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Tyrosine Phosphatases as Regulators of Skeletal Development and Metabolism

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Abstract The protein tyrosine kinases (PTK) and the protein tyrosine phosphatases (PTPs) are enzymes which play an integral role in tyrosine phosphorylation-dependent signaling cascades. By catalyzing the phosphorylation and dephosphorylation of cellular proteins, these enzymes direct the steady-state levels of specific phosphoproteins and ultimately dictate the functional state of all cells. The importance of this type of signaling in the skeleton is accepted but poorly understood. The contribution of the PTKs to signaling events in bone has been well studied but, in contrast, the regulation by PTPs is poorly defined. The recent identification of 107 genes within the human genome which encode members of the PTP superfamily emphasizes the need to consider the importance of these proteins in skeletal tissue. In this prospective, we will summarize the present state of our knowledge regarding the function of this enzyme superfamily, illustrating its relevance to the development and maintenance of the skeleton and highlighting future directions that should improve our understanding of these critical signaling molecules. J. Cell. Biochem. 96: 262–277, 2005.

Key words: tyrosine phosphatase; dual specificity phosphatase; skeleton; cartilage; limb bud; osteoblast; osteoclast

The tyrosine phosphorylation of cellular proteins is a critical signaling event in the regulation of all biological processes. The protein tyrosine kinases (PTKs), which catalyze the addition of a phosphate moiety, and the protein tyrosine phosphatases (PTPs), which remove that phosphate, are essential mediators of this type of signaling. These enzymes maintain a steady-state level of specific phosphotyrosine proteins which influence the functional state of the cell [Hunter, 1998]. Like other organ systems, the importance of this signaling mechanism in the development and maintenance of the skeleton has been accepted but is not fully understood. Many growth factors and cytokines that function as key regulators of bone formation mediate their actions by binding to receptors with intrinsic or associated PTK activity [Ostman and Bohmer, 2001]. Specific fibroblast growth factors (FGFs), for example, bind to the receptor tyrosine kinase, *Fgfr-2*, and are important for the proliferation of osteoprogenitors and subsequent osteoblast function during skeletogenesis [Naski and Ornitz, 1998; Yu et al., 2003]. Activity of the non-receptor tyrosine kinase c-src appears to be essential for cytoskeletal reorganization within the osteoclast, for osteoclast adhesion and subsequent bone resorption [Soriano et al., 1991; Tanaka et al., 1996; Miyazaki et al., 2004]. Much is known about these tyrosine kinase signaling cascades including their associated signaling

Abbreviations used: AER, apical ectodermal ridge; Akt/PKB, protein kinase B; CFU-F, colony forming unit fibroblast; ERK, extracellular signal regulated kinase; FAK, focal adhesion kinase; FGF, fibroblast growth factor; Fgfr-1/2, FGF receptor 1 or 2; FNIII, fibronectin type III repeat; HB-GAM, heparin binding growth associated molecule; I κB, inhibitor kappaB; JNK, c-jun N-terminal kinase; LAR, leucocyte antigen related molecule; MAPK, mitogen activated protein kinase; M-CSF, monocyte colony stimulating factor; MEK1, MAP kinase kinase 1; NF-κB, nuclear factor kappa B; PI(3)K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homology deleted on chromosome 10; PTH, parathyroid hormone; PTK, protein tyrosine kinase; PTN, pleiotropin; PTP, protein tyrosine phosphatase; Pyk2, proline-rich tyrosine kinase 2; PZ, progress zone; TRAF, tumor necrosis factor receptor associated factor; TRAP, tartrate-resistant acid phosphatase.

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molecules and the downstream biological effects of their activation. In contrast, the regulation of these cascades by tyrosine dephosphorylation catalyzed by PTPs is not as well studied. With the recent identification of 107 genes encoding PTPs within the human genome [Alonso et al., 2004] and the growing evidence that these molecules play an active, often dominant regulatory role, it is important for us to consider the relevance of the PTPs in skeletal tissue. The purpose of this review is to summarize our present knowledge regarding the function of this enzyme superfamily, illustrating its relevance to the development and maintenance of the skeleton and highlighting future directions that should improve our understanding of these critical signaling molecules.

BONE BIOLOGY BASICS

The skeleton of the vertebrate animal has evolved to provide structural support and give the organism the capacity for flexible, fluid locomotion. In addition, it is an important storage site for minerals such as calcium and serves an essential role in hematopoiesis, particularly in mammals. The development of this specialized tissue is initiated from three discrete cell lineages: (1) the cranial neural crest cells of the embryonic ectoderm which gives rise to jaw, teeth, auditory bones, and select skull bones of the craniofacial skeleton; (2) the paraxial mesoderm which gives rise to the skull, vertebrae, sternum, and ribs of the axial skeleton; and (3) the lateral plate mesoderm which gives rise to the limbs of the appendicular skeleton [Olsen et al., 2000; Karaplis, 2002]. Cells from these lineages migrate to the sites of future cartilage and bone tissue, forming dense aggregations of cells known as mesenchymal condensations which dictate the appropriate size and shape of the skeletal element. At these sites, the coordinated expression of critical gene products such as transcription factors, growth factors and adhesion molecules serves to specify three-dimensional positional information (e.g., anterior–posterior axis) for proper patterning of the limbs. In addition, these gene products help to drive the differentiation of these mesenchymal precursors to become the specialized cells of the developing cartilage and bone.

