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Review

Role of protein-tyrosine phosphatases in regulation of osteoclastic activity

M. H.-C. Sheng[a, b] and K.-H. W. Lau[a, b, c, *]

[a] Musculoskeletal Disease Center, Jerry L. Pettis Memorial VA Medical Center, 11201 Benton Street, Loma Linda, CA 92357 (USA), Fax: (909) 796 – 1680, e-mail: William.Lau@med.va.gov
[b] Department of Medicine, Loma Linda University, Loma Linda, CA 92350 (USA)
[c] Department of Biochemistry, Loma Linda University, Loma Linda, CA 92350 (USA)

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Abstract. Osteoclasts, the primary cell type mediating bone resorption, are multinucleated, giant cells derived from hematopoietic cells of monocyte-macrophage lineage. Osteoclast activity is, in a large part, regulated by protein-tyrosine phosphorylation. While information about functional roles of several protein-tyrosine kinases (PTK), including c-Src, in osteoclastic resorption has been accumulated, little is known about the roles of protein-tyrosine phosphatases (PTPs) in regulation of osteoclast activity. Recent evidence implicates important regulatory roles for four PTPs (SHP-1, cyt-PTP-ε, PTP-PEST, and PTP-oc) in osteoclasts. Cyt-PTP-ε, PTP-PEST, and PTP-oc are positive regulators of osteoclast activity, while SHP-1 is a negative regulator. Of these PTPs in osteoclasts, only PTP-oc is a positive regulator of c-Src PTK through dephosphorylation of the inhibitory phosphotyrosine-527 residue. Although some information about mechanisms of action of these PTPs to regulate osteoclast activity is reviewed in this article, much additional work is required to provide more comprehensive details about their functions in osteoclasts.

Keywords. Protein-tyrosine dephosphorylation, osteoclast, c-Src, bone resorption, protein-tyrosine phosphatases, PTP-oc, cytosolic PTP-ε, PTP-PEST, SHP-1.

Bone and bone remodeling

Bone is a vital, dynamic connective tissue comprising a complex mixture of organic and inorganic materials. The large majority of the organic matter is type I collagen that is the basic building block of the bone matrix fiber network. The inorganic matter is composed primarily of calcium phosphate in the form of hydroxyapatite crystals. The cellular component of the bone is made up of three functionally distinct cell types: The first cell type is the osteoblast, which is derived from osteoprogenitor cells of the mesenchymal lineage [1]. Osteoblasts are the principal cell type that mediates bone formation by synthesizing bone matrix and by promoting mineralization of the newly synthesized matrix [1]. The second cell type is the osteocyte, which is the star-shaped, terminally differentiated osteoblast that is fully entrapped by the bone matrix [2]. Osteocytes are well situated within bone matrix to detect mechanical loading stress [3], have the ability to communicate with other bone cells through an extensive network of cellular processes [4], and respond to mechanical stimuli [5, 6]. Thus,

* Corresponding author.

osteocytes are thought to be the primary mechanical sensory cells in bone [3, 7]. The third cell type is the multinucleated, giant osteoclast, whose exclusive function is to degrade (resorb) bone. Bone not only provides mechanical strength for locomotion and protection of internal organs, but also plays a central role in mineral homeostasis in that it serves as the reservoir for key minerals (calcium and phosphorus) needed for normal cellular and bodily functions.

One of the remarkable features of mammalian bone is its ability to "rejuvenate" or "renew" itself constantly by replacing damaged bone with new, mechanically sound bone through the process known as bone remodeling. Bone remodeling is accomplished by the collaborative and sequential actions of osteoclasts and osteoblasts that are collectively referred to as the Basic Multicellular Unit (BMU) [8]. The BMU remodeling process is initiated by the resorption activation phase, which involves recruitment of mononucleated osteoclast precursors from the circulation, infiltration of the bone lining cell layer, and fusion of the osteoclast precursors to form multinucleated pre-osteoclasts [9]. These pre-osteoclasts then affix themselves to the bone matrix by the use of a specialized adhesion structures known as podosomes [10] to form a sealing zone. During the resorption phase, the pre-osteoclast is activated and becomes a functionally active osteoclast to initiate bone resorption. The resorption phase ends with apoptosis of the osteoclast [11] (Fig. 1), which is immediately followed by the bone formation phase, in which resorption lacunae releases appropriate signals to recruit osteoprogenitor cells to the surface of resorption lacunae and to stimulate differentiation of osteoprogenitor cells into functional osteoblasts to lay down and mineralize new bone matrix to refill the resorption lacunae [12]. Under normal healthy situations, the increase in bone resorption is coupled to a compensatory increase in equal magnitude in bone formation, such that no net gain or loss of bone mass results at a given BMU site. In aged bone or under pathologic conditions, this tightly coupled relationship of bone formation to resorption becomes defective and the compensatory increase in bone formation fails to match the initial increase in bone resorption [13]. This leads to a net loss of bone mass during each cycle of bone remodeling, and eventually results in a significant bone loss. Although an insufficient compensatory increase in bone formation is implicated as an etiologic factor for aging-associated bone loss [14], excessive bone resorption is the primary cause of bone loss in a number of metabolic bone diseases, including postmenopausal osteoporosis [15–19]. Accordingly, osteoporosis and related bone-wasting diseases can be effectively treated with anti-resorptive therapies, such as bisphosphonates, calcitonin, and selective estrogen receptor modulators [20]. In addition, bone resorption is essential for all aspects of bone physiology, such as growth, remodeling, and even the repair of fractures. That bone resorption is essential for normal bodily function is evidenced by the disabling consequences of osteopetrosis, a rare genetic disease of insufficient bone resorption, leading to too much bone that is brittle and also inadequate hematopoiesis due to insufficient bone marrow spaces [21]. Thus, understanding the molecular mechanisms and regulation of osteoclast resorption is critical for the understanding of the pathophysiology and treatment of various metabolic bone wasting diseases.

Osteoclasts and bone resorption

Osteoclasts are terminally-differentiated, multinucleated, giant cells derived from the monocyte/macrophage hematopoietic cell lineage [22]. These cells are rich in tartrate-resistant acid phosphatase (TRACP) activity [23]. During bone resorption, mononucleated osteoclast precursor cells in the marrow are rapidly induced to fuse and differentiate into multinucleated TRACP-positive osteoclasts and the degree of multinucleation correlates with their capacity to resorb bone [24]. Key to the bone resorptive process is the ability of the osteoclast to form a microenvironment between itself and the underlying bone matrix with formation of a sealing zone via podosomes, which are a distinct type of adhesion structure [25], and also formation of a specialized membrane within the sealing zone, called the "ruffled border" [26]. Podosomes are punctuate structures that contain a cylindrical actin core and actin-associated adaptor proteins, including Wiscott-Aldrich syndrome protein (WASP), cortactin, gelsolin, leupaxin, and the Arp2/3 complex [27]. Podosomes in osteoclasts are highly dynamic [28]. The formation and maintenance of podosomes and the sealing barrier require that actin cytoskeleton and the adhesion substrates, particularly integrin $\alpha_v\beta_3$, be in absolute direct contact with the bone matrix surface [29]. The ruffled border membrane is rich in an electrogenic proton pump (H^+-ATPase) and a Cl^- channel (ClCN7). Protons, generated under the influence of carbonic anhydrase II, are "pumped" into the sealing zone through the ruffled-membrane-residing H^+-ATPase and ClCN7 to reduce the pH within the sealing zone to ~4.5 [30]. Lysosomal degradative enzymes, such as TRACP, cathepsin K, and matrix metalloproteases (MMPs) [MMP-9 and gelatinase], are also secreted into the sealing zone. These acidic lysosomal enzymes dissolve and digest the mineral

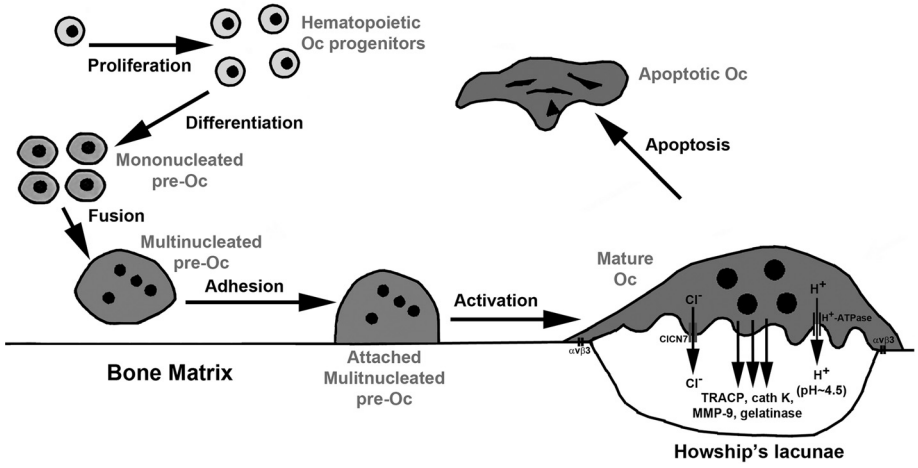

Figure 1. Schematic illustration of the life span of an osteoclast. The life span of an osteoclast begins with stimulation of the proliferation of hematopoietic osteoclast progenitors, which are then differentiated into mononucleated pre-osteoclasts. The mononucleated pre-osteoclasts are rapidly fused to form multinucleated, giant pre-osteoclasts, which then migrate to and attach to the bone surface through podosomes. The attached osteoclast is then activated and creates a microenvironment for bone resorption between itself and the underlying bone matrix by forming a sealing zone through reorganization of podosomes that are linked to $\alpha_v\beta_3$ integrin and a ruffled border. Through the H^+-ATPase proton pump and the ClCN7 chloride channel residing on the ruffled border, the activated osteoclast secretes protons and chloride ions into the sealing zone to reduce the localized pH to ~4.5. Various lysomsome enzymes, including TRACP, MMP9, cathepsin K, gelatinase, are also secreted to the sealing zone to initiate degradation of organic and inorganic components of the bone matrix to form a resorption lacuna. At the completion of the resorption process, the osteoclast rapidly undergoes apoptosis and comes off the bone surface.

and organic phases of bone matrix [31], creating saucer-shaped resorption cavities known as Howship's lacunae (Fig. 1). The essential role of the proton pump [32], Cl^- channel [33], and cathepsin K [34] in osteoclastic resorption is established by the fact that mutation or deletion of these genes each results in diseases of impaired resorption. Osteoclasts have a short life span and undergo apoptosis rapidly in the absence of appropriate effectors [35]. Thus, bone resorption is determined by not only the formation and activity of mature osteoclasts, but also their survival.

A large number of systemic hormones, e.g., parathyroid hormone, estrogen, 1,25-hydroxyvitamin D, calcitonin, circulating cytokines (interleukin-1, -6, -7, -11, -18, oncostatin M, tumor necrosis factor-α, interferon γ, prostaglandin E_2, and transforming growth factor-β), have been shown to have important regulatory roles in the formation, activation, and survival of osteoclasts [36]. Most of these regulators act through three cytokines locally produced by osteoblastic and marrow stromal cells: macrophage colony-stimulating factor (m-CSF), receptor activator of NFκB ligand (RANKL), and the soluble decoy receptor of RANKL, osteoprotegerin (OPG). Accordingly, m-CSF and RANKL are essential and sufficient for osteoclast formation, in that RANKL is the key osteoclastogenic cytokine, and m-CSF is needed for the proliferation, survival and, to some extent, differentiation of osteoclast precursors [37–39]. OPG is structurally very similar to the extracellular ligand binding domain of RANK (the cell surface receptor for RANKL), has a high binding affinity for RANKL, and is a potent competitive inhibitor of RANK for RANKL. Thus, the bioavailability of RANKL is determined by the ratio of RANKL and OPG. The roles of the RANKL/OPG axis and m-CSF on osteoclast differentiation and survival have been the focus of several review articles [40–42]. Thus, the scope of this review will be limited to regulation of osteoclast activity.

