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Rho GTPases in osteoclasts: orchestrators of podosome arrangement

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Abstract

Cells from the myeloid lineage, namely macrophages, dendritic cells and osteoclasts develop podosomes instead of stress fibers and focal adhesions to adhere and migrate. Podosomes share many components with focal adhesions but differ in their molecular organization, with a dense core of polymerized actin surrounded by scaffolding proteins, kinases and integrins. Podosomes are found either isolated both in macrophages and dendritic cells or arranged into superstructures in osteoclasts. When osteoclasts resorb bone, they form an F-actin rich sealing zone, which is a dense array of connected podosomes that firmly anchors osteoclasts to bone. It delineates a compartment in which protons and proteases are secreted to dissolve and degrade the mineralized matrix. Since Rho GTPases have been shown to control F-actin stress fibers and focal adhesions in mesenchymal cells, the question of whether they could also control podosome formation and arrangement in cells from the myeloid lineage, and particularly in osteoclasts, rapidly emerged. This article considers recent advances made in our understanding of podosome arrangements in osteoclasts and how Rho GTPases may control it.

Contents

Introduction

Rho family GTPases belong to the Ras superfamily of small GTP-binding proteins. Like Ras, they are activated by guanine nucleotide exchange factors (GEF) that catalyze exchange of GDP into GTP. Once bound to GTP, RhoGTPases adopt an active conformation to bind effectors that will transduce downstream signals. Small GTPases return in their inactive state by hydrolyzing GTP into GDP. Small GTP-binding proteins have limited intrinsic GTPase activity but GTPase activating proteins (GAP) accelerate GTP hydrolyzation rate leading to an inactive, GDP-bound GTPase. RhoGTPases are thus considered as molecular switches that need to be tightly regulated to ensure regular cell functions.

Rho GTPases are now well known to control F-actin and adhesion structure organization (for reviews, see Burridge and Wennerberg, 2004; Fukata et al., 2003). The current paradigm states that activation of RhoA induces stress fibers and focal adhesion assembly, whereas active Rac1 promotes lamellipodium and focal complexes formation and active Cdc42 induces actin polymerization to form thin protrusions named filopodia. Spatiotemporally coordinated activation of RhoA, Rac1 and Cdc42 is required for effective cell migration. Most studies have addressed the functions of Rho GTPases in cells developing F-actin stress fibers and focal adhesions. However, cells from the myeloid lineage namely macrophages, dendritic cells and osteoclasts develop podosomes to adhere and migrate and less is known about Rho GTPases function on podosomes. Podosomes are constitutive of cells from the myeloid lineage, but they have now been described in various cell types and they can be induced in response to growth factors, chemicals or expression of oncogenes (for review, see Linder and Aepfelbacher, 2003). Interestingly, although podosomes are the major F-actin structures found in macrophages and dendritic cells, only in osteoclasts podosomes can organize into superstructures referred to as podosome belt and sealing zone.

In this review, we describe the recent advances made in our understanding of podosome organization in osteoclasts and how Rho GTPases may control it. For clarity reason, we will use the term Rho GTPases when we will refer to the entire family of proteins and the term RhoA when we will describe the function of RhoA, B or C even if tools used in most studies do not allow discrimination between these three members.

Podosomes as structural units of osteoclast adhesions

Podosomes have been first described in cells transformed by the Rous Sarcoma Virus and referred to as electron-dense protrusions of the ventral plasma membrane of Src-transformed cells (David-Pfeuty and Singer, 1980; Tarone et al., 1985). Podosomes were then described as major adhesion structures found in monocytes and osteoclasts (Marchisio et al., 1984). They are formed by a F-actin core surrounded by a ring of scaffolding proteins such as vinculin, paxillin or talin. Interference-reflection microscopy indicated that the ring is in close proximity to the substratum suggesting that it mediates cell adhesive property (Marchisio et al., 1984). In agreement with that observation, integrins are localized at the ring and excluded from the core.