The existence and interaction of these specialized cell types is critical for de novo bone formation in the embryo as well as the continued maintenance and repair of the adult skeleton following normal mechanical stimulation, injury, or disease. These cells arise from mesenchymal and hematopoietic progenitors to become chondrocytes, osteoblasts, and osteoclasts [Aubin, 1998; Tosh and Slack, 2002]. The proliferation and differentiation of the chondrocyte from immature to hypertrophic cells is integral to the formation of the cartilage model of developing endochondral bones [Olsen et al., 2000; Karaplis, 2002]. This programmed maturation leads to type X collagen synthesis and calcification of the cartilage template, driving longitudinal growth of long bones during embryogenesis and early post-natal life. In the adult, unique types of chondrocytes remain within the articular and meniscal cartilage of the joints. The bone-forming cell, the osteoblast, invades the developing long bone, secretes a type I collagen-enriched matrix and ossifies that matrix to form the rigid, bony tissue [Olsen et al., 2000; Karaplis, 2002]. In addition, in the flat, intramembraneous elements such as the skull, osteoblasts form bony tissue without the presence of a cartilaginous template, differentiating from mesenchymal progenitors within these condensations. After becoming encased in its 'bony cage,' the mature osteoblast, known as the osteocyte, is thought to communicate the mechanical and hormonal status of its microenvironment, and serve a critical role in bone remodeling.

In contrast to the chondrocytes and osteoblasts which actively form skeletal tissue, the primary function of the bone resorbing cell known as the osteoclast is to break down or resorb bone. These cells are formed from hematopoietic precursors of the monocyte-macrophage lineage through the actions of factors, such as the receptor activator of NF-κB ligand (RANKL) and macrophage-colony stimulating factor (M-CSF), which are secreted by osteoblasts and other cells within the bone marrow microenvironment [Boyle et al., 2003]. It is the coupling between the osteoclast and the osteoblast that is critical in determining the effect of local or systemic hormonal modulators, such as interleukin-6 or parathyroid hormone, on bone metabolism. In general, these regulators act through the osteoblast, modulating the expression of molecules such as osteoprotegerin and RANKL which go on to affect osteoclastogenesis and osteoclast activation, thereby

controlling whether bone formation or bone resorption will be the ultimate physiological effect.

TYROSINE PHOSPHORYLATION AND BONE METABOLISM

As for all cells, the function of these skeletal cells is modulated by a myriad of hormonal and adhesion signals that ultimately result in changes in phosphorylation-dependent signaling cascades [Hunter, 2000]. The discovery in the early 1980s that tyrosine phosphorylation existed and was associated with normal and neoplastic cell growth prompted studies in osteoblasts to determine its importance and identify the enzyme(s) responsible. Interestingly, many of these early studies focused on tyrosine dephosphorylation, describing the existence of phosphotyrosyl phosphatase activity in normal bone tissue [William Lau et al., 1985] and reporting the extensive biochemical characterization of this activity [Lau et al., 1987, 1989a]. These studies showed that an inhibitor of phosphotyrosyl phosphatase activity, sodium orthovandate, could stimulate proliferation of primary osteoblasts in culture as well as elicit an increase in collagen synthesis and alkaline phosphatase activity, both indicators of osteoblast maturation and differentiation [Lau et al., 1988]. In effect, the orthovandate ions mimicked the actions of the fluoride ion; relevant since sodium fluoride has been used as a therapeutic agent for the treatment of osteoporosis [Lau et al., 1989b]. In some cases, this phosphotyrosyl phosphatase activity could be modulated by high cell density [Southey et al., 1995], contact with matrix proteins [Southey et al., 1995] and hormonal modulators of bone metabolism such as 1α,25-dihydroxyvitamin D3 and parathyroid hormone [Puzas and Brand, 1985; Southey et al., 1995]. Initially, it was hypothesized that alkaline phosphatase contributed to this phosphatase activity since this enzyme can dephosphorylate artificial phosphotyrosine substrates in vitro [Swarup et al., 1981, 1982; Puzas and Brand, 1985]. In addition, a 35 kDa tartrate-resistant acid phosphatase with sensitivity to vanadate and fluoride and specificity to phosphotyrosine had also been purported to be responsible for this activity [William Lau et al., 1985, 1987; Lau and Baylink, 2003]. However, the subsequent discovery of a family of tyrosine-specific enzymes with no sequence homology to the acid or alkaline phosphatases and distinct kinetic properties [Walton and Dixon, 1993] suggested that the PTPs were most likely the primary, physiologically relevant regulators of tyrosine phosphorylation in these bone cells.

THE PTP SUPERFAMILY

A decade after the first PTK, v-src, was identified, the first tyrosine-specific PTP, PTP1B, was purified [Charbonneau et al., 1989] and subsequently cloned from rat brain and human placenta [Chernoff et al., 1990; Guan et al., 1990]. Since this time, this superfamily of enzymes has grown, with member molecules classified by the presence of a consensus amino acid sequence, (I/V)HCSxGxGR(S/T)G, which delineates the active site within the signature catalytic PTP domain. Based on sequence information from the human genome, four main subfamilies are now recognized [Alonso et al., 2004]. The first and largest is the Class I Cysteine-based PTPs which includes the 'classical' tyrosine-specific enzymes as well as the dual-specificity phosphatases. This is the most diverse group exhibiting a wide range of substrate specificity from strict phosphotyrosine specificity (e.g., 'classical' PTPs) to phosphotyrosine/threonine (e.g., MAP kinase phosphatases (MKPs)) to phospholipids (e.g., PTENs and myotubularins). The Class II Cysteine-based PTPs includes a single human low molecular weight PTP (LMPTP) of unknown function which is related to the bacterial and yeast PTPs and appears to be phosphotyrosine specific [Bottini et al., 2002]. The Class III Cysteine-based enzymes include the cell cycle regulators (e.g., CDC25A) which activate the cyclin-dependent kinases (Cdks) by dephosphorylating phosphotyrosine/threonine residues within these proteins thus, enhancing cell cycle progression [Nilsson and Hoffmann, 2000]. The last and least well-defined subfamily is the Aspartate-based PTPs, known as the Eya genes. In contrast to the Cysteine-based PTPs, these proteins form a phosphoaspartate intermediate during catalysis, are reported to exhibit phosphotyrosine/serine phosphatase activity and appear to function in organogenesis [Li et al., 2003; Tootle et al., 2003].