Protein-tyrosine phosphorylation in regulation of osteoclast activity

Activation of osteoclastic resorption is initiated by several key processes that follow the fusion of mononucleated progenitors to form multinucleated osteoclasts. These processes include cytoskeletal reorganization, polarization, and construction of a sealing zone and ruffled borders, all of which involve the integrin-dependent reorganization [22]. Accordingly, the integrin signaling is a major regulatory mechanism of osteoclast activity. Integrins are a large family of cell-surface glycoproteins that act as receptors for extracellular matrix proteins and are involved in cell-cell, cell-matrix adhesion, as well as in signaling mechanisms of cell proliferation and differentiation. Each integrin is a heterodimer containing an α subunit

and a β subunit. The osteoclast attaches to bone surface predominantly through the $\alpha_v\beta_3$ vitronectin receptor. The extracellular domain of each subunit contributes to ligand binding, and the cytoplasmic domains of both subunits are essential for cytoskeletal organization, cell motility, survival, and signal transduction through phosphorylation and activation of downstream mediators, including but not limited to, Fak, c-Src, paxillin, vinculin, $p130^{Cas}$, Erk1/2, Syk, Vav, Rho, and Rac [27, 43, 44]. A recently identified novel osteoclastic protein, osteoactivin, has also been implicated to be a key downstream mediator of the integrin-mediated fusion and migration of osteoclasts [45].

As indicated, many of the downstream mediators of the integrin signaling in regulation of osteoclast activity are protein-tyrosine kinases (PTK) and/or regulated by protein-tyrosine phosphorylation. Protein-tyrosine phosphorylation is a reversible cellular regulatory process: The phosphorylation reaction is mediated by PTKs, whereas the dephosphorylation is catalyzed by protein-tyrosine phosphatases (PTPs). There is compelling evidence that protein-tyrosine phosphorylation has essential regulatory functions in the integrin signaling and also in the overall regulation of osteoclast activities. For example, the integrin-dependent adherence of osteoclasts to bone matrix results in increased tyrosine phosphorylation of a number of podosome-associated proteins, such as Pyk2, c-Src, paxillin, $p130^{Cas}$, Cbl, and several podosomal core proteins (WASP and cortactin) [43, 44, 46]. Phosphorylation of these proteins leads to osteoclast adhesion and migration to bone surface, reorganization of podosomes, formation of the sealing zone, and secretion of degradative enzymes needed for bone resorption. Protein-tyrosine phosphorylation also plays a regulatory role in other major signaling pathways in osteoclasts, such as those mediated by RANKL (via its receptor, RANK) and by m-CSF (through its receptor, c-Fms) [47, 48]. One of the most compelling pieces of evidence for the essentiality of protein-tyrosine phosphorylation in regulation of osteoclast activity is that osteoclasts of mice deficient in c-Src PTK are unable to form ruffled borders [49], display abnormal cytoskeletal structure, retard cell migration, and lack resorption activity [49, 50]. Ablation of another downstream PTK gene, Pyk2, in mice also leads to inactivation of osteoclasts and increased bone mass [51].

c-Src PTK activity in regulation of osteoclast activity

The c-Src is a member of a family of nine structurally similar, non-receptor PTKs that are associated with the cytoplasmic surface of cellular membranes. As mentioned, targeted disruption of the c-Src gene in mice led to a form of osteopetrosis, characterized by a normal (or even a slightly increased) number of, but totally inactive, osteoclasts without showing any obvious morphological or functional abnormalities in other tissues or cells [49, 50]. Thus, c-Src proto-oncogene is a key mediator of osteoclast activity. Although osteoclasts also express other members of this multigene family, such as c-Fyn, c-Lyn, c-Yes, c-Hck, and c-Fgr, the osteopetrotic abnormalities are unique to c-Src-deficient mice, as deletion of any one of these other genes of this family did not lead to osteopetrosis [49, 50]. An important clue that c-Src could be a key mediator of the integrin $\alpha_v\beta_3$-dependent cytoskeletal reorganization in osteoclasts and of their bone resorption activity came from the findings of the very similar osteoclastic phenotype of mice lacking the integrin $\alpha_v\beta_3$ gene and that seen in c-Src-deficient mice [52]. Both knockout mouse strains, despite a slight increase in osteoclast number *in vivo*, develop severe osteopetrosis that is due to an impaired bone resorption owing to dysfunctional cytoskeletal organization in osteoclasts, resulting in their inability to attach to bone surface, spread and migrate, as well as an absence of a ruffled border [50, 52]. The surprisingly similar defective cytoskeletal phenotypes of c-Src- and integrin $\alpha_v\beta_3$-deficient osteoclasts suggest a commonality of intracellular signaling mechanisms. In this regard, activation of c-Src PTK reduces the strength of association between the integrin and cytoskeleton, and mediates movement of integrin $\alpha_v\beta_3$ to the migratory machinery of the osteoclast in the form of lamellipodia. It also accelerates the disassembly of podosomes, most probably by phosphorylation of cortactin [53], leading to cytoskeletal reorganization and formation of the sealing zone. Thus, c-Src could be a major downstream mediator of the integrin signaling of osteoclast activity.

There is a large body of *in vitro* evidence that the PTK activity of c-Src is needed for the functional activity of mature osteoclasts. For example, active osteoclasts expressed high levels of c-Src PTK activity on ruffled border membranes [54] and in podosomes [55], and the c-Src PTK activity correlated with the number and activity of resorbing osteoclasts *in vivo* [50] and *in vitro* [56]. Activation of c-Src PTK activity is involved in regulation of osteoclast spreading and migration [57, 58]. Specific inhibitors of the c-Src PTK activity abolished bone resorption activity of osteoclasts *in vitro* [59 – 62]. In addition, the c-Src PTK activity in osteoclasts is increased by bone resorption activators (PTH [62, 63] and 1,25-dihydroxyvitamin D_3 [63, 64]) and reduced by resorption inhibitors (calcitonin [60] and alendronate [63]).

Despite the large body of evidence for an important role of c-Src PTK activity in regulation of normal osteoclast activities, the findings that transgenic expression of Y416F and K295R dominant-negative c-Src mutants with inactive PTK activity in c-Src deficient mice was able to partially rescue the osteopetrotic phenotype [65] challenge the conclusion that the PTK activity of c-Src is essential. Because the Src homology domain 2 (SH2) and SH3 domain of c-Src can: 1) function as adaptors regulating c-Src trafficking and membrane localization, 2) mediate interactions with downstream signaling proteins, and 3) recruit key signaling proteins to membrane receptor PTKs for phosphorylation [66], and because specific inhibitors of the c-Src SH2 domain effectively blocked osteoclast-mediated resorption [67], it was suggested that the SH2 and/or SH3 domains of c-Src (rather than its PTK activity) are essential for osteoclast activities. On the other hand, transgenic expression of Y416F or K295R dominant-negative c-Src mutants only partially rescued the osteopetrotic phenotype, and complete rescue of the osteopetrotic abnormalities required the c-Src PTK activity [65]. Down- or up-regulation of c-Src PTK activity through transgenic expression of the C-terminus Src kinase (Csk) in osteoclastic cells also, correspondingly, modulated their bone resorption activity *in vitro* and *in vivo* [58]. It has also been shown that not only the adaptor function, but also the PTK kinase activity of c-Src, are important in osteoclast resorption [68] and that transgenic expression of a truncated c-Src mutant lacking the PTK domain induced osteopetrosis in c-$Src^{+/-}$ mice and worsened osteopetrosis in c-$Src^{-/-}$ mice [69]. Thus, both the PTK activity and adaptor functions of c-Src are essential for osteoclast activity.

Regulation of c-Src PTK activity

All members of the c-Src PTK family have a similar molecular structure: the N-terminal segment includes the so-called SH4 domain that is a myristolylation and membrane-localization signal site (which is essential for its attachment to the membrane), as well as a 50- to 80-amino acids "unique" domain, which differs among members of the c-Src PTK family. This region is followed sequentially in the peptide chain by the SH3 domain that binds specific proline-rich motifs, the SH2 domain that binds phosphotyrosines in specific sequence contexts, and the PTK catalytic (also known as SH1) domain. These domains are followed by a short C-terminal sequence, which contains a key regulatory tyrosine residue (tyr^{527} for c-Src). The phosphorylation of this tyr^{527} is critical for maintaining c-Src in its inactive conformation (Fig. 2). Both the PTK and adaptor activities of c-Src are regulated by intramolecular interactions of the SH2 and SH3 domains [70]. When in the inactive state, the tyr^{527} residue is phosphorylated by Csk, which results in an intramolecular association between the phosphorylated tyr^{527} (PY527) and the SH2 domain that stabilizes the inactive, closed conformation. The SH3 domain is also involved in an intramolecular interaction with the linker region that connects the SH2 and kinase domains [71]. Dephosphorylation of PY527 by a PTP disrupts the intramolecular interaction, allowing the protein to acquire the open, active conformation. While activation of c-Src PTK activity is mediated primarily through dephosphorylation of PY527, both SH2 and SH3 intramolecular associations are involved in maintaining the enzyme in the inactive conformation, and proline-rich sequences that bind with high affinity to the SH3 domain have been shown to activate the PTK activity of members of the c-Src family [72]. In the active, open conformation, the tyr^{416} (not shown in Fig. 2) in the activation loop of the kinase domain is autophosphorylated, which leads to further upregulation of its PTK activity [71, 72]. Thus, both the PTK and adaptor activities of c-Src are regulated by its protein-tyrosine phosphorylation status: increased phosphorylation of tyr^{527} leads to inactivation, whereas increased phosphorylation of tyr^{416} results in activation, of its PTK activity. Conversely, the dephosphorylation of PY527 activates both its PTK and adaptor activities. Although it is now clear that Csk is the PTK that phosphorylates tyr^{527}, the PTP that dephosphorylates PY527 in osteoclasts has not been identified. It has been reported that treatment of chicken osteoclast precursor cells with 1,25-dihydroxyvitamin D_3 led to activation of the c-Src PTK activity through increasing expression of a c-Src-activating PTP [73]. Because mice deficient in c-Src exhibit a predominant phenotype of inactive osteoclasts [49], it is conceivable that there may be one or more PY527-specific PTPs in osteoclasts that are upregulated by at least some resorption effectors to dephosphorylate PY527 of c-Src. Although a number of specific c-Src PTK inhibitors have been developed [62, 67], and although it is conceivable that some of these c-Src PTK inhibitors could be developed into effective therapeutic agents for inhibiting osteoclastic resorption, knowledge of the identity and regulation of these c-Src PY527 PTPs in osteoclasts will be very helpful in further understanding of regulation of the integrin and/or c-Src signaling in osteoclast functions. It may even open up a new avenue for future development of novel therapeutic modalities to treat disorders with excessive osteoclastic resorption by targeting the c-Src PY527 PTP in osteoclasts.

