of integrins, leads to tyrosine phosphorylation of several cellular proteins, including members of the Src-family kinases, FAK, paxillin and tensin (2).

FAK appears to be a key signaling molecule which is recruited and activated by integrins as a major conduit for intracellular signal transduction. FAK is ubiquitously expressed in many tissues. It is comprised of a central kinase domain flanked by two large non-catalytic domains (44). The primary event in response to integrin-dependent activation is autophosphorylation of FAK at Tyr397 in the N-terminal domain which creates a high affinity binding site within FAK for Src or Fyn (45). The formation of these bipartite protein tyrosine kinase complexes parallels the assembly of focal adhesion contacts and the subsequent activation of intracellular signaling pathways. The C-terminal domain of FAK contains sequences required for focal adhesion localization, as well as binding sites for paxillin (46). In addition, Tyr925 in FAK serves as a binding site for the SH2 domain of the Grb-2 adaptor protein (47). The FAK C-terminal domain also contains two conserved proline-rich motifs. The motif proximal to the kinase domain binds to the SH3 domain of p130cas and the other one can bind to the SH3 domain of Graf (GTPase regulator associated with FAK) (48, 49). The association of FAK with these cytoskeletal and signaling molecules implies that FAK is involved in integrin-mediated signaling pathways which lead to cytoskeletal organization as well as stimulation of cell proliferation.

In addition to the direct interaction of FAK with Graf, activated Rho and other members of the Rho family of small GTP-binding proteins, Rac and Cdc42, also play a role in regulating the formation of focal adhesions and the actin cytoskeleton (50). Inhibition of Rho activity by C3 transferase, a bacterial exoenzyme that ADP ribosylates endogeneous Rho proteins, blocks the LPA-induced formation of focal adhesions and actin stress fibers (51, 52). Rho appears to be upstream of FAK, since C3 inhibition of Rho blocks both LPA-dependent and adhesion-dependent activation of FAK. Rho-GTP has been reported to bind to and to stimulate the activity of a phosphatidylinositol 4-phosphate 5'-kinase, which regulates the synthesis of phosphatidylinositol 4,5-bisphosphate (PIP2) (53). Interestingly, PIP2 has been suggested to regulate a number of protein-protein interactions that are central for the assembly of actin stress fibers and to the re-arrangement of the actin cytoskeleton (54).

The recent discovery of a structurally related protein tyrosine kinase, PYK2/CAK-beta/RAFTK, now defines a FAK family (55-57). PYK2 and FAK are structurally homologous (65%). PYK2 expression was detected predominantly in neuronal and hemopoeitic cells, including the human CD34(+) marrow cells, megakaryocytes, platelets and in various lymphocytic and myeloid cell lines (57, 58). In these cell types, PYK2 is co-expressed with FAK, but was suggested to play a role distinct from FAK (59-62). In megakaryocytes and B cells, PYK2 was shown to be activated and phosphorylated in an integrin-dependent manner and localized to "focal adhesion-like structure" (63, 64). *In vitro* association between PYK2 and the protein tyrosine kinases Src, Fyn, as well as p130cas, paxillin and Grb-2 was demonstrated (55, 59, 63-66). Recently, a role for PYK2 and Src in linking G-protein-coupled receptors with mitogen-activated protein kinase activation was also reported (59).

4. INTEGRINS IN OSTEOCLASTS

Osteoclasts are multinucleated, terminally differentiated cells which are responsible for degradation of mineralized matrix (67-69). During the bone resorption cycle, osteoclasts undergo differentiation, attachment to the calcified matrix, cytoskeletal organization to form the sealing zone and ruffled border, followed by the secretion of acid and lysosomal enzymes into the space beneath the ruffled border. In this section, we discuss the role of integrins in the regulation of two fundamental steps in osteoclast activity: osteoclast adhesion and cytoskeletal organization for cell migration and formation of sealing zones and ruffled borders.