Of all these subfamilies, it is predominantly the members of the Class I PTPs that have been examined in relation to their expression,

regulation, and function in the skeleton. Because of this, the 'classical' PTPs, which include the receptor and the intracellular enzymes, and the dual-specificity PTPs will be the focus of this review (Fig. 1). The receptor PTPs are highly unique because they are thought to directly couple an extracellular adhesive interaction to an intracellular tyrosine phosphorylation-dependent signaling cascade [Beltran and Bixby, 2003; Johnson and Van Vactor, 2003]. They accomplish this modulation through a striking diversity of extracellular domains and one or two intracellular PTP domains. Extracellular motifs such as fibronectin type III repeats (FNIII; Fig. 1), immunoglobulin-like domains (IgG-like) and carbonic anhydrase-like domains mediate homophilic or heterophilic interactions and regulate cell growth, migration, and differentiation. The intracellular PTPs possess a single PTP domain and unique non-catalytic domains which mediate their association with intracellular membranes (e.g., PTP1B), the cytoskeleton (e.g., PTP), and cellular phosphoproteins (e.g., SH2). By directing subcellular localization, these intracellular domains place these enzymes in the optimal microenvironment to regulate a variety of signaling cascades. The dual specificity PTPs include the mitogen-activated protein kinase phosphatases (MKPs) which exhibit phosphotyrosine and phosphothreonine phosphatase activity and show specificity for the MAP kinases, ERK, JNK, and p38. In the sections that follow, specific Class I PTP

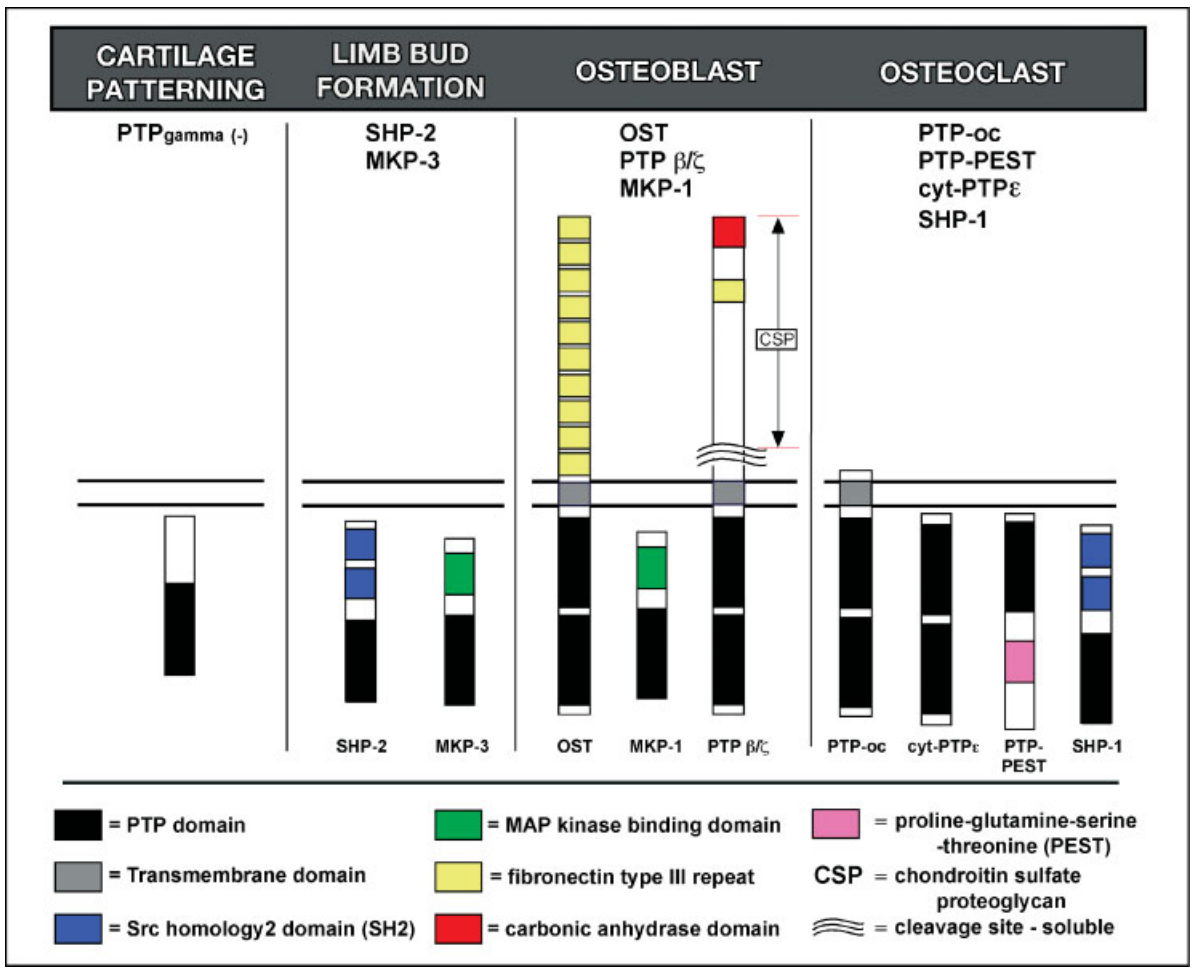

Fig. 1. Structural features of PTPs important during skeletogenesis. Select PTP molecules are pictured according to the process they are thought to regulate during skeletogenesis (i.e., cartilage patterning or limb bud formation) or the particular cellular function they are thought to regulate (i.e., that of the osteoblast or osteoclast). Key to symbols and block colors is given above.

molecules are discussed within the context of their relevance to skeletal development and maintenance. Figure 1 illustrates the structural features of the PTP molecules discussed. Table I summarizes the properties and the proposed function of these PTPs.