Although podosomes can be induced by cytokines, oncogenes or chemicals in many cell types, they constitutively form in cells from the myeloid lineage (for review see Linder, 2007). Osteoclasts possess unique podosomal organization since podosomes can assemble into superstuctures which consist of different kinds of podosome arrangements. Osteoclasts seeded on glass develop podosomes that are first grouped into clusters, which assemble into small podosome rings and eventually into a podosome belt. The frequency of podosome belt formation increases when osteoclasts are maintained in culture leading to the notion that changes in podosome arrangements are the result of maturation, starting from podosome clusters and evolving towards podosome belt (Destaing et al., 2003). When seeded on bone or bone-like substrate (hydoxyapatite-embedded collagen), osteoclasts polarize and develop a large and dense F-actin ring, called the sealing zone. It delineates a compartment in which osteoclasts secrete protons and proteases to dissolve and degrade the mineralized matrix. The sealing zone also mediates strong anchorage to the bone. In this structure, individual podosomes can not be distinguished by classical fluorescence microscopy raising the question of whether indeed all osteoclast adhesion structures evolve from a single substructure, the podosome (Saltel et al., 2004, reviewed in Jurdic et al., 2006). Recently, high resolution scanning electron microscopy combined with fluorescence microscopy unambiguously showed that the sealing zone constitutes densely interconnected arrays of podosome cores. Podosomes consist of bundled F-actin that forms a core to which radial actin fibers are connected, referred to as the F-actin cloud by Destaing et al. (2003). The density of podosome cores and the degree of inter-connectivity of radial fibers increases between each superstructures, being minimal in podosome clusters and maximal in the sealing zone (Luxenburg et al., 2007).

Therefore, the podosome appears to be the structural unit of F-actin structures found in osteoclasts and the degree of podosome "compaction" gives rise to different superstructures (Fig.1).

Rho GTPases control podosome arrangement in osteoclasts

In fibroblastic cell lines, activation of RhoA, Rac1 or Cdc42 induces the formation of specific F-actin and adhesion structures (for review, see Raftopoulou and Hall, 2004). The question then arose whether a specific Rho GTPase could control podosome formation.

The use of bacterial toxins known to inhibit Rho GTPases (for review see Aktories and Barbieri, 2005) has brought the first evidences that their activity was required for maintaining podosome organization. Treatment of osteoclasts with the exoenzyme C3 of *Clostridium botulinum*, which inhibits RhoA, RhoB and RhoC, blocks the resorbing activity of osteoclasts by disrupting the sealing zone (Zhang et al., 1995). In macrophage polykaryons, exoenzyme C3 causes cells to spread out and podosomes to disappear (Ory et al., 2000). Conversely, activation of RhoA can not induce podosome formation. Podosomes rather disappear or are redistributed away from the cell periphery when activated RhoA is microinjected or when RhoA is activated by osteopontin (Ory et al., 2000; Chellaiah et al., 2000).

Nonetheless, recent studies support a crucial function of RhoA in controlling podosome arrangement in osteoclast. Osteoclasts seeded on bone-like substrates devevelop an F-actin-rich sealing zone and adopt a polarized morphology by being thicker and less spread out than osteoclasts maintained on glass. This is correlated with high basal RhoA activity. When RhoA is inhibited, the sealing zone is disrupted. Instead, a podosome belt, a superstructure rarely seen in osteoclasts maintained on bone, is formed (Saltel et al., 2004). These observations suggest that RhoA may control sealing zone formation and that optimal RhoA activation is obtained when osteoclasts are seeded on bone. On the other hand, it also indicates that the podosome belt is formed when RhoA activation is minimal (Fig.1). Experiments with osteoclasts maintained on glass show that podosome belt formation is indeed accelerated when RhoA is inhibited (Destaing et al., 2005). It would thus be expected that RhoA activation would trigger sealing zone formation. However, microinjection of active RhoA is not sufficient to form a sealing zone in osteoclasts seeded on glass. Instead, the podosome belt is disrupted and podosome rings and clusters are formed (Ory et al., 2000; Destaing et al., 2005). Hence, RhoA activation is not sufficient by itself to promote sealing zone formation in the absence of signals emanating from the mineralized extracellular matrix.