4.1. Expression of integrins in osteoclasts

Osteoclasts express very high levels of the alpha v beta 3 integrin. The expression of this integrin in osteoclasts has been demonstrated in a variety of species, from birds to humans (70, 71). Mammalian osteoclasts also express other integrins at lower levels, such as the collagen/laminin receptor alpha 2 beta 1 and the vitronectin/fibronectin receptor alpha v beta 1 (72-74). Other integrins reported to be expressed in precursors of osteoclast-like cells generated *in vitro* from co-cultures of mouse bone marrow and osteoblasts, are Mac-1 (alpha M beta 2) and alpha 4 beta 1 (75, 76). Avian osteoclasts apparently express other integrins including the vitronectin receptor alpha v beta 5, the fibronectin receptor alpha 5 beta 1 and possibly beta 2 integrins (68, 77).

Adhesion of osteoclasts to the bone surface involves the interaction of integrins with extracellular matrix proteins within the bone matrix. Rat osteoclasts adhere in an alpha v beta 3-dependent manner to ECM proteins containing RGD sequences that are found in bone, such as osteopontin, bone sialoprotein and denatured collagen type I (78-80). More recently, it was reported that rat osteoclasts adhere to native collagen type I using alpha 1 beta 1 integrin, surprisingly in an RGD-dependent manner (81). Moreover, osteoclastic bone resorption is partially inhibited by both anti-alpha 2 and anti-beta 1 antibodies (82). On the other hand, soluble osteopontin and

RGD peptides inhibit avian osteoclast attachment and bone resorption. Collagen appears to make a minimal contribution to avian osteoclast adhesion and function (83).

4.2. The Vitronectin Receptor alpha v beta 3

During recent years, the vitronectin receptor alpha v beta 3 received considerable attention as a target for inhibition of bone resorption. Although alpha v beta 3 is known as a receptor for vitronectin, it binds to other RGD-containing ECM proteins, such as osteopontin, bone sialoprotein and to some extent fibronectin and a cryptic RGD-site in denatured collagen. Alpha v beta 3 integrin is expressed only in a limited number of cell types, and the cells with highest *in vivo* expression of alpha v beta 3 are the osteoclasts. Lower levels are found in platelets, megakaryocytes, some vascular smooth muscle, endothelium, kidney and placenta. This integrin is upregulated in certain pathologies, such as malignant melanoma, and in several *in vitro* cultured adherent cell lines. Alpha v-containing integrins, other than beta 3, are much more widely expressed in normal tissues (84). Interestingly, alpha v beta 3 has been recently shown to mediate cell-cell interaction by binding to two membrane-associated glycoproteins of the immunoglobulin superfamily, CD31/PCAM-1 and L1 adhesion molecule, the latter via an RGD sequence in one of its immunoglobulin domains (85, 86). In addition, alpha v beta 3 was demonstrated to bind an RGD sequence in adenoviral penton base coat protein (87). These latter findings suggest that this receptor may mediate a wide range of physiological and pathological processes.

4.3. Alpha v beta 3-mediated osteoclast function

The alpha v beta 3 integrin was suggested to play a major role in osteoclast function. Interference with alpha v beta 3, using a variety of approaches, leads to inhibition of bone resorption both *in vitro* and *in vivo*. The first evidence that alpha v beta 3 may play an important role in osteoclast function was obtained when a monoclonal antibody, 13C2, raised against osteoclasts was found to inhibit bone resorption *in vitro* (88). Later, Davies *et al*. (1989) identified the antigen of this antibody as the vitronectin receptor alpha v beta 3, which was subsequently demonstrated to be the most abundant integrin in osteoclasts (72, 89). Furthermore, osteoclastic bone resorption *in vitro* can be inhibited by RGD-containing peptides and proteins or by blocking antibodies to alpha v beta 3 (90-93).

Inhibition of alpha v beta 3 integrins can also block bone resorption *in vivo*. In the thyroparathyroidectomized (TPTX) model, co-infusion of echistatin and parathyroid hormone (PTH) completely blocked the PTH-induced increase in serum calcium (94). This observation was confirmed by King *et al*. (1994) using another RGD-containing disintegrin, kistrin (92). Echistatin was also shown to inhibit bone loss in the hypercalcemic mice maintained on a low calcium diet (95). In the bones of these animals, echistatin co-localizes with alpha v beta 3 integrins in osteoclasts (96). Recently, to obtain definitive proof that inhibition of bone resoption by integrin ligands was indeed mediated by alpha v beta 3, Crippes *et al*. (1996) demonstrated that infusion of anti-rat beta 3 integrin subunit antibody (mAb F11) blocked the effect of PTH on serum calcium in TPTX rats (97). Furthermore, recent *in vivo* studies demonstrated that echistatin or RGD-peptidomimetics inhibit bone resorption in ovariectomized rodents, probably by blocking the function of alpha v beta 3 integrins (98, 99). In these recent studies, the inhibition of bone loss was determined both by histomorphometry and bone density measurements.