CARTILAGE PATTERNING

PTPgamma and Chondrocytes

The migration and condensation of mesenchymal precursors to form the cartilaginous skeleton is one of the crucial early events in embryonic bone development. Whether they are eventually defined by intramembraneous or endochondral ossification or remain as cartilage, these initial condensations will determine the 'pattern' of the skeleton, dictating the size, shape, and position of each skeletal element [Karaplis, 2002]. Modulated by cell–cell/cell–matrix interactions and soluble signaling factors, these condensed mesenchymal cells will proliferate and differentiate to become chondroblasts or osteoblasts. The PTP known as PTP gamma (–) has been proposed to be an important regulator of chondrogenic patterning (Table I). Originally isolated as a splice variant of a group of PTPs related to the striatum-enriched phosphatase [STEP; Augustine et al., 2000b], this intracellular enzyme has a single phosphatase domain and has no recognizable homology to known protein motifs outside of this catalytic sequence (Fig. 1). In situ hybridization analysis of gestational day 15.5 and 17.5 mouse embryos showed that PTP $\gamma(-)$ is expressed in many skeletal tissues including craniofacial bones, skull, ribs, and vertebrae [Augustine et al., 2000a]. Functional studies using ex vivo cultures of day 11.5 first brachial arches revealed that knockdown of PTP $\gamma(-)$ expression disrupts normal patterning of Meckel's cartilage, with enlargement and enhanced proliferation of the dorsal segments and lack of chondrogenesis in ventral segments of the explant [Augustine et al., 2000a]. These studies suggest that this phosphatase may regulate the transition from a prechondrogenic to a chondrogenic phenotype within specific regions of a condensation. The mechanism by which PTP $\gamma(-)$ modulates proliferation and differentiation of these mesenchymal cells during the patterning of skeletal elements remains to be determined.

LIMB BUD DEVELOPMENT

SHP-2 and the Progress Zone

The formation of the vertebrate limb is one of the most complex and exquisite processes during embryogenesis. Originating from specific limb 'fields' within the embryo, mesenchymal cells of the lateral plate mesoderm proliferate underneath ectodermal tissue to create a 'bulge' known as the limb bud. By secreting factors such as FGF10, these cells induce the formation of the apical ectodermal ridge or AER [Mariani and Martin, 2003]. In turn, the AER serves as a critical signaling center, controlling the proliferation, differentiation, and survival of the underlying mesenchymal cells. Factors secreted by the AER, many also members of the FGF family (e.g., FGF8, FGF4), regulate the expression of genes which help dictate the anterior–posterior, proximal–distal, and dorsal–ventral axes, ensuring the proper development of each limb of the appendicular skeleton.

The PTP known as SHP-2 is thought to play an obligate role in this process. In vertebrates, two molecules have been characterized; SHP-1 expressed predominantly in hematopoietic cells and SHP-2 which is ubiquitously expressed [Neel et al., 2003]. Each molecule possesses two SH2 domains and a single PTP domain (Fig. 1; Table I). Originally identified in members of the Src family of non-receptor tyrosine kinases, the SH2 domains of these phosphatases bind to phosphotyrosyl residues promoting protein–protein interactions within the cell. These interactions help link specific receptors to downstream signaling molecules, modulating the intrinsic PTP activity of the SHP protein as well as the signaling pathway that these SHPs are regulating [Neel et al., 2003].

The initial indication that SHP-2 was relevant was based on studies to explore its role in hematopoiesis. Due to the lethality of loss of SHP-2 expression, researchers produced chimeras through aggregation of wild type or SHP-2 –/– murine embryonic stem (ES) cells with morula-stage wild type embryos. In addition to adverse effects on blood cell development, these chimeric animals exhibited abnormal limb features, shortened hind limbs and split lumbar vertebrae [Qu et al., 1998]. Chimeric analysis tracking individual cells within developing skeletal tissues showed that, in contrast to wild-type cells, little to no SHP-2 –/– mutant cells were present in somites and limb buds, in

TABLE I. Expression and Properties of PTP Superfamily Members in the Skeleton

	Gene (aliases)[a]	Species[b]	Domains[c]	Expression[d]	Proposed function
Receptor PTPs					
OST-PTP	*PTPrv* (Esp)	M, R	Ex: FNIII CD: 2	MC, PC, PO, DJ, OB $_{(P,L)}$	Differentiation of progenitors to osteoblast lineage
PTPbeta/zeta	*PTPrz1* (Phosphacan)	H, M, R	Ex: CA, CS CD: 2	OB $_{(L)}$	Osteoblast recruitment and adhesion in association with pleiotrophin
PTPepsilon	*PTPre*	H, M, R	Ex: Gly CD: 2	OC $_{(P)}$	Podosome formation and osteoclast-mediated resorption of bone
PTPoc	*PTPro* variant	Rbt	Ex: NH CD: 1	OC $_{(P)}$	Modulation of c-src-mediated regulation of osteoclast activity
Intracellular PTPs					
PTPgamma	*PTPrr* variant	M	NC: NH CD: 1	Miscellaneous skeletal elements	Chrondrocyte proliferation and patterning
PTP-PEST	*PTPn12*	H, M, R	NC: PEST CD: 1	OC $_{(P)}$	Regulation of multimeric protein complexes in podosomes of osteoclasts
SHP-1	*PTPn6* (hcph; me)	H, M, R	NC: SH2 (2) CD: 1	OC $_{(P)}$	Regulation of RANKL-mediated osteoclast formation
SHP-2	*PTPn11*	H, M, R, Ch	NC: SH2 (2) CD: 1	LBM, OB $_{(P,L)}$ OC $_{(P)}$	Modulation of cells within the Progress zone of the developing limb bud
Dual specificity PTPs					
MKP-1	*DUSP1* (CL100; 3CH134)	H, M, R	CD: 1	LB, OB $_{(L,P)}$	Modulation of glucocorticoid- and PTH-induced inhibition of osteoblast proliferation
MKP-3	*DUSP6* (Pyst1; rVH6)	H, M, R, Ch	CD: 1	LBM, OB $_{(L)}$	Regulation of FGF8 signaling in the developing limb bud