Although much less is know concerning Rac1 and Cdc42 in the control of podosome formation, macrophage polykaryons also have disorganized podosomes when activated or dominant negative Rac1 are microinjected (Ory et al., 2000). In addition, expression of activated Cdc42 leads to podosome and podosome belt disruption in macrophages and osteoclasts respectively (Linder et al., 1999, Chellaiah, 2005).

Thus, unlike F-actin and adhesion structures in mesenchymal cells, activation of neither RhoA, Rac or Cdc42 can induce podosome assembly in cells from the myeloid lineage. But modulating their activities clearly impinges on podosome organization.

What else beyond RhoA, Rac1 and Cdc42 in osteoclasts?

The Rho family GTPases is composed of 20 members (Boureux et al., 2007). Most studies have addressed the function of RhoA, Rac1 and Cdc42 on F-actin organization and cell adhesion. Since these GTPases can not induce podosome formation but rather regulate their arrangement, we attempted to get a better picture of which Rho GTPases are expressed in osteoclasts. Using quantitative PCR analysis, we established the expression profile during the differentiation process of osteoclasts of 18 Rho GTPases. RhoBTBs were excluded to restrict the study to the low molecular weight Rho GTPases. Interestingly, among the 18 GTPases, only RhoU/Wrch-1 was upregulated whereas other Rho GTPases were either not expressed (RhoH, TCL/RhoJ, Rac3, RhoD, Rnd3) or expressed at constant levels (Brazier et al., 2006). RhoU is related to Rac and Cdc42 and like them, it binds and activates PAK1. But RhoU possesses additional domains at its N- and C-termini: RhoU N-terminal extension is involved in binding to SH3-containing proteins such as Nck1 and Grb2 (Saras et al., 2004; Shutes et al., 2004) whereas the C-terminal extension participates to the correct subcellular localization of RhoU (Ory et al., 2007). Its subcellular localization is also in part driven by the addition of palmitoyl motif rather than a prenyl group, which is usually added on the CAAX box of Rho GTPases (Berzat et al., 2005). The palmitoylation localizes RhoU to endomembranes that partially colocalize with endosomes (Berzat et al., 2005).

When we overexpressed RhoU in osteoclasts, we found that it localizes to the podosome belt of osteoclasts seeded on glass, around the actin core of individual podosomes and to the sealing zone of osteoclasts seeded on bone-like substrate. This localization is dependent on the ability of RhoU to bind to effectors since a RhoU mutant unable to bind the Cdc42/Rac interaction domain of PAK is only found on vesicles (Ory et al., 2007; HB, unpublished data). Like many components of podosomes, RhoU is also found in focal

adhesions where it alters their dynamics and, in consequence, the migration rate of fibroblasts (Ory et al., 2007; Chuang et al., 2007). The C-terminal extension of RhoU and residues critical for effector binding are required for targeting RhoU to focal adhesions (Ory et al., 2007). Whether RhoU affects podosome organization of osteoclasts remains to be addressed but the fact that RhoU may activate the RhoA pathway (Saras et al., 2004) and localizes to osteoclast podosomes (Ory et al., 2007) identifies it as an attractive candidate for the control of podosome arrangement in osteoclasts.

Effectors recruited and activated by RhoU still need to be identified. In vitro, RhoU can interact with and activate proteins from the p21-activated kinase (PAK) family. Nothing is known about the function of PAK proteins in osteoclasts, but a recent study shed light on their potential role in the control of podosome formation in macrophages. PAK4 localizes to podosome cores in macrophages. In these cells, PAK4 kinase activity regulates the size of individual podosomes suggesting that PAK4 activity may interfere with F-actin polymerization. In addition, PAK4 mutants impaired in GTPase binding diminish the number of podosome per cell (Gringel et al., 2006). Since Rho GTPases affect PAK4 localization rather than its activity (Abo et al., 1998), it suggests that Rho GTPases may act to correctly localize PAK proteins to podosome in order to modulate their dynamic and organization.