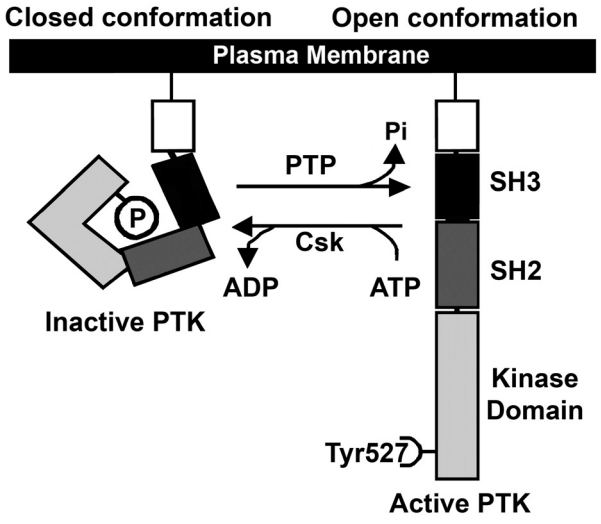

Figure 2. Regulation of the c-Src PTK activity. This is a schematic picture of the molecular structures of the closed (inactive) and open (active) conformation of c-Src PTK. The restrained (closed) conformation of c-Src is stabilized by the intramolecular interactions among the kinase domain, the SH2/SH3 domains, and the phosphorylation of the tyr^{527} (PY527) residue at the C-terminal tail, mediated by the C-terminus Src kinase (Csk). Displacement of SH2 and/or SH3 domains, either by C-terminal tail PY527 dephosphorylation or by competitive binding of optimal SH2/SH3 domain ligands, allows the kinase domain to open, exposing tyr^{416} to phosphorylation (not shown). The dephosphorylation of PY527 is catalyzed by a protein-tyrosine phosphatase (PTP).

PTPs

Although it was once assumed that protein phosphatases are non-regulatory, housekeeping enzymes that largely play counter-regulatory roles to protein kinases in regulation of the overall protein phosphorylation status of key signaling proteins, it is now well established that protein phosphatases (including PTPs) also play key regulatory roles in various signal transduction pathways of cell proliferation, differentiation, and functions [74]. There are more than 100 members of the PTP superfamily in the human genome; all of which contain one or two cytosolic PTP catalytic domains of ~240 residues that include the (I/V)HCSXGXXGR(S/T)G signature motif [75]. The hydroylsis of the phosphotyrosine residue by PTPs is mediated by a two-step, displacement reaction mechanism, in which the key cysteine residue residing in the core of PTP catalytic domain first forms a covalent intermediate with the phosphate group of the substrate, leading to breakage of the bond between this phosphate group and the protein tyrosine moiety and the release of the dephosphorylated protein substrate. The second step involves hydrolysis of the cyteine-phosphate bond, resulting in the release of the inorganic phosphate and regeneration of the active site of the enzyme [76]. While most PTPs have broad substrate specificities, the substrate specificity of several PTPs is relatively narrow [77].

Structurally, PTPs are divided into two major classes: transmembrane PTPs and cytosolic PTPs. Each class may be further divided into several subclasses, based on sequence similarities and presence of specific functional domains [75]. Most transmembrane PTPs contain an extracellular domain, a transmembrane domain, and one or two cytosolic PTP catalytic domains. However, a few transmembrane PTPs lack an extracellular domain. The functional role of extracellular domains of transmembrane PTPs is not well understood, but they may act as the ligand binding domain for receptor-associated PTPs. When there are two catalytic domains, the enzymatic activity is often associated with the first, proximal domain with the second, distal domain showing weak or no enzymatic activity [78]. This distal PTP domain is thought to perform primarily regulatory functions [78]. In contrast, cytosolic PTPs are typically composed of a single PTP catalytic domain that is flanked by other functional domains, which serve to regulate the subcellular localization of the PTP and/or binding for adaptor proteins.

Regulatory PTPs in osteoclasts

The importance of PTPs in the regulation of osteoclast formation and activity has been recognized since the discovery of the potent ability of non-specific PTP inhibitors, i.e., orthovanadate, phenylarsine oxide, and bisphosphonates, to suppress osteoclast formation and resorption activity *in vitro* [79]. A number of well known PTPs have been reported to have modulatory actions on osteoclast formation and activities. Many of these PTPs, such as the dual-specificity phosphatase MAPK phosphatase-1 (MKP-1) and SHP-2, a cytosolic Src homology 2 (SH2) domain-containing PTP, alter osteoclast formation and differentiation by acting as the counteracting enzyme activity to the PTK-mediated phosphorylation of key PY-containing signaling proteins. For example, MKP-1 dephosphorylates and inactivates MAPKs, leading to suppression of m-CSF- and/or RANKL-mediated proliferation and differentiation of osteoclast progenitors [80]. Conversely, SHP-2 is recruited to the m-CSF receptor (c-Fms) and other receptor PTKs via the Grb2-associated familiy of binding proteins to dephosphorylate (and thereby inhibit) the RasGAP activity responsible for suppressing Ras function [81]. This results in prolongation of Erk1/2 activation and leads to enhanced m-CSF- and/or RANKL-mediated proliferation and/or differentiation of osteoclast precursor cells. Because the emphasis of this review is on

regulation of osteoclast activity, the following discussion will be limited to only those PTPs that affect osteoclast activity, with a focus on those that may be responsible for dephosphorylation of PY527 of c-Src in osteoclasts. Several PTPs (SHP-1, cytosolic PTP-ε, PTP-PEST, and PTP-oc) have been shown to be key regulators of osteoclast activity [82, 83]. As shown in Figure 3, these four classes of PTPs are structurally quite different: three are cytosolic PTPs and one is a transmembrane PTP. The differences in their molecular structure, and also presumably intracellular localization, raise the possibility that these PTPs may each have different functional role, or participate in different aspects of regulation, in osteoclast activity.

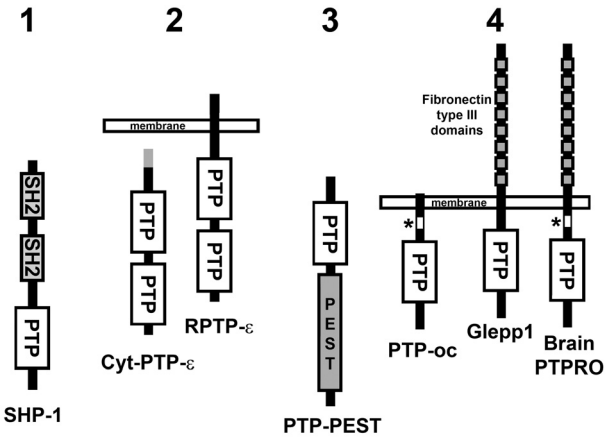

Figure 3. Schematic illustrations of the molecular structure of the four classes of PTPs (shown as 1 to 4) that have been implicated in the regulation of osteoclast activity. 1) SHP-1 is a member of cytosolic PTP superfamily. It has two N-terminal tandem SH2 domains followed by a single PTP catalytic domain and a C-terminal tail. 2) Cytosolic PTP-ε (Cyt-PTP-ε) is a truncated cytosolic isoform of the transmembrane receptor-associated PTP-ε (RPTP-ε). Cyt-PTP-ε and RPTP-ε both contains two PTP catalytic domains in the cytosolic region. Cyt-PTP-ε lacks the extracellular and transmembrane domains but it has a short, unique, 12 amino acid residues insert (shown as a light grey box) at its N-terminus, when compared to RPTP-ε. 3) PTP-PEST is a cytosolic PTP, which contains a C-terminal domain rich in proline (P), glutamic acid (E), serine (S), and threonine (T) residues; collectively known as the PEST domain. 4) PTP-oc is a truncated variant of the transmembrane PTP, Glepp1 (also known as PTPRO). Glepp1 contains a large extracellular domain that contains eight fibronectin type III domains (dark grey boxes) and sixteen N-glycosylation sites (not shown). PTP-oc completely lacks an extracellular domain, except for eight amino acid residues. In contrast to the renal Glepp1, PTP-oc contains a 28 amino acid-long juxtamembrane sequence insert due to alternative splicing of exon 17 of the renal Glepp1 (indicated by an asterisk). However, this insert is present in the brain PTPRO isoform.

SHP-1

SHP-1 is a cytosolic SH2 domain-containing PTP and is expressed predominantly in cells of hematopoietic lineage [84], which include osteoclast progenitor cells [22]. SHP-1 contains two tandem SH2 domains at the N-terminus, followed by a single PTP catalytic domain and a C-terminal tail [85] (Fig. 3). The N-terminal SH2 domains of SHP-1 can interact intramolecularly with its catalytic domain, which forces SHP-1 to assume an inactive conformation. This intramolecular interaction can be 'relieved' by the binding of phosphotyrosine-containing interacting proteins to the SH2 domains [85]. The binding of specific phosphotyrosine-containing docking proteins to the SH2 domains can also alter subcellular localization of SHP-1 [85], thus providing a unique means for targeted recruitment or sequestration of SHP-1 to a specific subcellular location. There is evidence that the C-terminal tail of SHP-1 also helps to regulate its PTP activity by forming inter- and intra-molecular interactions, which are facilitated by the proline-rich motifs and phosphorylatable tyrosine and serine residues at the C-terminal tail [85].

SHP-1 binds and dephosphorylates several phosphotyrosine-containing signaling proteins that are recruited to c-Fms (receptor of m-CSF) upon m-CSF activation; the net effect being a decrease in the phosphorylation levels of these signaling proteins and a diminished m-CSF signaling [86]. Studies with spontaneous homozygous mutation of SHP-1 in viable (mev^-/mev^-) or lethal motheaten (me^-/me^-) mice that either have substantially reduced levels of, or are deficient in, SHP-1 activity, respectively, offer the most persuasive argument that SHP-1 is an important negative regulator of osteoclast differentiation and activity. Accordingly, both motheaten mutant strains developed severe osteoporosis due to an increase in the number of highly active osteoclasts [87, 88]. The macrophages and osteoclast precursors of these SHP-1-deficient mice are hyper-responsive to m-CSF, a characteristic that leads to an increased numbers of apoptosis-resistant osteoclasts and their precursors [88], suggesting that SHP-1 is a negative regulator of the m-CSF-induced proliferation and/or differentiation of osteoclast precursors. SHP-1 is also associated with the complex containing TRAF6 in a RANKL-dependent manner in osteoclastic cells, exerting a negative effect in RANKL-RANK-TRAF6 signaling. This then leads to suppression of the RANKL-mediated osteoclast differentiation and/or activity [89]. The association of TRAF6 with RANK was increased in bone marrow macrophages of motheaten mice and in RAW264 cells overexpressing an inactive SHP-1 mutant, indicating that SHP-1 also acts as a negative regulator of the RANKL signaling by controlling the binding of TRAF6 to RANK.

While there is no direct evidence connecting SHP-1 to the regulation of integrin- and/or c-Src-mediated

cytoskeletal reorganization and/or cell adhesion and migration, there is circumstantial evidence that SHP-1 participates in the integrin-mediated signaling. For example, the integrin β_1-induced SHP-1 activation in T cells appeared to have a major suppressive role in T cell receptor signaling [90]. Macrophages [91] and neutrophils [92] of motheaten mice were hyper-adhesive and responded to integrin engagement more strongly than cells from wild-type mice, suggesting that SHP-1 may antagonize integrin-mediated cell adhesion. Thus, it is conceivable that SHP-1 may play a negative role in osteoclast activity. Because SHP-1 is a negative regulator of osteoclast formation and activity, this PTP would not be the c-Src PY527 PTP that mediates the integrin- and/or c-Src-induced activation of osteoclasts.

Cytosolic PTP-ε (cyt-PTP-ε)

Cytosolic PTP-ε is a cytosolic isoform of the receptor PTP-ε, which is a member of the transmembrane receptor-type PTP superfamily that contains two PTP catalytic domains (Fig. 3). The PTP-ε gene has two alternative promoters, yielding a transmembrane (RPTP-ε) and a cytosolic (cyt-PTP-ε) isoforms [93]. Two additional, but less abundant, isoforms of cytosolic PTP-ε (p67 and p65 PTP-ε) are produced by an alternative translation start codon and by proteolytic cleavage of cyt-PTP-ε [94]. The expression profiles of RPTP-ε and cyt-PTP-ε were non-overlapping; thus, RPTP-ε and cyt-PTP-ε are rarely found in the same cell type. In bone, the expression of cyt-PTP-ε is restricted to osteoclasts and is not expressed in osteoblasts. There is no detectable expression of RPTP-ε in either bone cell type [95].