[a]Gene codes = accepted gene designation according to National Center for Biotechnology Information (NCBI); (aliases) = other published names used. Gene code variant indicates an isoform of this gene which has not been assigned a specific code.
[b]Species for which mRNA transcripts have been cloned. H, human; M, mouse; Rbt, rabbit; R, rat; Ch, chicken.
[c]Predicted domains of each protein listed as "location/type of domain: description." Ex, extracellular domain; CD, consensus catalytic phosphatase domain; NC, non-catalytic domains. Abbreviations: FNIII, fibronectin type III repeats; CA, carbonic anhydrase; CS, chondroitin sulfate proteoglycan; Gly, glycosylation sites; NH, no known sequence homology; PEST, proline-glutamine-serine-threonine rich; SH2, Src homology-2.
[d]Expression (mRNA or protein) reported within specific skeletal cells or structures. Abbreviations: DJ, developing joint space; LB, long bone; LBM, limb bud mesenchyme; MC, mesenchymal condensations; OB, osteoblast (P, primary cells; L, cell lines); OC, osteoclast; PC, perichondrium; PO, periosteum.

particular within the progress zone (PZ), the region of proliferating mesenchyme directly beneath the AER [Saxton et al., 2000]. Additional chimeric analyses with FGF receptor 1 (Fgfr1) −/− ES cells revealed a similar lack within this region. These mutant cells were capable of a mitogenic response to FGFs but exhibited abnormal adhesive properties with enhanced and mislocalized focal contacts as well as atypical, homotypic aggregation in vitro [Saxton et al., 2000]. These studies suggest that SHP-2 is required for proper functioning of cells in the PZ where it may regulate cell migration and adhesion. The signaling pathway involved may be initiated through the Fgfr1. It is possible that in cells of the PZ this phosphatase acts upstream of ERK as a positive regulator, interacting with and dephosphorylating inhibitors of Fgfr1-induced MAPK activation. This scenario has been shown in models of lens and retinal development and neurite outgrowth [Gotoh et al., 2004; Hanafusa et al., 2004].

MKP-3 and FGF Signaling

Following the formation of the AER, cells of this region secrete FGF8 which, through the modulation of the MAPK and PI(3)K pathways, mediates cell proliferation and cell death of the underlying mesenchymal cells. Studies examining chick, mouse, and zebrafish limb/fin development have shown that FGF8 can induce and is necessary for the expression of the dual-specificity PTP known as mitogen activated protein kinase phosphatase 3 (MKP-3) in the mesenchyme [Kawakami et al., 2003; Farooq and Zhou, 2004]. This PTP is known to selectively dephosphorylate and inactivate the MAPK, ERK1/2 (Fig. 1; Table I). Inactivation of ERK 'turns off' or downregulates MAPK signaling in many types of cells. Within the limb bud, the AER exhibits the highest levels of phosphorylated, activated ERK suggesting heightened activity of this cascade where, interestingly, MKP3 expression is normally suppressed during development. Viral overexpression within the AER of a mutant MKP3 molecule, which sequesters ERK and inhibits MAPK signaling, disrupts normal limb bud formation. Artificial induction of MAPK/ERK signaling by overexpressing a constitutively active Mek1 molecule results in enhanced cell death within the mesenchyme. Likewise, suppression of MKP-3 expression using siRNA leads to enhanced levels of phosphorylated ERK and increased cell death. These results strongly support a critical role of this PTP in mediating the anti-apoptotic effects of the secreted AER growth factor, FGF8, on the underlying mesenchyme of the developing limb. The activity of MKP3 in these cells appears to modulate MAPK/ERK activity and thereby prevent cell death.

THE OSTEOBLAST

OST, Osteoprogenitors, and Differentiation

Originating from the mesoderm and the neural crest of the developing embryo, the mesenchymal stem cells are multipotent cells capable of giving rise to bone-forming osteoblasts, as well as chondrocytes, myoblasts, and adipocytes. In the adult, a related population of stem cells exists, known as CFU-F (colony forming unit-fibroblast), which is localized within the bone marrow stroma and has the capacity to form new osteoblast cells. Driven by signals within their microenvironment, these stem cells will progress through multiple steps of increasingly restricted progeny to form progenitors committed to osteoblast development and bone formation [Aubin, 1998]. Although the potential for plasticity exists within the osteogenic lineage, these osteoprogenitors are generally thought to proceed through a linear sequence from progenitor to preosteoblast, osteoblast, and then bone lining cells or osteocytes. This temporal progression of osteoblast differentiation has been divided into three main phases: (i) proliferation—cells express genes associated with cell proliferation (e.g., H4 histone, c-fos, c-myc) as well as those encoding the predominant bone matrix protein, type I collagen; (ii) matrix development and maturation—cells continue expression of collagen and initiate expression of differentiation-associated genes such as alkaline phosphatase, required for subsequent matrix mineralization; and (iii) mineralization—upregulation of osteocalcin, osteopontin, and bone sialoprotein gene expression with the deposition of mineral hydroxyapatite salts composed of calcium and phosphorous.