Rho GTPase signaling and osteoclast adhesion

Integrins are extracellular matrix receptors that activate signaling pathways upon ligand binding. The major integrin found in osteoclasts is $\alpha_v\beta_3$, a ligand for vitronectin and osteopontin. Mice in which the β_3 gene has been deleted present a mild osteosclerotic phenotype due to osteoclast defects (McHugh et al., 2000). This may be a consequence of impaired osteoclast migration and reduced activation of Rho GTPases in response to growth factors. Indeed, macrophage stimulating factor (M-CSF) or hepatocyte growth factor (HGH) induces cytoskeletal remodeling of osteoclast by activating both RhoA and Rac proteins. As a consequence, osteoclast migration is increased (Faccio et al., 2003). In β_3 -/- osteoclasts, RhoA and Rac activation is impaired and osteoclast migration in response to M-CSF or HGF is defective. Reexpression of full length β_3 integrin restores both migration and Rho GTPases activation, whereas a mutant β_3 integrin lacking the C-terminal domain or bearing a Ser752 to Pro mutation does not (Faccio et al., 2003). It shows that RhoA and Rac are activated downstream of β_3 integrin and that this is essential for osteoclast migration and function.

In addition, β_3 integrin binds to Src and Pyk2 (Pfaff and Jurdic, 2001; Butler and Blystone, 2005; Ross and Teitelbaum, 2005), two tyrosine kinases critical for osteoclast biology, which may also regulate the function of Rho GTPases. Src and Pyk2 (a tyrosine kinase highly expressed in osteoclasts and related to the focal adhesion kinase FAK) ensure normal podosome assembly and disassembly cycle when osteoclasts adhere (Duong et al., 1998; Sanjay et al., 2001). Src null mice have a severe osteopetrotic phenotype (Soriano et al., 1991). Osteoclasts can not form a sealing zone and podosomes in Src -/- osteoclasts are less dynamics and structurally disorganized (Destaing et al. 2007). On the other hand, the deletion of Pyk2 in mice leads to increased bone mass due to osteoclast defects. Interestingly, Pyk2 null osteoclast seeded on glass can not form a podosome belt because of increased basal RhoA activity. Podosome belt formation in Pyk2-/- osteoclasts can be restored by RhoA inhibition but not by a constitutively activated Src, despite its potential role in down regulating RhoA activity (Gil-Henn et al., 2007; Flevaris et al., 2007; Arthur et al., 2000; Fincham et al., 1999). Indeed, Pyk2 and Src may form a complex in which p190 RhoGAP is recruited, phosphorylated and activated in a Src-dependent manner (Arthur et al., 2000; Zrihan-Licht et al., 2000). It suggests that in osteoclasts seeded on glass, once activated, integrin $\alpha_v\beta_3$ may spatially coordinate Src/Pyk2/RhoGAP multimolecular complexes, and Pyk2 may serve to localize GAPs correctly and control Src-dependent RhoA inactivation. Interestingly, recent findings have reported a Src-dependent interaction between RhoU and Pyk2 in porcine aortic endothelial cells (Ruusala and Aspentröm, 2007). In these cells, RhoU induces Pyk2- and Src-dependent stress fiber dissolution. RhoU binding to Pyk2 requires its proline rich region, but similarly to what we found (Ory et al., 2007), RhoU-dependent effects on the actin cytoskeleton also requires an intact effector binding site (Ruusala and Aspentröm, 2007).