The conclusion that cyt-PTP-ε has key regulatory roles in osteoclast resorption was based on observations that targeted deletion of the receptor RPTP-ε gene in mice yielded an increased trabecular bone mass phenotype, which was due to defects in the bone resorption activity of mature osteoclasts [95]. Because osteoclasts do not express the transmembrane RPTP-ε isoform and targeted deletion of RPTP-ε also led to complete absence of cyt-PTP-ε in osteoclasts [95], the osteoclastic phenotype in this knockout mouse strain was due to deficient cyt-PTP-ε expression in osteoclasts [82, 83]. This increased trabecular bone mass phenotype was most prevalent in young adult female mutant mice [95], but the reason for this sex-related preference of the bone phenotype is unclear. Consistent with a defective bone resorption, the PTP-ε-deficient mice showed a marked reduction in serum C-telopeptide, a biomarker of bone resorption. Osteoclasts derived from bone-marrow osteoclast precursors of these cyt-PTP-ε-deficient mice exhibited poor bone resorption activity *in vitro*. The impaired bone resorption ability of the mutant osteoclasts appeared to be associated with reduced ability to adhere to bone surface and defective formation of an actin sealing ring [95]. There were no abnormalities in osteoblasts or in bone formation rate in the mutant mice. Thus, the cyt-PTP-ε appears to be a positive regulator of osteoclast activity. PTP-ε-deficient mice are also defective in the mobilization of hematopoietic progenitor cells from the bone marrow niche to the ciruclation; a process that involves osteoclastic resorption [96]. However, the precise role of PTP-ε in the hematopoietic progenitor cell mobilization process is unclear.

The molecular mechanism by which cyt-PTP-ε affects osteoclast activity remains to be determined. Although it has been reported that RPTP-ε can dephosphorylate c-Src in Neu-induced mammary tumor cells [97], loss of cyt-PTP-ε expression did not appear to disrupt the c-Src signaling pathway in osteoclasts of mutant mice [95]. In addition, while osteoclasts of c-Src-deficient mice were completely unable to form ruffled borders and to resorb bone [49, 50], osteoclasts of PTP-ε-deficient mutant mice developed ruffled borders and were still able, albeit with markedly reduced ability, to resorb bone. Thus, cyt-PTP-ε is not likely to be a major PY527 c-Src PTP in osteoclasts.

PTP-PEST

PTP-PEST is also a member of the cytosolic PTP superfamily. It is a ubiquitous 88 kDa protein, which has an N-terminal PTP catalytic domain and a C-terminal domain that is rich in proline, glutamic acid, serine, and threonine residues (PEST) [98] (Fig. 3). This PEST region is capable of interacting with several molecules found in the podosomal complexes, such as paxillin, leupaxin, Pyk2, $p130^{Cas}$, Grb2, c-Src, and PSTPIP [99–103]. PTP-PEST also interacts with WASP indirectly through PTP-PEST interacting protein (PSTPIP) [101, 104], in that PSTPIP functions as a scaffold protein between PTP-PEST and WASP [105]. WASP integrates signals from Rho, Cdc42, and kinases that bind to the Arp2/3 complex and stimulates Arp2/3-dependent actin polymerization. Phosphorylation of WASP at tyr^{291}, which is mediated by c-Src PTK in osteoclasts [106], is essential for osteoclast resorption *in vitro* [107] and increases the actin polymerization activity through the Arp2/3 complex [108, 109]. The PY291 of WASP is a substrate of PTP-PEST, and the interaction among PTP-PEST, PSTPIP, and WASP allows PTP-PEST to dephosphorylate WASP [103]. The dephosphorylation of PY291 of

WASP leads to inhibition of cell spreading and membrane protrusion, and promotion of focal adhesion turnover as well as cell migration [110, 111]. In addition to PSTPIP and WASP, several phosphotyrosine-containing podosomal proteins, such as $p130^{Cas}$, paxillin, and Pyk2, are also substrates of PTP-PEST [111–113].

Immunostaining of murine osteoclasts revealed that PTP-PEST is localized predominantly to the podosome structures and peripheral sealing zone of resorbing osteoclasts along with leupaxin, gelsolin, WASP, c-Src, Pyk2, and PSTPIP [99, 101, 114]. Thus, PTP-PEST is probably a key podosomal PTP that participates in regulating osteoclast adhesion to bone matrix and formation of a sealing zone. The most supportive evidence for an important regulatory role for PTP-PEST in osteoclast activity is the finding that siRNA-mediated knockdown of PTP-PEST expression in osteoclasts markedly reduced formation of podosomal structures at the cell periphery and reduced their bone resorption activity *in vitro* [101]. Further studies are needed to provide precise mechanistic information about the role of PTP-PEST in the formation and function of podosomes and the sealing zone in osteoclasts. On the other hand, although these *in vitro* findings indicate that PTP-PEST is a positive regulator of osteoclast activity, there is recent evidence that PTP-PEST is a negative regulator of intestinal cell migration [115]. Thus, additional work is needed to definitively confirm that PTP-PEST is indeed a positive regulator of osteoclast activity *in vivo*. PTP-PEST is a substrate of c-Src PTK. There is no report that PTP-PEST can dephosphorylate PY527 of c-Src in osteoclasts or any other cell types. Conversely, there is recent evidence that PTP-PEST can dephosphorylate the stimulatory PY416 residue of c-Src in intestinal cells, leading to an inhibition of its PTK activity [115]. Therefore, PTP-PEST is also unlikely to be a PY527 c-Src PTP in osteoclasts.

PTP-oc

PTP-oc belongs to the superfamily of transmembrane PTPs and was identified from a rabbit osteoclast cDNA library through molecular cloning [116]. This PTP is structurally unique in that, unlike most transmembrane PTPs, it has only a very short (8 amino acid residues) extracellular domain, lacks a signal peptide proximal to the N-terminus, contains a single PTP catalytic domain, and is relatively small (405 residues) (Fig. 3). Because it lacks a significant extracellular domain, PTP-oc is not a typical receptor-associated PTP. It contains no SH2 or SH3 domains or other adaptor binding domains [116]; thus, it probably does not function as an adaptor protein. Because this transmembrane PTP is expressed predominantly in hematopoietic cells [B lymphocytes and cells of monocyte-macrophage lineage (including osteoclast precursors)] and in mature osteoclasts [116, 117], this unique PTP was referred to as the osteoclastic PTP, PTP-oc [116].

With the exception of the 28 amino acid residues insert (indicated by the * in Fig. 3) in its cytosolic region proximal to the transmembrane domain, PTP-oc shows complete sequence identity with the transmembrane and cytosolic domains of a renal receptor PTP, termed glomerular epithelial protein 1 (Glepp1) (Fig. 3), which is also known as PTPRO (receptor PTP type O), PTP-ϕ, RPTP-BK, Cryp-2, or PTPRO-FL [118–123]. Both PTP-oc and Glepp1 are derived from parts of the same genomic structure on human chromosome 12p12-p13 [124] or on mouse chromosome 6 [125]. The human Glepp1 gene contains 27 exons spanning over 223 kb. Exon 17, which corresponds to the insert of 28 residues (residue 66–93) of PTP-oc [116], is not expressed in the kidney Glepp1 mRNA [124] but is expressed in the mRNA of brain Glepp1 isoform [125]. By contrast, the human PTP-oc gene spans ~56 kb encoded by 15 exons (corresponding to exon 13–27 of the Glepp1 gene) [126]. The PTP-oc mRNA transcription start site is located at the last 217 bases of intron 12 of Glepp1. Thus, the first exon of the PTP-oc gene contains the 217-bp intronic sequence of intron 12 of Glepp1. The start codon of the PTP-oc gene begins from the last 4 bases of exon 14 of Glepp1, and the stop codon of both genes is located near the end of exon 26. Although the first exon of the human PTP-oc gene exists as a single exon, this exon is split into two exons (interrupted by a short intron) in the murine PTP-oc gene [127]. Accordingly, PTP-oc is in fact a truncated variant of Glepp1 and, therefore, is also referred to as PTPROt or PTP-U2S [117, 128]. However, PTP-oc is not a splicing variant of Glepp1 and its expression is driven by a distinct, alternative, cell-type-specific, intronic promoter (within intron 12 of Glepp1) [126, 127, 129]. This genomic arrangement of PTP-oc and Glepp1 is reminiscent of the two distinct promoters driving cell-type-specific expression of RPTP-ε and cyt-PTP-ε, respectively [93]. The basal elements of the intronic promoter of murine PTP-oc have been characterized and compared with that of the human PTP-oc gene [127], and cell-type-specificity of the intronic PTP-oc promoter has been confirmed *in vitro* [126, 127].

There is substantial *in vitro* evidence that PTP-oc may function as a positive regulator of osteoclast activity and that its molecular mechanism is in part mediated through activation of c-Src PTK by dephosphorylating its inhibitory PY527 residue. Accordingly, suppression

of PTP-oc expression in rabbit osteoclasts with a specific phosphorothioated PTP-oc antisense oligodeoxynucleotide decreased basal and PTH- or 1,25-dihydroxyvitamin D_3-stimulated bone resorption activity, which was accompanied by an increase in PY527 level of c-Src [130]. Osteoclast-like cells derived from two precursor cell lines (U937 cells [131] and RAW264.7 cells [132]) with stable overexpression of wild-type PTP-oc created significantly larger and deeper resorption pits *in vitro*. Conversely, the size and depth of resorption pits created by phosphatase-deficient PTP-oc-overexpressing osteoclast-like cells were much smaller than those by corresponding control osteoclast-like cells. Overexpression of wild-type PTP-oc activated, while transgenic expression of the phosphatase-deficient PTP-oc mutant or treatment with PTP-oc siRNA inhibited the c-Src PTK activity in these cells [131, 132]. Upregulation of PTP-oc expression by certain resorption activators in rabbit osteoclasts led to dephosphorylation of PY527 of c-Src and cellular PTP-oc expression levels correlated strongly with c-Src PTK activation [133]. Consistent with the premise that PY527 of c-Src is a cellular substrate for PTP-oc, the synthetic c-Src PY527 peptide is a good substrate for the recombinant PTP-oc *in vitro* [133]. Conversely, there has been no report showing that SHP-1, PTP-ε, or PTP-PEST was capable of dephosphorylating the synthetic c-Src PY527 peptide *in vitro*. A recent preliminary GST-PTP-oc pull down experiment also showed that PY527-c-Src was pulled down by the GST-wild-type-PTP-oc fusion protein, but not by the GST-phosphatase-deficient PTP-oc fusion protein in osteoclastic cells (unpublished observation). Therefore, PTP-oc is a potential PY527 c-Src PTP in osteoclastic cells.

The most compelling *in vivo* evidence that PTP-oc is an important positive regulator of osteoclast activity is the recent preliminary characterization of transgenic mice with targeted overexpression of PTP-oc in cells of osteoclastic lineage using the TRACP exon 1C promoter (which is highly specific for osteoclasts [134]) to drive transgene expression [135]. Young adult male F2 progenies of three independent transgenic lines showed that transgenic mice had significantly shorter femurs and lower bone volume, bone area, cortical and trabecular bone mineral content (BMC) and BMD in the femur. Histomorphometric analyses at secondary spongiosa of the femur or at metaphysis of L4 vertebrae confirmed that young adult male transgenic mice exhibited marked decreases in trabecular surface, % trabecular area, and trabecular number, and increases in trabecular separation and osteoclast numbers per bone surface length. Consistent with an increased bone resorption, the serum C-telopeptide level was higher in young adult male F2 transgenic mice than in wild-type littermates. There were no differences in the dynamic bone formation parameters between the young adult male transgenic mice and age- and sex-matched wild-type littermates (unpublished observations). *In vitro*, the average resorption pit area per pit created by marrow-derived osteoclasts of transgenic mice was greater than those by osteoclasts of wild-type littermates. The increased bone resorption activity of transgenic osteoclasts was associated with lower PY-527 levels of c-Src and increased tyrosine phosphorylation levels of paxillin (unpublished observations). It is noteworthy that the osteoclast and bone phenotypes of the PTP-oc transgenic mice, as in the case of PTP-ε-deficient mice [95], also showed sex-related preferences. However, contrary to PTP-ε-deficient mice, which showed a more prevalent increased trabecular bone mass phenotype in young adult female mutant mice, the decreased trabecular bone mass and increased bone resorption phenotypes were greater and much more prominent in young adult male than age-matched female transgenic mice, as the bone and osteoclastic resorption phenotypes were not readily detectable in young adult female transgenic mice (unpublished observations). The mechanistic reason(s) for the sex-related differences in bone phenotype in PTP-oc transgenic mice is unclear and remains to be determined.