A receptor PTP known as osteotesticular PTP (OST; also embryonic stem cell phosphatase or Esp) is hypothesized to have a role in this osteoblast commitment and differentiation (Fig. 1; Table I). Originally cloned from rat

primary osteoblasts and osteosarcoma cell lines (UMR 106), the OST molecule has a unique structure, possessing 10 fibronectin type III-like (FNIII) repeats and two intracellular PTP domains [Mauro et al., 1994; Lee et al., 1996; Fig. 1]. Classified as a Type III receptor molecule, the only other member of this subgroup in mammalian species that is similar in structure to OST is the density-enriched tyrosine phosphatase known as DEP-1 which has 8 FNIII extracellular domains and a single PTP domain [Ostman et al., 1994]. The rat and mouse *OST* gene have a very restricted tissue distribution, expressed in a regulated manner primarily in bone, ovary, and testes [Lathrop et al., 1999; Morrison and Mauro, 2000]. By 12.5 days post coitum during development of the embryonic skeleton, transcripts encoding this PTP can be localized within mesenchymal condensations of skeletal templates [Yunker et al., 2004]. Subsequently, expression is restricted to the perichondrium, the layer of undifferentiated mesenchymal progenitors surrounding all endochondral skeletal elements. Once ossification begins, OST is also expressed within the bone collar and periosteum as well as within putative osteoblasts along the chondro-osseous border and the cortical and trabecular surfaces of long bones [Yunker et al., 2004]. In adult rodents, OST expression is only detectable in a subpopulation of articular chondrocytes [Yunker et al., 2002] and in cells of the bone marrow cavity. Interestingly, preliminary studies indicate that OST expression within bone marrow cells is greatly enhanced following exercise (L. Yunker, J. Delaney, D. Rowe and L. Mauro, unpublished observations) suggesting that this PTP may be an early marker of osteoprogenitors present in the marrow.

In vitro studies have proven that the expression of OST is regulated in cells of the osteoblast lineage and may be an important gene involved in osteoblast differentiation. In primary and immortalized calvarial cultures, levels of OST mRNA and protein are nondetectable in the proliferating pre-osteoblast and increase 5–10 fold in the differentiating osteoblast [Mauro et al., 1994; Chengalvala et al., 2001; Wheeler et al., 2002]. Once expression of the matrix protein, osteocalcin, is evident and mineralization has initiated, OST expression declines to the basal, proliferation levels paralleling the expression of the alkaline phosphatase gene. This upregulation in OST expression appears to be somewhat specific to this member of the PTP family since the expression of another receptor PTP, LAR, as well as that of the intracellular PTP, PTP1B, is unchanged during this process [Wheeler et al., 2002]. The mechanism driving this dramatic increase in OST in maturing osteoblasts is, in part, the osteoblast-specific, transcriptional activation of the *OST* gene which is dependent on cell density as well as the attainment of the differentiated osteoblast phenotype [Wheeler et al., 2002]. Suppression of OST expression in such differentiating cultures results in a $\sim$80% reduction in bone nodules and a $\sim$60% decrease in secreted osteocalcin suggesting that this PTP has a critical role during this progression from preosteoblast to mineralizing osteoblast [Chengalvala et al., 2001]. In addition to regulated expression during differentiation, hormonal modulators of bone metabolism, such as parathyroid hormone (PTH), have a potent effect on OST expression. Acute PTH stimulation of osteoblast-like cells (10 nM PTH; $\geq$10 min) causes a dramatic increase in OST mRNA, detected in studies using both Northern blot and microarray analysis [Mauro et al., 1994, 1996; Qin et al., 2003].

The highly specific and unique localization of OST in vivo to mesenchymal osteoprogenitors, the regulated expression of this PTP during bone development and osteoblast differentiation and its modulation by physiologically relevant regulators of bone metabolism are all strong evidence for a putative role of OST in bone formation. In addition, the exciting possibility that this molecule could serve as a much-needed marker of mesenchymal progenitor cells adds practical value to such a role. Of course, further research is necessary to establish the physiological substrates of this PTP, to determine the signaling pathways it regulates and to clarify the ultimate effect of OST activity on bone cell function. Toward this end, the recent development of a 'knock-in' LacZ mouse model lacking the *OST* gene should prove helpful [Dacquin et al., 2004]. The caveat in all of these studies is whether such research is relevant to human bone development and metabolism since recent published studies indicate that human *OST* is a pseudogene and that no functional human *OST* ortholog exists [Cousin et al., 2004]. This is quite surprising, as a human ortholog has been detected for all the other rodent molecules within the PTP superfamily. It is hypothesized that this gene may have been

modified following the divergence between Artiodactyls and Primates since (i) ESTs encoding putative OST orthologs have been isolated from domesticated pigs and cows and; (ii) the chromosomal region, 1q32.1, where the human OST pseudogene is found is thought to be a frequent site of gene rearrangements and innovation during evolution [Cousin et al., 2004]. The question remains as to what receptor PTP may compensate for OST activity in humans since such strong evidence exists for its importance in rodent models. Considering the similarities in its expression during cell differentiation and high cell density, it would be interesting to determine if the structurally related type III receptor PTP, DEP-1, could fulfill such a role.