Although $\alpha_v\beta_3$ appears to control RhoA and Rac activation in migrating osteoclasts, its function on matrix-dependent remodeling of podosomes is unlikely. Indeed, $\alpha_v\beta_3$ is in a low-affinity state when localized around podosomes (Faccio et al., 2002). Upon activation, $\alpha_v\beta_3$ leaves podosome and redistributes to newly formed protrusions resembling lamellipodia (Faccio et al., 2002; 2003). There is thus a discrepancy between the sites of $\alpha_v\beta_3$ activity (and by extension RhoA) and the sites of podosome remodeling that is supposed to occur for osteoclasts to move. But, by still unclear mechanisms, $\alpha_v\beta_3$ –dependent RhoA activation may regulate other extracellular matrix receptors, which could have critical functions for podosome assembly and osteoclast migration. Osteopontin (OPN) is an extracellular matrix

protein that can bind to both the hyaluronan receptor CD44 and $\alpha_v\beta_3$ integrin. OPN-deficient osteoclasts are hypomotile and defective in bone resorption, the amount of CD44 associated to the plasma membrane and exposed to the extracellular space is reduced (Chellaiah et al., 2003b). This may be due to reduced activation of the RhoA/ROCK pathway in absence of OPN secreted by osteoclasts (Chellaiah et al., 2003 a,b). Interestingly, OPN is sufficient to restore normal cell motility and bone resorption by OPN -/- osteoclast whereas vitronectin another ligand of $\alpha_v\beta_3$ integrin is not. In that context, OPN treatment stabilizes CD44 at the plasma membrane by activating the RhoA/ROCK pathway. This occurs through an $\alpha_v\beta_3$ – dependent pathway since ROCK activation is inhibited by a blocking antibody directed against $\alpha_v\beta_3$ integrins but not by one directed against CD44 (Chellaiah et al., 2003a,b). These experiments thus highlight a cross-talk between $\alpha_v\beta_3$ and CD44 signaling that remains to be better defined, but which may coordinate osteoclast migration and podosome rearrangement (see below).

WASp, a Cdc42 effector and a key organizer of podosomes

Rho GTPases mediate their effect through binding and activation of multiple effectors. Although little is known about most effectors of Rho GTPases in osteoclast biology, recent reports are pointing out to the Wiscott-Aldrich syndrom protein (WASp) as a key player in podosome organization of myeloid cells. WASp (expressed in hematopoietic tissues) and N-WASP (ubiquitous) contain several domains that regulate their activity and localization (for review, see Burns et al., 2004). By binding to Cdc42, to phosphatidyl inositol (4,5) bisphosphate (PI(4,5)P2) and to the actin nucleating Arp2/3 complex, WASP nucleates actin polymerization (Rohatgi et al., 1999). In macrophages from WAS patients in which WASp is truncated and not functional, podosome formation and macrophage chemotaxis are dramatically impaired (Zicha et al., 1998; Linder et al., 1999). Critical function of WASp is supported by analysis of dendritic cells or osteoclasts coming from WASp (Burns et al., 2001; Calle et al., 2004) and WIP (WASp interacting protein) null mice (Chou et al., 2006; Chabadel et al., 2007). As WIP binds and protects WASp from calpain-dependent degradation (de la Fuente et al., 2007), WASp expression levels are highly reduced in WIP null cells. The absence (WASp null cells) or reduced amounts (WIP null cells) of WASp protein result in defective podosome assembly. Recovery of normal WASp level by proteases inhibitors or, more surprisingly, by clustering of CD44 receptor, restores levels of WASp and normal

podosome formation (Chou et al., 2006; Chabadel et al., 2007). Interestingly, deletion of WIP only affects podosome core formation while the F-actin cloud is still present in osteoclasts (Chabadel et al., 2007). It thus defines two independent domains inside the podosome, the podosome core which coincides with the localization of actin polymerization regulating proteins (WASp, WIP, Arp2/3, gelsolin) and the F-actin cloud to which scaffolding proteins are recruited (paxillin, vinculin, talin).