Contrary to PTP-oc, Glepp1 is highly expressed in podocytes of kidney and neurons, and to lesser extents in spleens and hematopoietic cells [116]. As shown in Fig. 3, Glepp1 is a receptor-associated PTP, which has a large extracellular domain containing eight fibronectin type III domains and several glycosylation sites [118–123], which are signature motifs of a ligand binding site. Glepp1 and PTP-oc not only display cell-type-specific expression, but also show tissue-specific functions. Studies with Glepp1 knockout mice (through targeted disruption of exon 1) revealed that knocking out Glepp1 expression altered the podocyte structure and adversely affected functions of the kidney [136], without a significant bone or osteoclast phenotype. Therefore, unlike PTP-oc, Glepp1 does not appear to have an essential regulatory function in osteoclasts.

Although there is strong circumstantial evidence that PTP-oc acts in part through activation of c-Src PTK, the precise molecular mechanism of PTP-oc that leads to activation of osteoclast activity remains elusive. On the one hand, because SH2 and SH3 domains of c-Src can serve as adaptors to recruit signaling proteins of the integrin $\alpha_v\beta_3$ signaling to regulate osteoclast attachment and migration [65], and because the c-Src-mediated phosphorylation of key downstream signaling proteins, such as Pyk2 and c-Cbl, are needed

for osteoclast attachment and migration [57], it is possible that PTP-oc may regulate osteoclast activity through c-Src-dependent upregulation of the integrin $\alpha_v\beta_3$ signaling. However, because the regulatory roles of PTP-oc in osteoclast adhesion and migration to bone surface, cytoskeletal reorganization, and/or formation of the ruffled border have not been investigated, the assumption that PTP-oc-mediated upregulation of c-Src PTK in osteoclasts would enhance the integrin $\alpha_v\beta_3$-dependent osteoclast adhesion and migration, and/or cytoskeletal reorganization needs to be established. On the other hand, there is overwhelming evidence that c-Src PTK mediates multiple signaling pathways to regulate osteoclast activity. For example, the activated c-Src in osteoclasts could migrate to mitochondria to phosphorylate cytochrome c oxidase, which is required for osteoclastic resorption [137]. PTP-oc could upregulate osteoclast activity in part through promoting survival of osteoclasts and/or precursors via c-Src-dependent activation of NFκB and JNK2 [132]. Thus, the possibility that PTP-oc/c-Src signaling may also act through integrin-independent mechanisms to enhance osteoclast activity cannot be overlooked. Moreover, recent studies have identified several novel cellular substrates for PTP-oc, such as neuronal pentraxin with chromo domain (NPCD) [138], Syk [139], and Eph receptors [140]. Two of these novel substrates have been postulated to be important regulators of osteoclasts [141, 142]. Therefore, it is conceivable that PTP-oc may also act through c-Src-independent pathways to regulate osteoclast activity.

In addition to regulation of osteoclast activity, there is *in vitro* evidence that PTP-oc may also regulate the RANKL-dependent osteoclast differentiation [131, 132]. The most compelling evidence that PTP-oc regulates osteoclast differentiation is that knocking out PTP-oc gene expression without affecting Glepp1 expression in RAW264.7 cells *in vitro* by targeted deletion of the PTP-oc promoter prevented the RANKL-mediated osteoclastic differentiation of RAW264.7 cells [143]. PTP-oc has been reported to have a regulatory role in the differentiation of B-lymphocytes [119] and leukemia cells [128]. However, because knocking out c-Src expression in mice did not reduce the number of osteoclasts on bone surface [49], it is unlikely that the enhancing effects of PTP-oc on osteoclast differentiation is mediated through c-Src-dependent mechanisms. On the other hand, because PTP-oc can also dephosphorylate other cellular substrates, such as NPCD [138], Syk [139], and Eph receptors [140], it is possible that the enhancing effects of PTP-oc on osteoclast differentiation are mediated through c-Src PTK-independent mechanisms through these or yet-to-identified cellular substrates of PTP-oc in osteoclasts. At any rate, current *in vivo* and *in vitro* evidence strongly suggest that PTP-oc, but not the related Glepp1, is an important positive regulator of osteoclastic resorption.

Concluding remarks

It is now quite clear that regulation of osteoclast activity is highly complex and involves a number of signaling mechanisms that are mediated largely through protein-tyrosine phosphorylation [22, 35, 41, 43]. However, although significant information has been accumulated about the identity, regulation, and molecular mechanisms of various PTKs involved, including c-Src PTK, relatively little is known about the identity and functional roles of PTPs in the regulation of osteoclast activity. Accordingly, while it is evident that at least four structurally, and perhaps also functionally, distinct PTPs (i.e., SHP-1, cyt-PTP-ε, PTP-PEST, and PTP-oc) have essential roles in regulating osteoclast activity, a number of basic but very important issues about these PTPs in osteoclasts remain unresolved. For example, the spatial and temporal expression patterns of each of these PTPs genes in mature osteoclasts and their precursors have not been delineated. Subcellular localizations of many of these PTPs in osteoclasts have also not been clearly established. Such information could provide important insights into potential functional significance and/or regulation of these enzymes in osteoclasts. In addition, it appears that these four PTPs have relatively broad substrate specificities. Some of the cellular substrates of these PTPs have been tentatively identified. For example, PY527 of c-Src appears to be a major cellular substrate for PTP-oc in osteoclasts, and several podosomal proteins are key cellular substrates for PTP-PEST. Detailed knowledge of substrate specificities of these PTPs in osteoclasts is still lacking. Understanding the more detailed spectrum of physiological substrates of these PTPs in osteoclasts would provide valuable information not only about their functional roles in osteoclast activity, but also their potential molecular mechanisms of action. Moreover, there is very little information about the regulation of these PTPs in osteoclasts. Knowledge of regulation of these PTPs in osteoclasts is critical to the overall understanding of regulation of osteoclast activity. It may even open up a new avenue for future development of novel osteoclastic PTP-inhibitor-based therapeutic modalities to treat disorders with excessive osteoclastic resorption. Accordingly, clarification of these basic but essential issues will advance our understanding of the overall contribution of these PTPs in regulation of osteoclast

activity at the molecular level and also help identify pathways and candidate targets for future pharmaceutical intervention of osteoclastic resorption. Finally, anecdotal studies in other cell systems suggest that there are likely additional PTPs that are essential for osteoclast activity. In this regard, PTP-λ is shown to be a PY527 PTP in chicken pre-B cells [144]. PTP-1B has been shown to be a positive regulator of the integrin signaling in platelets [145]. PTP20 has also been reported to induce actin cytoskeleton reorganization in rat ovarian granulose cells by dephosphorylating p190 RhoGAP [146], a key mediator of the integrin signaling mechanism. The cytoskeleton-associated low molecular weight PTP has been shown to play a key regulatory role in the cytoskeleton rearrangement and cell adhesion and migration through dephosphorylation of p190 RhoGAP [147]. Consequently, the possibility that these additional PTPs also play essential regulatory role in osteoclast activity should not be overlooked and must be explored. Understanding the identity, functional roles, and regulation of additional PTPs that are essential for osteoclastic resorption will yield a more complete picture of the role of PTPs in osteoclastic resorption at the molecular level.

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1 Wlodarski, K. H. (1990) Properties and origin of osteoblasts. Clin. Orthop. Relat. Res. 252, 276–93.
2 Polig, E., and Jee, W. S. (1990) A model of osteon closure in cortical bone. Calcif. Tissue Int. 47, 261–269.
3 Aarden, E. M., Burger, E. H., and Nijweide, P. J. (1994) Function of osteocytes in bone. J. Cell. Biochem. 55, 287–299.
4 Aubin, J. E., Liu, F., Malaval, L., and Gupta, A. K. (1995) Osteoblast and chondroblast differentiation. Bone 17, 77S-83S.
5 Dodds, R. A., Ali, N., Pead, M. J., and Lanyon, L. E. (1993) Early loading-related changes in the activity of glucose 6-phosphate dehydrogenase and alkaline phosphatase in osteocytes and periosteal osteoblasts in rat fibulae in vivo. J. Bone Miner. Res. 8, 261–267.
6 Cheng, B., Zhao, S., Luo, J., Sprague, E., Bonewald, L. F., and Jiang, J. X. (2001) Expression of functional gap junctions and regulation by fluid flow in osteocyte-like MLO-Y4 cells. J. Bone Miner. Res. 16, 249–259.
7 Lanyon, L. E. (1993) Osteocytes, strain detection, bone modeling and remodeling. Calcif. Tissue Int. 53, S102-S017.
8 Parfitt, A. M. (2002) Physiologic and pathogenetic significance of bone histomorphometric data. In: Disorders of Bone Mineral Metabolism, 2nd ed. pp. 469–485, Coe, F. L., and Favus, M. J. (ed.), Lippincott Williams and Wilkins, Baltimore, MD, USA.
9 Roodman, G. D. (1999) Cell biology of the osteoclast. Exp. Hematol. 27, 1229–1241.
10 Geiger, B., Bershadsky, A., Pankov, R., and Yamada, K. M. (2001) Transmembrane crosstalk between the extracellular matrix-cytoskeleton crosstalk. Nat. Rev. Mol. Cell Biol. 2, 793–805.
11 Reddy, S. V. (2004) Regulatory mechanisms operative in osteoclasts. Crit. Rev. Eukaryot. Gene Expr. 14, 255–270.
12 Baron, R., Vignery, A., and Tran Van, P. (1980) The significance of lacunar erosion without osteoclasts: studies on the reversal phase of the remodeling sequence. Metab. Bone Dis. Rel. Res. 2S, 35–40.
13 Aguila, H. L., and Rowe, D. W. (2005) Skeletal development, bone remodeling, and hematopoiesis. Immunol. Rev. 208, 7–18.
14 Nicolas, V., Prewett, A., Bettica, P., Mohan, S., Finkelman, R. D., Baylink, D. J., and Farley, J. R. (1994) Age-related decreases in insulin-like growth factor-I and transforming growth factor-β in femoral cortical bone from both men and women: implications for bone loss with aging. J. Clin. Endocrinol. Metab. 78, 1011–1016.
15 Ivey, J. L., and Baylink, D. J. (1981) Postmenopausal osteoporosis: proposed roles of defective coupling and estrogen deficiency. Metab. Bone Dis. Relat. Res. 3, 3–7.
16 Parisien, M., Silverberg, S. J., Shane, E., Dempster, D. W., and Bilezikian, J. P. (1990) Bone disease in primary hyperparathyroidism. Endocrinol. Metab. Clin. North Am. 19, 19–34.
17 Mosekilde, L., and Melsen, F. (1978) A tetracycline-based histomorphometric evaluation of bone resorption and bone turnover in hyperthyroidism and hyperparathyroidism. Acta Med. Scand. 204, 97–102.
18 Kraut, J. A. (1995) The role of metabolic acidosis in the pathogenesis of renal osteodystrophy. Adv. Ren. Replace. Ther. 2, 40–51.
19 Krane, S. M., and Simon, L. S. (1987) Metabolic consequences of bone turnover in Paget's disease of bone. Clin. Orthop. Relat. Res. 217, 26–36.
20 Levine, J. P. (2006) Pharmacologic and nonpharmacologic management of osteoporosis. Clin. Cornerstone 8, 40–53.
21 De Vernejoul, M. C., and Benichou, O. (2001) Human osteopetrosis and other sclerosis disorders: recent genetic developments. Calcif. Tissue Int. 69, 1–6.
22 Miyamoto, T., and Suda, T. (2003) Differentiation and function of osteoclasts. Keio J. Med. 52, 1–7.
23 James, I. E., Dodds, R. A., Lee-Rykaczewski, E., Eichman, C. F., Connor, J. R., Hart, T. K., Maleeff, B. E., Lackman, R. D., and Gowen, M. (1996) Purification and characterization of fully functional human osteoclast precursors. J. Bone Miner. Res. 11, 1608–1618.
24 Fallon, M. D., Teitelbaum, S. L., and Kahn, A. J. (1983) Multinucleation enhances macrophage-mediated bone resorption. Lab. Invest. 49, 159–164.
25 Geiger, B., Bershadsky, A., Pankov, R., and Yamada, K. M. (2001) Transmembrane crosstalk between the extracellular matrix-cytoskeleton crosstalk. Nat. Rev. Mol. Cell Biol. 2, 793–805.
26 Teti, A., Marchisio, P. C., and Zallone, A. Z. (1991) Clear zone in osteoclast function: role of podosomes in regulation of bone-resorbing activity. Am. J. Physiol. 261, C1-C7.
27 Luxenburg, C., Geblinger, D., Klein, E., Anderson, K., Hanein, D., Geiger, B., and Addadi, L. (2007) The architecture of the adhesive apparatus of cultured osteoclasts: from podosome formation to sealing zone assembly. PLoS ONE 2, e179.
28 Destaing, O., Saltel, F., Geminard, J. C., Jurdic, P., and Bard, F. (2003) Podosomes display actin turnover and dynamic self-organization in osteoclasts expressing actin-green fluorescent protein. Mol. Biol. Cell 14, 407–416.
29 Stenbeck, G., and Horton, M. A. (2000) A new specialized cell-matrix interaction in actively resorbing osteoclasts. J. Cell Sci. 113, 1577–1587.
30 Teitelbaum, S. L., and Ross, F. P. (2003) Genetic regulation of osteoclast development and function. Nat. Rev. Genet. 4, 638–649.
31 Delaisse, J. M., Andersen, T. L., Engsig, M. T., Henriksen, K., Troen, T., and Blavier, I. (2003) Matrix metalloproteinases (MMP) and cathepsin K contribute differently to osteoclastic activities. Microsc. Res. Tech. 61, 504–513.