Although the above findings indicate that alpha v beta 3 integrin has an important rate limiting function in osteoclasts, its mechanism of action in the osteoclast is far from fully understood. Several studies have demonstrated that alpha v beta 3 integrin plays a role in the initial adhesion events in osteoclasts (74, 89, 100). Recognition of extracellular matrix components by osteoclasts is an important step in initiating osteoclast function (89, 100, 101). Integrin-mediated cell adhesion to vitronectin, fibronectin or collagen was reported to induce cell spreading and actin rearrangement in osteoclasts (82), while addition of RGD-containing peptides or disintegrins (such as echistatin or kistrin) blocked osteoclast adhesion to bone surfaces or caused retraction in adhered osteoclasts (90, 92).

Expression of alpha v beta 3 integrin could also be detected in mononucleated tartrate-resistant acid phosphatase (Trap) positive cells during *in vitro* osteoclastogenesis in culture, and RGD-containing disintegrins, such as echistatin, inhibited the formation of multinucleated Trap (+) cells. This observation suggested that alpha v beta 3 may play a role in osteoclast differentiation *in vitro* (102), possibly by interfering with the migration of osteoclast precursors necessary for the fusion.

The presence of the RGD sequence in the bone matrix protein osteopontin led to the suggestion that attachment at the sealing zone may be mediated by integrins. It was reported that the vitronectin receptor alpha v beta 3 is enriched in clear zones of resorbing osteoclats (101, 103-105), and in podosomes of osteoclasts plated on glass (106). However, not all studies detected the presence of alpha v beta 3 in the sealing zone membrane apposed to the bone surface (96, 100, 107). In those studies, the vitronectin receptor was found to localize to the basolateral membranes, intracellular vesicles and ruffled border of the resorbing osteoclasts. Regardless of the precise localization of alpha v beta 3, integrins probably do not form the tight seal, according to their proposed structural features (70). Interestingly, in the echistatin-treated mice, where bone resorption was inhibited, the number of osteoclasts on the bone surface was increased rather than decreased and their electron microscopic morphology was found to be normal (95, 99). Inhibition of integrin function thus did not cause the detachment of osteoclasts from the bone surface, or changes in clear zone or ruffled borders (95). Alpha v beta 3 ligands (antagonists) may therefore inhibit bone resorption by a different mechanism *in vivo*, such as blocking integrin-mediated osteoclast migration.

5. INTEGRIN DEPENDENT SIGNAL TRANSDUCTION IN OSTEOCLASTS

Oteoclasts respond to integrin ligands, RGD-containing peptides and proteins, by changes in intracellular calcium and protein tyrosine phosphorylation. In rat, mouse, and human osteoclasts, RGD-containing peptides trigger a transient increase in intracellular calcium (108-110). Furthermore, RGD-induced calcium signaling in osteoclasts is unaffected by inhibition of either G-protein or tyrosine kinase activity (111). In parallel with changes in intracellular calcium, integrin-dependent signaling pathways also induce tyrosine phosphorylation and cytoskeletal reorganization in osteoclasts.

Insights into these signal transduction pathways have been provided by findings related to c-Src. Soriano *et al*. reported that the targeted disruption of c-src in mice induces osteopetrosis (112). Osteoclasts express high levels of c-Src and Src(-/-) mice have inactive osteoclasts that lack ruffled border (113). These observations indicated that signaling mediated by c-Src tyrosine kinase is probably involved in the polarization of osteoclasts. The product of the proto-oncogene c-Cbl, a 120 kDa protein, is tyrosine phosphorylated in response to the activation of various signaling pathways, including M-CSF stimulation in monocytes and v-Src transformation in fibroblasts. Tanaka *et al*. (1996) reported that the level of tyrosine phosphorylation of c-Cbl immunoprecipitated from Src(-/-) osteoclasts is markedly reduced, compared with that found in wild-type osteoclasts. Furthermore, c-Src is found to associate and co-localize with c-Cbl in the membranes of intracellular vesicles in osteoclasts (114).