Interestingly, OPN but not vitronectin is sufficient to restore normal podosome organization in WIP-/- osteoclasts suggesting that WASp function can be regulated by both the $\alpha_v\beta_3$ integrins and CD44 (Chellaih, 2005; Chellaih et al., 2007; Chabadel et al., 2007). Indeed, OPN increases $\alpha_v\beta_3$–dependent activity of the RhoA/ROCK pathway and CD44 exposure to the plasma membrane. It suggests that OPN may indirectly stabilize WASp to locally regulate F-actin polymerization (Linder et al., 2000; Chellaiah, 2005). WASp activation and subsequent actin polymerization could be further facilitated by RhoA-dependent formation of PI(4,5)P$_2$ that binds, activates and localizes WASp to podosomes in osteoclasts (Chellaiah, 2005). Thus, OPN may coordinate a functional network between $\alpha_v\beta_3$, CD44 and RhoA to synchronize osteoclast migration and bone resorption. It would be interesting to analyze Rho GTPases signaling and podosome rearrangements induced by OPN in $\beta_3^{-/-}$ osteoclasts to better address signaling events that are CD44 dependent. Finally, whereas WASp-/- mice have increased bone mass due to defective osteoclasts (Calle et al., 2004), CD44-/- mice have no clear bone phenotype. However, they show increased bone loss in a model of TNF-dependent chronic inflammatory arthritis (Hayer et al., 2005). This indicates that CD44 is dispensable for normal osteoclast function but may have a regulatory function in inflammatory diseases.

Exchange factors and osteoclast biology

Rho GTPases can be potentially activated by 69 GEFs with Dbl homology domain (diffuse B-cell lymphoma oncogene; for review, see Rossman et al., 2005) and 11 GEFs with CZH domains (CDM-zizimin1 homology; for review see Meller et al., 2005). Vav proteins (Vav1, Vav2 and Vav3) belong to the Dbl family. Vav1 is mainly expressed in hematopoietic tissues whereas Vav2 and Vav3 have broader patterns of expression (for review see Turner and Billadeau, 2002). Gene targeting of Vav in mice revealed that Vav3 is crucial for osteoclast function. Vav3-/- mice have increased bone mass because of impaired resorptive

function of osteoclasts. In vitro, Vav3-/- cells show disrupted podosome belt which is correlated with defects in Rac or Src activation in response to M-CSF or adhesion to the extracellular matrix, respectively (Faccio et al., 2005).

By using DNA microarrays, we analyzed the expression profile of the 80 GEFs and found that 2 GEFs were upregulated during osteoclastogenesis, one from the Dbl family (Arhgef8/Net1) and the other one from the CZH family (Dock5). Knocking down the expression of Net1 or Dock5 in osteoclast precursors dramatically impairs formation of multinucleated cells indicating that these 2 GEFs are critical for osteoclast differentiation and/or function in vitro (Brazier et al., 2006). Interestingly, preventing the expression of Dock5 in zebrafish impairs myoblast fusion (Moore et al., 2007) indicating that Dock5 may be involved in the process of osteoclast fusion.

Finally, αPIX, an exchange factor for Rac and Cdc42, localizes to podosome cores in macrophages and regulates the arrangement and number of podosomes. The recruitment of αPIX requires binding to GIT1 and its function on podosome arrangement is dependent on a functional GEF domain (Gringel et al., 2006). GIT proteins (GIT1 and GIT2) possess Arf GAP activity and can bind to paxillin. In addition to their potential enzymatic activity on Rho and Arf GTPases, PIX and GIT proteins are essential to the recruitment of signaling complexes including Rac1 and PAK1. They also integrate signals from Src and FAK to regulate focal adhesion dynamics (Brown et al., 2005; ten Klooster et al., 2006). The multimolecular complex PIX-GIT-PAK may thus be important for regulating Rho GTPases and podosome organization in osteoclast.