32 Michigami, T., Kageyama, T., Satomura, K., Shima, M., Yamaoka, K., Nakayama, M., and Ozono, K. (2002) Novel mutations in the a3 subunit of vacuolar H^+-adenosine triphosphatase in a Japanese patient with infantile malignant osteopetrosis. Bone 30, 436–439.
33 Frattini, A., Pangrazio, A., Susani, L., Sobacchi, C., Mirolo, M., Abinun, M., Andolina, M., Flanagan, A., Horwitz, E. M., Mihci, E., Notarangelo, L. D., Ramenghi, U., Teti, A., Van Hove, J., Vujic, D., Young, T., Albertini, A., Orchard, P. J., Vezzoni, P., and Villa, A. (2003) Chloride channel ClCN7 mutations are responsible for severe recessive, dominant, and intermediate osteopetrosis. J. Bone Miner. Res. 18, 1740–1747.
34 Motyckova, G., Weilbaecher, K. N., Horstmann, M., Rieman, D. J., Fisher, D. Z., and Fisher, D. E. (2001) Linking osteopetrosis and pycnodysostosis: regulation of cathepsin K expression by the microphthalmia transcription factor family. Proc. Natl. Acad. Sci. USA 98, 5798–5803.
35 Tanaka, S., Nakamura, I., Inoue, J., Oda, H., and Nakamura, K. (2003) Signal transduction pathways regulating osteoclast differentiation and function. J. Bone Miner. Metab. 21, 123–133.
36 Lee, S. K., and Lorenzo, J. (2006) Cytokines regulating osteoclast formation and function. Curr. Opin. Rheumatol. 18, 411–418.
37 Pixley, F. J., and Stanley, E. R. (2004) CSF-1 regulation of the wandering macrophage: Complexity in action. Trends Cell Biol. 14, 628–638.
38 Suda, T., Takahashi, N., Udagawa, N., Jimi, E., Gillespie, M. T., and Martin, T. J. (1999) Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. Endocr. Rev. 20, 345–357.
39 Boyle, W. J., Simonet, W. S., and Lacey, D. L. (2003) Osteoclast differentiation and activation. Nature 423, 337–342.
40 Teitelbaum, S. L. (2000) Bone resorption by osteoclasts. 289, 1504–1508.
41 Blair, H. C., Robinson, L. J., and Zaidi, M. (2005) Osteoclast signaling pathways. Biochem. Biophys. Res. Commun. 328, 728–738.
42 Asagiri, M., and Takayanagi, H. (2007) The molecular understanding of osteoclast differentiation. Bone 40, 251–264.
43 Ross, F. P., and Teitelbaum, S. L. (2005) $\alpha_v\beta_3$ and macrophage colony-stimulating factor: partners in osteoclast biology. Immunol. Rev. 208, 88–105.
44 Teitelbaum, S. L. (2005) Osteoclasts and integrins. Ann. N. Y. Acad. Sci. 1068, 95–99.
45 Sheng, M. H.-C., Wergedal, J. E., Mohan, S., and Lau, K.-H. W. (2008) Osteoactivin is a novel osteoclastic protein and plays a key role in osteoclast differentiation and activity. FEBS Lett. 582, 1451–1458.
46 Nakamura, I., Duong le, T., Rodan, S. B., and Rodan, G. A. (2007) Involvement of $\alpha_v\beta_3$ integrins in osteoclast function. J. Bone Miner. Metab. 25, 337–344.
47 Wada, T., Nakashima, T., Hiroshi, N., and Penninger, J. M. (2006) RANKL-RANK signaling in osteoclastogenesis and bone disease. Trends Mol. Med. 12, 17–25.
48 Roth, P., and Stanley, E. R. (1992) The biology of CSF-1 and its receptor. Curr. Top. Microbiol. Immunol. 181, 141–167.
49 Soriano, P., Montgomery, C., Geske, R., and Bradley, A. (1991) Targeted disruption of the c-src proto-oncogene leads to osteoporosis in mice. Cell 64, 693–702.
50 Boyce, B. F., Yoneda, T., Lowe, C., Soriano, P., and Mundy, G. R. (1992) Requirement of $pp60^{c\text{-}src}$ expression for osteoclasts to form ruffled borders and resorb bone in mice. J. Clin. Invest. 90, 1622–1627.
51 Gil-Henn, H., Destaing, O., Sims, N. A., Aoki, K., Alles, N., Neff, L., Sanjay, A., Bruzzaniti, A., De Camilli, P., Baron, R., and Schlessinger, J. (2007) Defective microtubule-dependent podosome organization in osteoclasts leads to increased bone density in $Pyk2^{-/-}$ mice. J. Cell Biol. 178, 1053–1064.
52 McHugh, K. P., Hodivala-Dilke, K., Zheng, M. H., Namba, N., Lam, J., Novack, D., Feng, X., Ross, F. P., Hynes, R. O., and Teitelbaum, S. L. (2000) Mice lacking β_3 integrins are osteosclerotic because of dysfunctional osteoclasts. J. Clin. Invest. 105, 433–440.
53 Wu, H., Reynolds, A. B., Kanner, S. B., Vines, R. R., and Parsons, J. T. (1991) Identification and characterization of a novel cytoskeleton-associated $pp60^{src}$ substrate. Mol. Cell. Biol. 11, 5113–5124.
54 Tanaka, S., Takahashi, N., Udagawa, N., Sasaki, T., Fukui, Y., Kurokawa, T., and Suda, T. (1992) Osteoclasts express high levels of $p60^{c\text{-}src}$, preferentially on ruffled border membranes. FEBS Lett. 323, 85–89.
55 Ochoa, G. C., Slepnev, V. I., Neff, L., Ringstad, N., Takei, K., Daniell, L., Kim, W., Cao, H., McNiven, M., Baron, R., De Camilli, P. (2000) A functional link between dynamin and the actin cytoskeleton at podosomes. J. Cell Biol. 150, 377–389.
56 Yoneda, T., Niewolna, M., Lowe, C., Izbicka, E., and Mundy, G. R. (1993) Hormonal regulation of $pp60^{c\text{-}src}$ expression during osteoclast formation *in vitro*. Mol. Endocrinol. 7, 1313–1318.
57 Sanjay, A., Hougton, A., Neff, L., DiDomenico, E., Bardelay, C., Antoine, E., Levy, J., Gailit, J., Bowtell, D., Horne, W. C., and Baron, R. (2001) Cbl associates with Pyk2 and Src to regulate Src kinase activity, $\alpha_v\beta_3$ integrin-mediated signaling, cell adhesion, and osteoclast motility. J. Cell Biol. 152, 181–195.
58 Miyazaki, T., Takayanagi, H., Isshiki, M., Takahashi, T., Okada, M., Fukui, Y., Oda, H., Nakamura, K., Hirai, H., Kurokawa, T., and Tanaka, S. (2000) *In vitro* and *in vivo* suppression of osteoclast function by adenovirus vector-induced csk gene. J. Bone Miner. Res. 15, 41–51.
59 Hall, T. J., Schaeublin, M., and Missbach, M. (1994) Evidence that c-src is involved in the process of osteoclastic bone resorption. Biochem. Biophys. Res. Commun. 199, 1237–1244.
60 Yoneda, T., Lowe, C., Lee, C.-H., Gutierrez, G., Niewolna, M., Williams, P. J., Izbicka, E., Uehara, Y., and Mundy, G. R. (1993) Herbimycin A, a $pp60^{c\text{-}src}$ tyrosine kinase inhibitor, inhibits osteoclast bone resorption in vitro and hypercalcemia *in vivo*. J. Clin. Invest. 91, 2791–2795.
61 Blair, H. C., Jordan, S. E., Peterson, T. G., and Barnes, S. (1996) Variable effects of tyrosine kinase inhibitors on avian osteoclastic activity and reduction of bone loss in ovariectomized rats. J. Cell. Biochem. 61, 629–637.
62 Missabach, M., Jeschke, M., Feyen, J., Muller, K., Glatt, M., Green, J., and Susa, M. (1999) A novel inhibitor of the tyrosine kinase Src suppresses phosphorylation of its major cellular substrates and reduces bone resorption *in vitro* and in rodent models *in vivo*. Bone 24, 437–439.
63 Lau, K.-H. W., Wu, L.-W., Sheng, M. H.-C., Amoui, M., Suhr, S. M., and Baylink, D. J. (2006) A unique osteoclastic transmembrane protein-tyrosine phosphatase is a potential positive regulator of the c-src protein-tyrosine kinase activity, a mediator of osteoclast activity. J. Cell. Biochem. 97, 940–955.
64 Chappel, J., Ross, F. P., Abu-Amer, Y., Shaw, A., and Teitelbaum, S. L. (1997) 1,25-dihydroxyvitamin D_3 regulates $pp60^{c\text{-}src}$ activity and expression of a $pp60^{c\text{-}src}$ activating phosphatase. J. Cell. Biochem. 67, 432–438.
65 Schwartzberg, P. L., Xing, L., Hoffmann, O., Lowell, C. A., Garrett, L., Boyce, B. F., Varmus, H. E. (1997) Rescue of osteoclast function by transgenic expression of kinase-deficient Src in $Src^{-/-}$ mutant mice. Genes Dev. 11, 2835–2844.
66 Schwartzberg, P. (1998) The many faces of Src: multiple functions of a prototypical tyrosine kinase. Oncogene 17,1463–1468.
67 Violette, S. M., Guan, W., Bartlett, C., Smith, J. A., Bardelay, C., Antoine, E., Rickles, R. J., Mandine, E., van Schravendijk, M. R., Adams, S. E., Lynch, B. A., Shakespeare, W. C., Yang,

M., Jacobsen, V. A., Takeuchi, C. S., Macek, K. J., Bohacek, R. S., Dalgarno, D. C., Weigele, M., Lesuisse, D., Sawyer, T. K., Baron, R. (2001) Bone-targeted Src SH2 inhibitors block Src cellular activity and osteoclast-mediated resorption. Bone 28, 54–64.