Two independent studies reported the presence of FAK in osteoclasts and indicated that this molecule is possibly involved in osteoclastic bone resorption. FAK "knock out" mice were embryonic lethal, and shed no further light on the role of this molecule in osteoclast function. More recently, we and others identified PYK2 as a major adhesion-dependent tyrosine kinase in osteoclasts both *in vivo* and *in vitro* (115, 116). Ligation of alpha v beta 3 integrin by substrate-engagement or by antibody-mediated clustering increased PYK2 tyrosine phosphorylation. Moreover, in adherent osteoclasts, PYK2 was found to tightly associate with c-Src via its SH2 domain. In osteoclasts derived from Src(-/-) mice, which do not form actin rings and do not resorb bone, tyrosine phosphorylation and kinase activity of PYK2 are makedly reduced (117). Moreover, upon adhesion, PYK2 translocates into the Triton X-100 insoluble cytosketal fraction. PYK2 localizes to the podosomes and the sealing zones in resorbing osteoclasts plated on bone. The Src-dependent tyrosine phosphorylation of PYK2 is suggested to be involved in the adhesion-induced formation of the sealing zone, a pre-requisite for osteoclastic polarization.

Another integrin-dependent signaling molecule recently characterized in osteoclasts is $p130^{cas}$ (Cas, Crk associated substrate), shown to be highly tyrosine phosphorylated upon osteoclast adhesion to extracellular matrix proteins. In addition, $p130^{cas}$ localizes to the actin ring formed in osteoclasts on glass and to the sealing zone on bone (118). In adhering osteoclasts, $p130^{cas}$ is found to be associated with PYK2, suggesting that this adaptor molecule participates in the integrin-PYK2 signaling pathway (119).

In avian osteoclasts, alpha v beta 3 is associated with the signaling molecule phosphatidyl inositol 3-kinase (PI3-K), c-src and FAK (120) . In that system, interaction of alpha v beta 3 with osteopontin resulted in increased PI3-K activity and association with Triton-insoluble gelsolin (121). In murine osteoclasts formed in culture, PI3-K was found to translocate into the cytoskeleton upon osteoclast attachment to the bone surface (122). In addition, wortmannin, the potent inhibitor of PI3-K, inhibits mammalian osteoclastic bone resorption *in vitro* and *in vivo* (123, 124).

Another group of proteins found to regulate osteoclast function, are related to the Rho protein, a member of the family of small guanine nucleotide binding proteins that control cytoskeletal function (125). How Rho fits into the adhesion signaling pathway is presently not well understood. However, clostridium botulinum derived ADP-ribosyltransferase (C3 coenzyme), a potent inhibitor of Rho activation, inhibits actin ring and pit formation in osteoclasts, suggesting that Rho plays a role in bone resorption by regulating the cytoskeletal organization in osteoclasts.

This review discussed the role of integrin receptors in osteoclast activity. The finding that inhibition of alpha v beta 3 integrin leads to inhibition of bone resorption *in vivo* in rodent models has defined an important role for this receptor. However, many key questions remain to be answered regarding the down stream signaling pathways initiated by the alpha v beta 3 integrin and their physiological consequences. A summary on the signaling molecules involved in osteoclast adhesion and polarization known to date are illustrated in figure 1. Attempts to integrate the information, as described above, into a comprehensive model are hampered by the fact that many players have not yet been identified. A simplified model (figure 2), based on the current state of knowledge, suggests approximately the following sequence of events: osteoclasts' contact with mineralized bone extracellular matrix induces alpha v beta 3 receptor clustering and the initiation of intracellular signals that lead to phosphorylation of the adhesion kinase PYK2 by c-Src to which it binds via the SH2 domains. Implicit in this interaction is tyrosine phosphorylation of PYK2, either by the enzyme itself or some other kinase. This step, and additional as yet unidentified signals, possibly coming from osteoblast lineage cells, start a positive feedback cascade which causes actin reorganization and inclusion of several proteins in the cytoskeleton, including PI3-kinase, vinculin, paxillin, Rho and probably additional kinases and phosphatases. The protein-protein interactions involve phosphorylations and binding via SH2/SH3 domains probably occuring simultaneously rather than divided into upstream and downstream events. This leads to the formation of the actin rings and the actin-rich attachment