Concluding remarks

Much progress has been made in understanding the arrangement of podosomes in osteoclasts, but many questions remain open, especially on how the sealing zone of osteoclasts is formed and regulated. It is now clear that Rho GTPases and mineralized matrix play a critical regulatory function in actin cytoskeleton organization of osteoclasts. However, how the mineralized matrix or mineral by itself may affect RhoGTPase activity and then osteoclast function is an open question. An emerging theme is that adhesion strength and substrate stiffness regulate cell migration and adhesion structure organization (de Rooij et al., 2005; Gupton and Waterman-Storer, 2006). In turn, cells adjust their internal stiffness to match that of their substrate (Solon et al., 2007). In fibroblasts, distribution and life span of podosomes is affected by substrate rigidity and increased stiffness favors the formation of individual podosomes (Collin et al., 2006). The chemical and mechanical properties of bone or bone-like matrix are likely to affect osteoclast adhesive properties by modifying Rho GTPases signaling cascades. For example, the ultrastructural organization of the sealing zone, which is encountered only on bone-like substrate, suggests that the compaction of podosome cores could be the result of a RhoA-dependent increase in contractility mediated by the acto-myosin system that may connect podosome cores together. Spreading of osteoclasts following myosin inhibition by blebbistatin and high levels of activated RhoA in osteoclast maintained on bone-like substrate supports that hypothesis (Chabadel et al., 2007). This implies that RhoA has to be correctly localized and able to interact with its downstream effector ROCK. In addition, cytoskeletal tension and cell adhesion strength regulates RhoGTPase binding to their effector. Cells restrained in their spreading for instance display high RhoA but low ROCK activity (Bhadriraju et al., 2007). This suggests that mechanical properties of the substratum may impinge on RhoA signaling by preventing or restricting binding of the GTPase to a subset of downstream effectors. Thus, in order to better understand how Rho GTPases regulate podosome arrangement, we need to know where Rho GTPases are indeed activated in osteoclast and how efficient is the coupling of Rho GTPases with their downstream effectors.

Finally, the primary function of osteoclasts is to dissolve and resorb the mineralized matrix. This leads to high calcium concentration into the resorption lacunae. Part of the calcium is eliminated by transcytosis (Berger et al., 2001) but the inner part of the sealing zone is exposed to high calcium concentration as compared to the outer part. Many proteins critical for podosome organization are regulated by calcium. For example, Pyk2 can be

activated by calcium (Sanjay et al., 2001) and calpains are Ca2+-dependent proteases critical for osteoclasts function (Marzia et al., 2006) that may cleave β3 integrin (Flevaris et al., 2007), RhoA (Kulkarni et al., 2002), Pyk2 (Marzia et al., 2006) and WASp (de la Fuente et al., 2007). Change in calcium concentration affects podosome organization of osteoclast (Miyauchi et al., 1990) and knocking out the calcium transporter TRPV5 generates inactive osteoclasts (van der Eedern et al., 2005). It would thus be interesting to evaluate the consequences of change in calcium concentration during bone resorption on Rho GTPase signaling and how this may affect podosome arrangement.

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Figure legends

Schematic representation of podosome arrangements in osteoclasts. When seeded on glass, podosomes can assemble into clusters, podosome rings or a podosome belt. The density of podosome cores in each structure is depicted in the rectangle. The maximum density is achieved in the sealing zone, when osteoclasts are seeded on bone. RhoA controls podosome arrangement and RhoA activation is maximal on bone. Whereas the podosome belt can form at minimal RhoA activity on either glass or bone, the sealing zone only assembles on mineralized matrix. RhoA activation on glass results in podosome ring or cluster formation indicating that mineralized matrix may provide an additional signal for osteoclasts to form a sealing zone.