68 Miyazaki, T., Sanjay, A., Neff, L., Tanaka, S., Horne, W. C., and Baron, R. (2004) Src kinase activity is essential for osteoclast function. J. Biol. Chem. 279, 17660–17666.

69 Xing, L., Venegas, A. M., Chen, A., Garrett-Beal, L., Boyce, B. F., Varmus, H. E., and Schwartzberg, P. L. (2001) Genetic evidence for a role for Src family kinases in TNF family receptor signaling and cell survival. Genes Dev. 15, 241–253.

70 Boggon, T. J., and Eck, M. J. (2004) Structure and regulation of Src family kinases. Oncogene 23, 7918–7927.

71 Xu, W., Harrison, S. C., and Eck, M. J. (1997) Three-dimensional structure of the tyrosine kinase c-Src. Nature 385, 595–602.

72 Moarefi, I., LaFevre-Bernt, M., Sicheri, F., Huse, M., Lee, C. H., Kuriyan, J., and Miller, W. T. (1997) Activation of the Src-family tyrosine kinase Hck by SH3 domain displacement. Nature 385, 650–653.

73 Lau, K.-H. W., and Baylink, D. J. (1993) Phosphotyrosyl protein phosphatases: potential regulators of cell proliferation and differentiation. Crit. Rev. Oncogenesis 4, 451–471.

74 Alonso, A., Sasin, J., Bottini, N., Friedberg, I., Osterman, A., Godzik, A., Hunter, T., Dixon, J., and Mustelin, T. (2004). Protein tyrosine phosphatases in the human genome. Cell 117, 699–711.

75 Chappel, J., Ross, F. P., Abu-Amer, Y., Shaw, A., and Teitelbaum, S. L. (1997) 1,25-dihydroxyvitamin D_3 regulates $pp60^{c\text{-}src}$ activity and expression of a $pp60^{c\text{-}src}$ activating phosphatase. J. Cell. Biochem. 67, 432–438.

76 Barford, D., Das, A. K., and Egloff, M. P. (1998) The structure and mechanism of protein phosphatases: insights into catalysis and regulation. Annu. Rev. Biophys. Biomol. Struct. 27, 133–164.

77 Zhang, Z. Y. (2002) Protein tyrosine phosphatases: structure and function, substrate specificity, and inhibitor development. Annu. Rev. Pharmacol. Toxicol. 42, 209–234.

78 Andersen, J. N., Mortensen, O. H., Peters, G. H., Drake, P. G., Iversen, L. F., Olsen, O. H., Jansen, P. G., Andersen, H. S., Tonks, N. K., and Møller, N. P. (2001) Structural and evolutionary relationships among protein tyrosine phosphatase domains. Mol. Cell. Biol. 21, 7117–7136.

79 Schmidt, A., Rutledge, S. J., Endo, N., Opas, E. E., Tanaka, H., Wesolowski, G., Leu, C. T., Huang, Z., Ramachandaran, C., Rodan, S. B., and Rodan, G. A. (1996) Protein-tyrosine phosphatase activity regulates osteoclast formation and function: inhibition by alendronate. Proc. Natl. Acad. Sci. U.S.A. 93, 3068–3073.

80 Ducruet, A. P., Vogt, A., Wipf, P., and Lazo, J. S. (2005) Dual specificity protein phosphatases: therapeutic targets for cancer and Alzheimer's disease. Annu. Rev. Pharmacol. Toxicol. 45, 725–750.

81 Gu, H., and Neel, B. G. (2003) The 'Gab' in signal transduction. Trends Cell Biol. 13, 122–130.

82 Granot-Attas, S., Knobler, H., and Elson, A. (2007) Protein tyrosine phosphatases in osteoclasts. Crit. Rev. Eukaryot. Gene Expr. 17, 49–71.

83 Granot-Attas, S., and Elson, A. (2008) Protein tyrosine phosphatases in osteoclast differentiation, adhesion, and bone resorption. Eur. J. Cell Biol. 87, 479–490.

84 Frearson, J. A., and Alexander, D. R. (1997) The role of phosphotyrosine phosphatases in haematopoietic cell signal transduction. Bioassays 19, 417–427.

85 Poole, A. W., and Jones, M. L. (2005) A SHPing tale: perspectives on the regulation of SHP-1 and SHP-2 tyrosine phosphatases by the C-terminal tail. Cell. Signal. 17, 1323–1332.

86 Neel, B. G., Gu, H., and Pao, L. (2003) The 'shp'ing news: SH2 domain-containing tyrosine phosphatases in cell signaling. Trends Biochem. Sci. 28, 284–293.

87 Umeda, S., Beamer, W. G., Takagi, K., Naito, M., Hayashi, S., Yonemitsu, H., Yi, T., and Shultz, L. D. (1999) Deficiency of SHP-1 protein-tyrosine phosphatase activity results in heightened osteoclast function and decreased bone density. Am. J. Pathol. 155, 223–233.

88 Aoki, K., Didomenico, E., Sims, N. A., Mukhopadhyay, K., Neff, L., Houghton, A., Amling, M., Levy, J. B., Horne, W. C., and Baron, R. (1999) The tyrosine phosphatase SHP-1 is a negative regulator of osteoclastogenesis and osteoclast resorbing activity: increased resorption and osteopenia in me^v/me^v mutant mice. Bone 25, 261–267.

89 Zhang, Z., Jimi, E., and Bothwell, A. L. (2003) Receptor activator of NF-κB ligand stimulates recruitment of SHP-1 to the complex containing TNFR-associated factor 6 that regulates osteoclastogenesis. J. Immunol. 171, 3620–3626.

90 Mary, F., Moon, C., Venaille, T., Thomas, M. L., Mary, D., and Bernard, A. (1999) Modulation of TCR signaling by β1 integrins: role of the tyrosine phosphatase SHP-1. Eur. J. Immunol. 29, 3887–3897.

91 Roach, T. I., Slater, S. E., White, L. S., Zhang, X., Majerus, P. W., Brown, E. J., and Thomas, M. L. (1998) The protein tyrosine phosphatase SHP-1 regulates integrin-mediated adhesion of macrophages. Curr. Biol. 8, 1035–1038.

92 Kruger, J., Butler, J. R., Cherapanov, V., Dong, Q., Ginzberg, H., Govindarajan, A., Grinstein, S., Siminovitch, K. A., and Downey, G. P. (2000) Deficiency of Src homology 2-containing phosphatase 1 results in abnormalities in murine neutrophil function: studies in motheaten mice. J. Immunol. 165, 5847–5859.

93 Tanuma, N., Nakamura, K., and Kikuchi, K. (1999) Distinct promoters control transmembrane and cytosolic protein tyrosine phosphatase epsilon expression during macrophage differentiation. Eur. J. Biochem. 259, 46–54.

94 Gil-Henn, H., Volohonsky, G., Toledano-Katchalski, H., Gandre, S., and Elson, A. (2000) Generation of novel cytoplasmic forms of protein tyrosine phosphatase epsilon by proteolytic processing and translational control. Oncogene 19, 4375–4384.

95 Chiusaroli, R., Knobler, H., Luxenburg, C., Sanjay, A., Granot-Attas, S., Tiran, Z., Miyazaki, T., Harmelin, A., Baron, R., and Elson, A. (2004) Tyrosine phosphatase epsilon is a positive regulatory of osteoclast function *in vitro* and *in vivo*. Mol. Biol. Cell 15, 234–244.

96 Kollet, O., Dar, A., Shivtiel, S., Kalinkovich, A., Lapid, K., Sztainberg, Y., Tesio, M., Samstein, R. M., Goichberg, P., Spiegel, A., Elson, A., and Lapidot, T. (2006) Osteoclasts degrade endosteal componets and promote mobilization of hematopoietic progenitor cells. Nat. Med. 12, 657–664.

97 Gil-Henn, H., and Elson, A. (2003) Tyrosine phosphatase-ε activates Src and supports the transformed phenotype of Neu-induced mammary tumor cells. J. Biol. Chem. 278, 15579–15586.

98 Yang, Q., Co, D., Sommercorn, J., and Tonks, N. K. (1993) Cloning and expression of PTP-PEST. A novel, human, nontransmembrane protein tyrosine phosphatase. J. Biol. Chem. 268, 6622–6628.

99 Gupta, A., Lee, B. S., Khadeer, M. A., Tang, Z., Chellaiah, M., Abu-Amer, Y., Goldknopf, J., and Hruska, K. A. (2003) Leupaxin is a critical adaptor protein in the adhesion zone of the osteoclast. J. Bone Miner. Res. 18, 669–685.

100 Shen, Y., Lyons, P., Cooley, M., Davidson, D., Veillette, A., Salgia, R., Griffin, J. D., and Schaller, M. D. (2000) The noncatalytic domain of protein-tyrosine phosphatase-PEST targets paxillin for dephosphorylation in vivo. J. Biol. Chem. 275, 1405–1413.

101 Chellaiah, M. A., Kuppuswamy, D., Lasky, L., and Linder, S. (2007) Phoshphorylation of a Wiscott-Aldrich syndrome protein-associated signal complex is critical in osteoclast bone resorption. J. Biol. Chem. 282, 10104–10116.

102 Sahu, S. N., Khadeer, M. A., Robertson, B. W., Núñez, S. M., Bai, G., and Gupta, A. (2007) Association of leupaxin with Src in osteoclasts. Am. J. Physiol. Cell Physiol. 292, C581-C590.