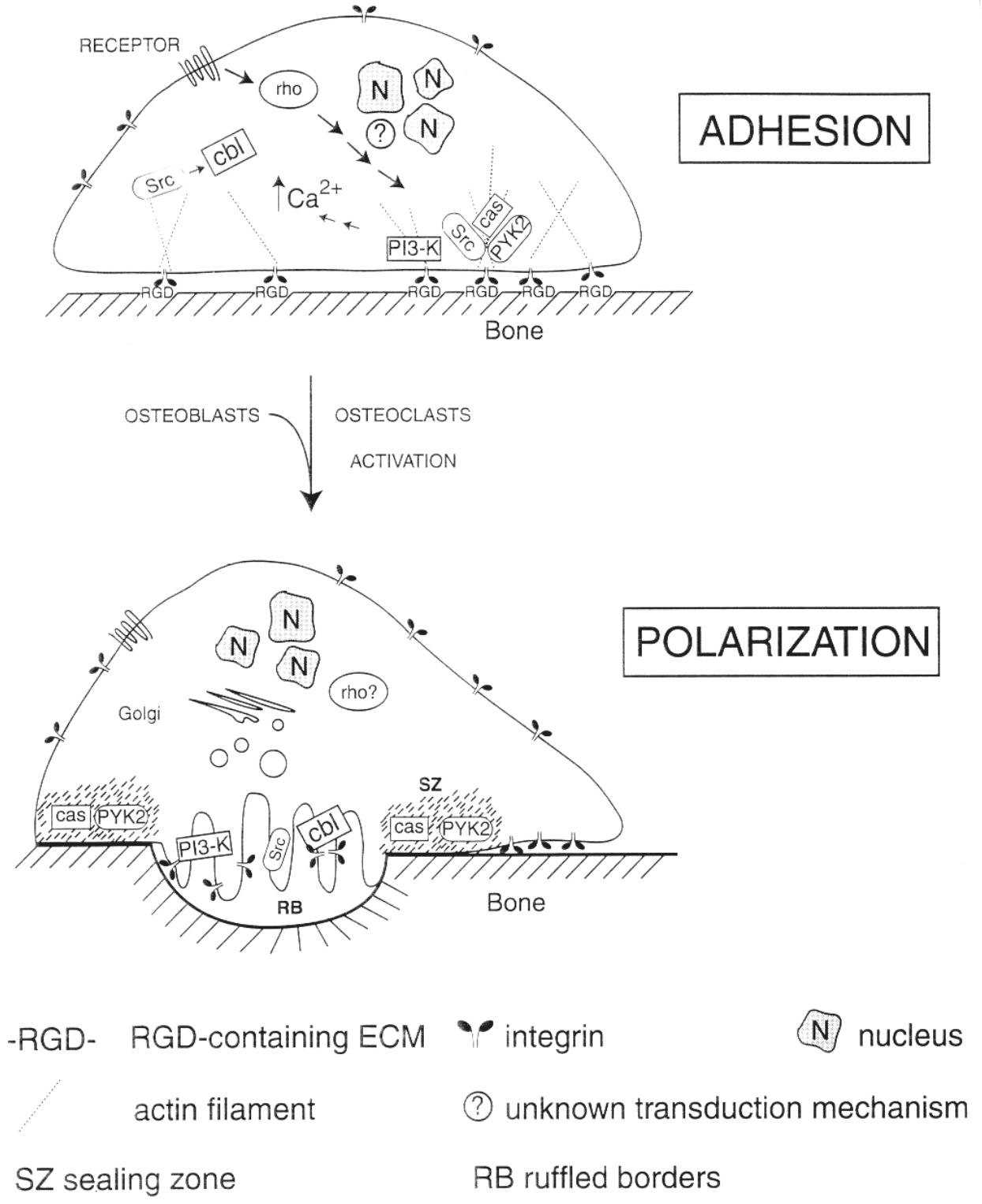

Figure 1. This figure summarizes current information and hypotheses regarding the role of alpha v beta 3 integrins and potential molecules participating in the adhesion-dependent signaling pathway(s) involved in osteoclast bone resorption.