Figure1

Rho activity

podosome clusters → podosome rings → podosome belt ← *Exoenzyme C3* → sealing zone

glass

mineralized matrix
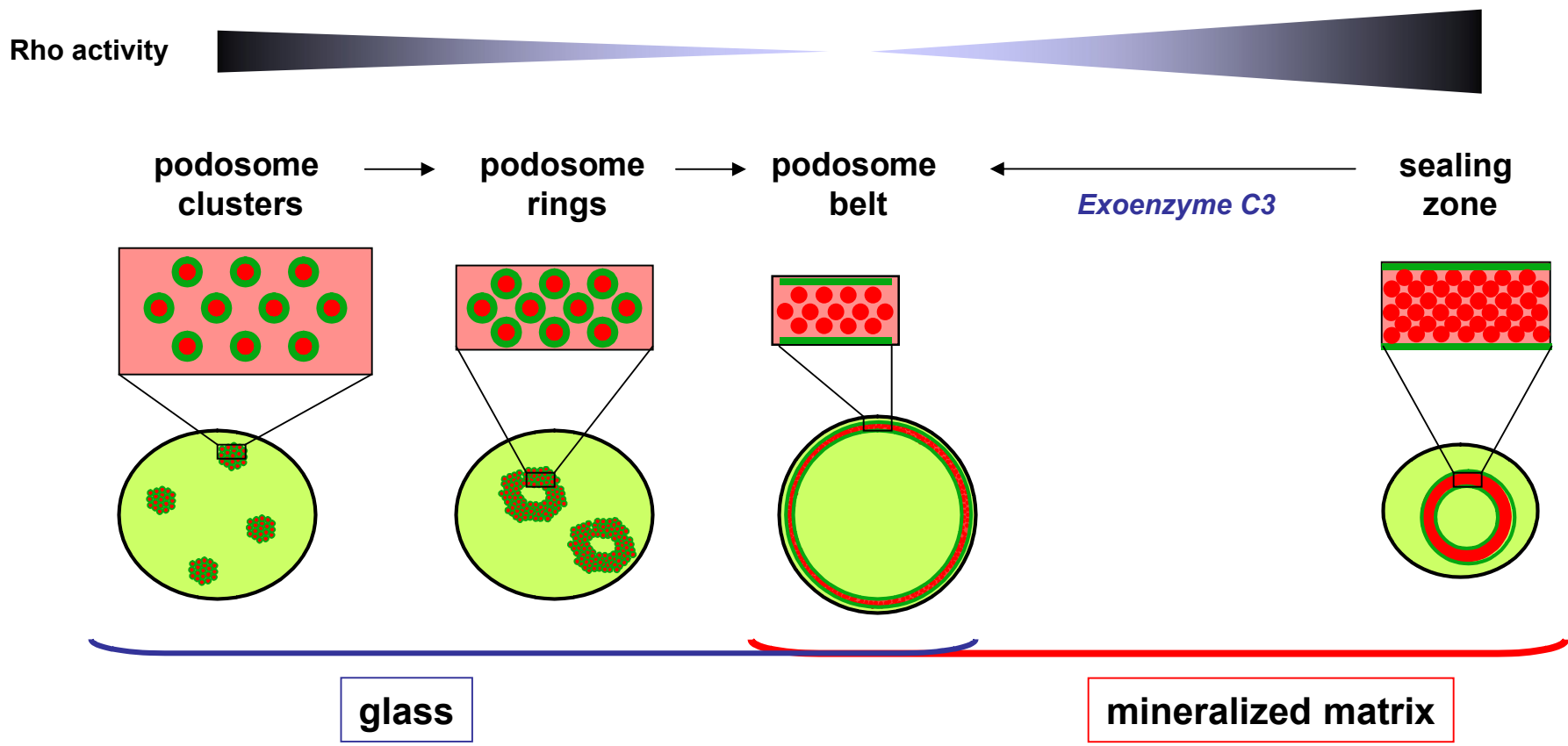
● podosome core (F-actin, CD44, WASp, Arp2/3, cortactin, gelsolin)

— Integrin b3, vinculin, talin, paxillin

▮ F-actin cloud, myosin