103 Côté, J. F., Chung, P. L., Theberge, J. F., Halle, M., Spencer, S., Lasky, L. A., and Tremblay, M. L. (2002) PSTPIP is a substrate of PTP-PEST and serves as a scaffold guiding PTP-PEST toward a specific dephosphorylation of WASP. J. Biol. Chem. 277, 2973–2986.
104 Dowbenko, D., Spencer, S., Quan, C., and Lasky, L. A. (1998) Identification of a novel polyproline recognition site in the cytoskeletal associated protein, prolin serine threonine phosphatase interacting protein. J. Biol. Chem. 273, 989–996.
105 Badour, K., Zhang, J., Shi, F., Leng, Y., Collins, M., and Siminovitch, K. A. (2004) Fyn and PTP-PEST-mediated regulation of Wiskott-Aldrich syndrome protein (WASp) tyrosine phosphorylation is required for coupling T cell antigen receptor engagement to WASp effector function and T cell activation. J. Exp. Med. 199, 99–112.
106 Cory, G. O., Garg, R., Cramer, R., Ridley, A. J. (2002) Phosphorylation of tyrosine 291 enhances the ability of WASp to stimulate actin polymerization and filopodium formation. Wiskott-Aldrich Syndrome protein. J. Biol. Chem. 277, 45115–45121.
107 Ma, T., Samanna, V., and Chellaiah, M. A. (2008) Dramatic inhibition of osteoclast sealing ring formation and bone resorption in vitro by a WASP-peptide containing pTyr294 amino acid. J. Mol. Signal 3, 4.
108 Torres, E., and Rosen, M. K. (2006) Protein-tyrosine kinase and GTPase signals cooperate to phosphorylate and activate Wiskott-Aldrich syndrome protein (WASP)/neuronal WASP. J. Biol. Chem. 281, 3513–3520.
109 Miki, H., and Takenawa, T. (2003) Regulation of actin dynamics by WASP family proteins. J. Biochem. 134, 309–313.
110 Larsen, M., Tremblay, M. L., and Yamada, K. M. (2003) Phosphatases in cell-matrix adhesion and migration. Nat. Rev. Mol. Cell Biol. 4, 700–711.
111 Jamieson, J. S., Tumbarello, D. A., Halle, M., Brown, M. C., Tremblay, M. L., and Turner, C. E. (2005) Paxillin is essential for PTP-PEST-dependent regulation of cell spreading and motility: a role for paxillin kinase linker. J. Cell Sci. 118, 5835–5847.
112 Angers-Loustau, A. Côté, J. F., Charest, A., Dowbenko, D., Spencer, S., Lasky, L. A., and Tremblay, M. L. (1999) Protein tyrosine phosphatase-PEST regulates focal adhesion disassembly, migration, and cytokinesis in fibroblasts. J. Cell Biol. 144, 1019–1031.
113 Lyons, P. D., Dunty, J. M., Schaefer, E. M., and Schaller, M. D. (2001) Inhibition of the catalytic activity of cell adhesion kinase β by protein-tyrosine phosphatase-PEST-mediated dephosphorylation. J. Biol. Chem. 276, 24422–24431.
114 Chellaiah, M. A., Biswas, R. S., Yuen, D., Alvarez, U. M., and Hruska, K. A. (2001) Phosphatidylinositol 3,4,5-triphosphate directs association of Src homology 2-containing signaling proteins with gelsolin. J. Biol. Chem. 276, 47434–47444.
115 Mathew, S., George, S. P., Wang, Y., Siddiqui, M. R., Srinivasan, K., Tan, L., and Khurana, S. (2008) Potential molecular mechanism for c-Src kinase-mediated regulation of intestinal cell migration. J. Biol. Chem. 283, 22709–22722.
116 Wu, L.-W., Baylink, D. J., and Lau, K.-H. W. (1996) Molecular cloning and expression of a unique rabbit osteoclastic phosphotyrosyl phosphatase. Biochem. J. 316, 515–523.
117 Aguiar, R. C. T., Yakushijin, Y., Kharbanda, S., Tiwari, S., Freeman, G. J., and Shipp, M. A. (1999) PTPROt: an alternatively spliced and developmentally regulated B-lymphoid phosphatase that promotes G0/G1 arrest. Blood 94, 2403–2413.
118 Thomas, P. E., Wharram, B. L., Goyal, M., Wiggins, J. E., Holzman, L. B., and Wiggins, R. C. (1994) GLEPP1, a renal glomerular epithelial cell (podocyte) membrane protein-tyrosine phosphatase. Identification, molecular cloning, and characterization in rabbit. J. Biol. Chem. 269, 19953–19962.
119 Semiya, H., Sawabe, T., Inazawa, J., and Tsuruo, T. (1995) Cloning, expression and chromosomal localization of a novel gene for protein tyrosine phosphatase (PTP-U2) induced by various differentiation-inducing agents. Oncogene 10, 1731–1738.
120 Bodden, K., and Bixby, J. L. (1996). CRYP-2: a receptor-type tyrosine phosphatase selectively expressed by developing vertebrate neurons. J. Neurobiol. 31, 309–324.
121 Tagawa, M., Shirasawa, T., Yahagi, Y., Tomoda, T., Kuroyanagi, H., Fujimura, S., Sakiyama, S., and Maruyama, N. (1997) Identification of a receptor-type protein tyrosine phosphatase expressed in postmitotic maturing neurons: its structure and expression in the central nervous system. Biochem. J. 321, 865–871.
122 Motiwala, T., Ghoshal, K., Das, A., Majumder, S., Weichenhan, D., Wu, Y. Z., Holman, K., James, S. J., Jacob, S. T., and Plass, C. (2003) Suppression of the protein tyrosine phosphatase receptor type O gene (PTPRO) by methylation in hepatocellular carcinomas. Oncogene 22, 6319–6331.
123 Andersen, J. N., Jansen, P. G., Echwald, S. M., Mortensen, P. H., Fukada, T., Del Vecchio, R., Tonks, N. K., Møller, N. P. (2004) A genomic perspective on protein tyrosine phosphatases: gene structure, pseudogenes, and genetic disease linkage. FASEB J. 18, 8–30.
124 Wiggins, R. C., Wiggins, J. E., Goyal, M., Wharram, B. L., and Thomas, P. E. (1995) Molecular cloning of cDNAs encoding human GLEPP1, a membrane protein tyrosine phosphatase: characterization of the GLEPP1 protein distribution in human kidney and assignment of the GLEPP1 gene to human chromosome 12p12-p13. Genomics 27, 174–181.
125 Bischof, J. M., and Wevrick, R. (2005) Genome-wide analysis of gene transcription in the hypothalamus. Physiol. Genomics 22, 191–196.
126 Amoui, M., Baylink, D. J., Tillman, J. B., and Lau, K.-H. W. (2003) Expression of a structurally unique osteoclastic protein-tyrosine phosphatase is driven by an alternative intronic, cell-type-specific promoter. J. Biol. Chem. 278, 44273–44280.
127 Yang, J.H., Amoui, M., Strong, D.D., and Lau, K.-H.W. (2007) Characterization and comparison of the intronic promoter of murine osteoclastic protein-tyrosine phosphatase, PTP-oc, with the human PTP-oc promoter. Arch. Biochem. Biophys. 465, 72–81.
128 Wang, Z., Bhattacharya, N., Meyer, M. K., Seimiya, H., Tsuruo, T., Tonani, J. A., and Magnuson, N. S. (2001) Pim-1 negatively regulates the activity of PTP-U2S phosphatase and influences terminal differentiation and apoptosis of monoblastoid leukemia cells. Arch. Biochem. Biophys. 390, 9–18.
129 Jacob, S. T., and Motiwala, T. (2005) Epigenetic regulation of protein tyrosine phosphatases: potential molecular targets for cancer therapy. Cancer Gene Ther. 12, 665–672.
130 Suhr, S. M., Pamula, S., Baylink, D. J., and Lau, K.-H. W. (2001) Antisense oligodeoxynucleotide evidence that a unique osteoclastic protein-tyrosine phosphatase is essential for osteoclastic resorption. J. Bone Miner, Res. 16, 1795–1803.
131 Amoui, M., Suhr, S. M., Baylink, D. J., and Lau, K.-H. W. (2004) An osteoclastic protein-tyrosine phosphatase may play a role in the differentiation and activity of human monocytic U-937 cell-derived, osteoclast-like cells. Am. J. Physiol. Cell Physiol. 287, C874-C884.
132 Amoui, M., Sheng, M. H.-C., Chen, S.-T., Baylink, D. J., and Lau, K.-H. W. (2007) A transmembrane osteoclastic protein-tyrosine phosphatase regulates osteoclast activity in part by promoting osteoclast survival through c-Src-dependent activation of NFκB and JNK2. Arch. Biochem. Biophys. 463, 47–59.
133 Lau, K.-H. W., Wu, L.-W., Sheng, M. H.-C., Amoui, M., Suhr, S. M., and Baylink, D. J. (2006) A unique osteoclastic transmembrane protein-tyrosine phosphatase is a potential positive regulator of the c-src protein-tyrosine kinase activity, a mediator of osteoclast activity. J. Cell. Biochem. 97, 940–955.
134 Walsh, N. C., Cahill, M., Carninci, P., Kawai, J., Okazaki, Y., Hayashizaki, Y., Hume, D. A., and Cassady, A. I. (2003)

Multiple tissue-specific promoters control expression of the murine tartrate-resistant acid phosphatase gene. Gene 307, 111–123.

135 Sheng, M. H.-C., Amoui, M., Srivastava, A. K., Wergedal, J. E., and Lau, K.-H. W. (2007) Transgenic overexpression of osteoclastic protein-tyrosine phosphatase, PTP-oc, in cells of osteoclastic lineage led to increased bone resorption and marked reduction in trabecular bone mass and density in adult mice. J. Bone Miner. Res. 22 (Suppl. 1), S84, abstract # O112.

136 Wharram, B. L., Goyal, M., Gillespie, P. J., Wiggins, J. E., Kershaw, D. B., Holzman, L. B., Dysko, R. C., Saunders, T. L., Samuelson, L. C., and Wiggins, R. C. (2000) Altered podocyte structure in GLEPP1 (Ptpro)-deficient mice associated with hypertension and low glomerular filtration rate. J. Clin. Invest. 106, 1281–1290.

137 Miyazaki, T., Neff, L., Tanaka, S., Horne, W. C., and Baron, R. (2003) Regulation of cytochrome c oxidase activity by c-Src in osteoclasts. J. Cell Biol. 160, 709–718.

138 Chen, B., and Bixby, J. L. (2005) A novel substrate of receptor tyrosine phosphatase PTPRO is required for nerve growth factor-induced process outgrowth. J. Neurosci. 25, 880–888.

139 Chen, L., Juszczynski, P., Takeyama, K., Aguiar, R. C., and Shipp, M. A. (2006) Protein tyrosine phosphatase receptor-type O truncated (PTPROt) regulates SYK phosphorylation, proximal B-cell-receptor signaling, and cellular proliferation. Blood 108, 3428–3433.

140 Shintani, T., Ihara, M., Sakuta, H., Takahashi, H., Watakabe, I., and Noda, M. (2006) Eph receptors are negatively controlled by protein tyrosine phosphatase receptor type O. Nature Neurosci. 9, 761–769.

141 Zou, W., Kitaura, H., Reeve, J., Long, F., Tybulewicz, V. L., Shattil, S. J., Ginsberg, M. H., Ross, F. P., and Teitelbaum, S. L. (2007) Syk, c-Src, the αvβ3 integrin, and ITAM immunoreceptors, in concert, regulate osteoclastic bone resorption. J. Cell Biol. 176, 877–888.

142 Mundy, G. R., and Elefteriou, F. (2006) Boning up on ephrin signaling. Cell 126, 441–443.

143 Yang, J. H., Amoui, M., and Lau, K.-H. W. (2007) Targeted deletion of the osteoclast protein-tyrosine phosphatase (PTP-oc) promoter prevents RANKL-mediated osteoclastic differentiation of RAW264.7 cells. FEBS Lett. 581, 2503–2508.

144 Fang, K. S., Sabe, H., Saito, H., and Hanafusa, H. (1994) Comparative study of three protein-tyrosine phosphatases. Chicken protein-tyrosine phosphatase λ dephosphorylates c-Src tyrosine 527. J. Biol. Chem. 269, 20194–20200.

145 Arias-Salgado, E. G., Haj, F., Dubois, C., Moran, B., Kasirer-Friede, A., Furie, B. S., Furie, B., Neel, B. G., Shattil, S.J. (2005) PTP-1B is an essential positive regulator of platelet integrin signaling. J. Cell Biol. 170, 837–845.

146 Shiota, M., Tanihiro, T., Nakagawa, Y., Aoki, N., Ishida, N., Miyazaki, K., Ullrich, A., Miyazaki, H. (2003) Protein tyrosine phosphatase PTP20 induces actin cytoskeleton reorganization by dephosphorylation p190 RhoGAP in rat ovarian granulose cells stimulated with follicle-stimulating hormone. Mol. Endocrinol. 17, 534–549.

147 Chiarugi, P., Cirri, P., Taddei, L., Giannoni, E., Camici, G., Manao, G., Raugei, G., and Rampoin, G. (2000) The low M_r protein-tyrosine phosphatase is involved in Rho-mediated cytoskeleton rearrangement after integrin and platelet-derived growth factor stimulation. J. Biol. Chem. 275, 4640–4646.

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