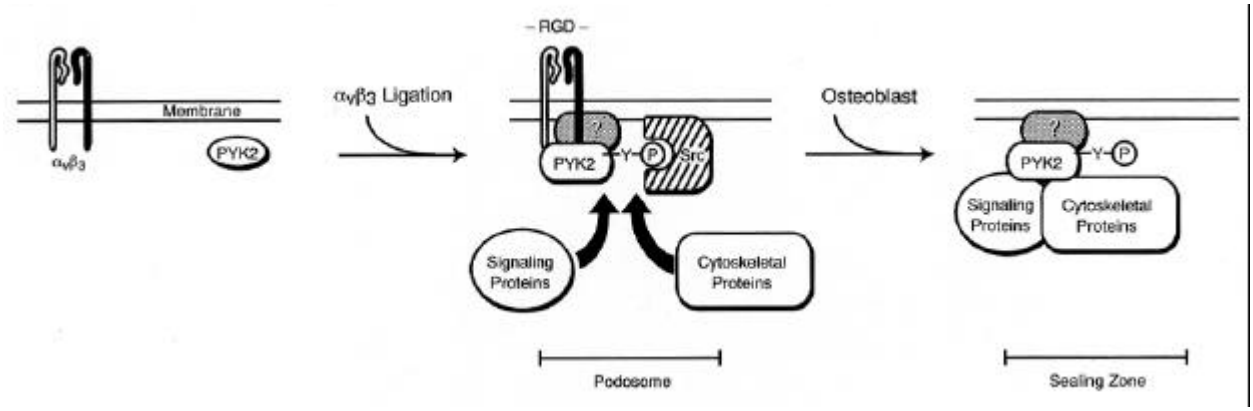

Figure 2. PYK2 participation in the activation of osteoclast-like cells. A schematic diagram depicting the involvement of PYK2 in organizing podosomes in osteoclasts. Ligation of alpha v beta 3 integrin results in recruitment and tyrosine phosphorylation (-Y-) of PYK2, which enables the binding of Src to PYK2. Assembly of mature podosomes requires additional recruitment of unidentified signaling proteins, and cytoskeletal proteins, such as paxillin, vinculin and F-actin. The nature of PYK2 interaction with the integrin cytoplasmic domains or with the membrane is not clear (represented by a boxed ?). Osteoblast-dependent effects enhance PYK2 association with the actin cytoskeleton and its kinase activity, leading to the further organization of podosomes into sealing zone in resorbing osteoclasts. Formation of mature sealing zone does not require the presence of alpha v beta 3 integrin and Src, but may require PYK2 for recruitment of other signaling cytoskeletal proteins.

zone, which contains many of these proteins, but not c-Src. At the same time, there is extensive vesicular traffic involving the microtubules, during which vesicles are inserted into the membrane to form the ruffled border, circumscribed by the attachment zone. This probably brings the proton ATPase to the cell surface and leads to the acidification that causes mineral dissolution and the release of lysosomal enzymes that digest the matrix proteins. The degradation products are taken up by other vesicles and are transported to the other side of the cell, another aspect of cytoskeletal function. The signals that usually terminate osteoclast activity during bone remodeling have not yet been identified.

6. PERSPECTIVE

In the sequence of events enumerated above, the alpha v beta 3 integrin was shown in experimental *in vitro* and *in vivo* studies to play a rate-limiting role in osteoclastic bone resorption. Exposure of isolated osteoclasts in culture to alpha v beta 3 ligands, peptides and non-peptides, as well as the injection of such ligands to rodents, effectively blocked bone loss in secondary hyperparathyroidism and estrogen deficiency. The demonstration that small molecular weight, non-peptides can produce this effects raises the possibility of developing therapeutic agents that could prove effective in diseases associated with increased bone resorption, such as osteoporosis, by interfering with the interaction of the alpha v beta 3 osteoclast integrin with its *in vivo* ligand.

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