PTPbeta/zeta (PTPβ/ζ) and Osteoblast Recruitment

During bone development or remodeling, osteoblast precursors must be recruited to the site of osteoid deposition, attach to the surface, and differentiate to become a mature, bone-producing cell. Chemotactic signals such as diffusible factors as well as haptotactic signals such as immobilized proteins are thought to mediate this recruitment and subsequent attachment to the bone matrix. The interaction between the matrix associated HB-GAM [heparin-binding growth-associated molecule; also known as pleiotrophin or osteoblast specific factor-1 (OSF-1)] and the cell-surface proteoglycan, N-syndecan, may be an important regulatory mechanism for this process [Imai et al., 1998]. Pleiotropin (PTN) is highly expressed by osteoblast-like cells (e.g., UMR 106) and at sites of osteoid deposition such as within mineralized cartilage and epiphyseal growth plates of developing bone and at sites of fracture healing and adjuvant-induced arthritic injury [Tare et al., 2002a]. N-syndecan, the putative receptor for PTN, is also abundantly expressed and colocalizes with PTN in these cells and tissues. In vitro studies have supported the importance of this PTN/syndecan interaction, showing specific migratory behavior of UMR 106 cells which can be competed with both soluble PTN or N-syndecan. In addition, supporting in vivo studies have shown that overexpression of PTN in transgenic mice results in enhanced bone formation with increased volume of cortical, cancellous, and intramembraneous bone [Imai et al., 1998; Tare et al., 2002b].

The mechanism by which PTN and N-syndecan mediate osteoblast recruitment may involve signaling through the receptor PTP known as PTP beta/zeta (PTPβ/ζ; Fig. 1 and Table I). This protein possesses two PTP domains and an extracellular domain with a carbonic anhydrase-like domain and a FNIII domain. This extracellular domain can be synthesized, cleaved, and secreted as a chondroitin sulfate proteoglycan and can be incorporated into the extracellular matrix. Similar to N-syndecan, PTPβ/ζ is expressed by osteoblast-like cells, has been identified as another receptor for PTN and has high affinity for both PTN and a homologous protein known as midkine [Rauvala et al., 2000]. In vitro studies with UMR 106 cells have shown that inhibition of overall PTP activity using orthovandate inhibits midkine-induced migration whereas engagement of PTPβ/ζ molecules using anti- PTPβ/ζ antibodies results in enhanced cell migration [Qi et al., 2001]. Interestingly, binding of PTN to PTPβ/ζ suppresses its phosphatase activity and enhances β-catenin phosphorylation, providing a putative connection between PTP activity and a downstream signaling cascade which mediates cell adhesion [Meng et al., 2000]. The intriguing connection between PTN and PTPβ/ζ will require a greater focus on the function of PTPβ/ζ in osteoblast recruitment and adhesion. Future examination of osteoblasts from PTPβ/ζ-deficient mice, in particular, in states of hormonal activation and bone injury, may prove informative.

MKP-1 and Osteoblast Signaling

The balance between the proliferation and differentiation of an osteoblast has an important influence on the amount of bone formation during development and during the remodeling of healthy or diseased bone. Local and systemic secretion of cytokines, growth factors, and hormones provide the regulatory signals to the osteoblast to expand the pool of osteoblast precursors, promote differentiation to a mature, bone-forming phenotype or modulate osteoclast-mediated resorption. Parathyroid hormone is one such signal which serves as a critical systemic regulator of calcium balance and bone metabolism. Secreted by the parathyroid gland, PTH can have catabolic or anabolic effects on the bone by acting directly on the osteoblast or indirectly on the osteoclast [Swarthout et al., 2002]. Studies have suggested

that chronic, high dose exposure to this hormone leads to enhanced bone resorption whereas intermittent administration of PTH leads to enhanced bone formation. The underlying signaling mechanisms mediating these strikingly different effects on osteoblast function are not well understood.

Recent studies have provided evidence to suggest that the dual specificity PTP, known as MKP-1, may be an important mediator of PTH signaling (Fig. 1, Table I). Gene expression profiling of PTH-stimulated osteoblasts (e.g., primary and osteosarcoma cells) has revealed about 125 known genes that are regulated. These include *MKP-1*, whose expression increases ~2 fold within 1 h following PTH treatment [Qin et al., 2003]. Similar to MKP-3, which has been shown to be relevant in limb bud formation, MKP-1 is also recognized as a major regulator of MAP kinase signaling and exhibits a broader enzymatic specificity, dephosphorylating several major MAPKs, including ERK, p38, and JNK. In such a role, MKP-1 may serve as a suppressor of MAPK signaling in PTH-stimulated osteoblasts, promoting cell cycle arrest and subsequent differentiation, potentially contributing to the anabolic effect of PTH. In vitro studies with UMR 106 osteoblasts have shown that MKP-1 expression increases after PTH stimulation, and that there is a parallel decrease in ERK phosphorylation suggestive of MAPK inactivation [Qin et al., 2005]. The profile of cell cycle proteins suggest cell cycle arrest with an increase in the cyclin-dependent kinase inhibitor, $p21^{Cip}$ and a decrease in cyclin D. These shifts in expression are also observed after in vivo PTH administration. Overexpression of MKP-1 in this cell line results in a 9% increase in the percentage of cells in G_1 and a comparable decrease of cells in S phase. Taken together, it is feasible that the anti-proliferative effects of PTH are mediated by upregulation of MKP-1 expression, resulting in inhibition of the MAPK/ERK pathway and subsequent arrest of cell cycle progression. Other studies have suggested that MKP-1 is not responsible for the observed PTH-induced attenuation of ERK phosphorylation, but rather acts on another MAPK, JNK, and that the inhibition of the MAPK/ERK pathway is through transcription-independent mechanisms [Homme et al., 2004]. Studies utilizing antisense or RNA interference methodologies to suppress MKP-1 expression should help clarify the role of MKP-1 and the relative contributions of the MAPK/ERK and MAPK/JNK pathways. This approach has been used successfully in preadipocyte and endothelial cell models [Chandrasekharan et al., 2004; Sakaue et al., 2004]. In addition, the availability of MKP-1-deficient mice would be another valuable tool for these studies [Dorfman et al., 1996].

Glucocorticoids are another hormonal signal of interest because of their potent effects on osteoblast function. Research has focused on furthering our understanding of the condition known as glucocorticoid-induced osteoporosis, a common complication of long-term use of synthetic glucocorticoids for the treatment of a variety of disease conditions [Canalis et al., 2004]. The action of glucocorticoids to decrease bone formation and functional osteoblast numbers with this condition is thought to be the result of this hormone's capacity to inhibit osteoblast proliferation and enhance apoptosis. In vitro studies with mouse stromal (MBA-15.4) and human preosteoblast (MG-63) cell lines have revealed that treatment with the highly potent glucocorticoid, dexamethasone (Dex), results in an inhibition of proliferation following serum stimulation and a marked decrease in ERK phosphorylation following TPA stimulation [Engelbrecht et al., 2003]. In addition, total phosphatase activity of these cells increases with Dex treatment while inhibition of cellular PTPs using sodium orthovanadate completely reverses the Dex-induced inhibition of proliferation. Since these data suggested involvement of PTPs, expression analysis of MKP-1 and MKP-3 was conducted and revealed a dramatic increase in both MKP-1 mRNA and protein. It is hypothesized that the up-regulation of MKP-1 suppresses MAPK/ERK activation, providing a rapid mechanism accounting for at least some of the direct inhibitory effects of glucocorticoids on osteoblast proliferation. Studies in other cell types have shown that glucocorticoids can also attenuate the degradation of MKP-1 protein adding another potential facet to their regulation of bone. Interestingly, in vivo inhibition of PTPs using vanadium supplementation can prevent the steroid-induced loss of bone mineral density and bone strength in a rat model [Hulley et al., 2002]. However, similar to PTH signaling, it remains to be proven that MKP-1 is the PTP responsible and that it is a necessary and indispensable component of glucocorticoid effects on osteoblast function.

THE OSTEOCLAST

PTPoc, c-src, and Osteoclast Differentiation

A PTP known as osteoclastic protein tyrosine phosphatase (PTP-oc) was recently identified [Wu et al., 1996] and is thought to regulate osteoclast function (Fig. 1, Table I). With the exception of a stretch of amino acids at the cytoplasmic juxtamembrane region, this PTP-oc protein is thought to be a truncated variant of the renal receptor PTP known as glomerular epithelial protein-1 [GLEPP1; Thomas et al., 1994], sharing complete sequence identity with the intracellular domain of GLEPP1. Although both are products of the *PTPro* gene, the transcription of PTP-oc is driven by an alternative, intronic promoter [Amoui et al., 2003] which appears to confer cell-specific expression in osteoclast precursors as well as mature osteoclasts. Putative repressor binding sites within this promoter may be important for this tissue specificity.

Studies with in vitro osteoclast cultures suggest that this PTP may be essential for bone resorption, potentially by regulating osteoclast proliferation and differentiation. Suppression of PTP-oc expression in primary rabbit osteoclast cultures using antisense oligonucleotides results in a marked reduction in both basal and hormone-stimulated (PTH or vitamin D3) resorption with no change in numbers of TRAP-positive osteoclasts [Suhr et al., 2001]. In addition, the observed increase in the phosphorylation levels of intracellular c-src at the regulatory tyrosine residue, tyr-527, suggests that PTP-oc may normally regulate c-src activity in the osteoclast. In other studies, overexpression of wild-type, catalytically active PTP-oc (WT-PTP-oc) was associated with enhanced bone resorption activity of U-937 cell-derived "osteoclast-like" cells as well as increased numbers of TRAP-positive osteoclasts (i.e., mature osteoclasts capable of resorption) The proliferative effects of PTP-oc overexpression are supported by increased [^{3}H] thymidine incorporation by WT-PTP-oc cells and an increased percentage of these cells in S phase of the cell cycle. Wild type PTP-oc overexpression is also associated with enhanced NF-κβ nuclear translocation and increased transcriptional activity of an NF-κβ reporter gene. In addition, levels of phosphorylated c-src PY-527 were reduced to 25% of control cells. In all, these results strongly support a putative role of PTP-oc in osteoclast proliferation and differentiation. This PTPs regulation of src-dependent or NF-κβ-dependent signaling pathways and the identity of its physiological substrate(s) remains to be clarified. It would also be interesting to determine if osteoclasts from the GLEPP1-deficient mouse express the cytosolic form of the *PTP-oc* gene and if a defect in osteoclast function exists in these animals [Wharram et al., 2000].

PTP-PEST, cyt-PTPε, and Podosomes

Podosomes are specialized adhesions found in activated osteoclasts. These adhesions are a precursor to the dense actin ring: a structure which delineates the sealing zone of the osteoclast and forms the compartment between the surface of the bone and the ruffled border of the osteoclast. Podosomes consist of a multimeric complex of surface and intracellular proteins which are assembled following the interaction of extracellular matrix proteins, such as osteopontin, with the $\alpha_v\beta_3$ integrin receptor, resulting in a structural link between the bone matrix and the cytoskeleton. The proper assembly and disassembly of the podosomes is essential for osteoclast motility and bone resorption.

Studies suggest that PTP-PEST may participate in the regulation of podosome formation. This phosphatase possesses an N terminal proline-glutamic acid-serine-threonine (PEST)-rich region as well as a single PTP domain (Fig. 1, Table I). Immunofluorescence studies have shown that PTP-PEST is colocalized within the podosome, in close association with such molecules as the cytoskeletal proteins gelosin, leupaxin, paxillin, vinculin, and talin; the tyrosine kinases Pyk2, FAK, and c-src; and the lipid kinase PI3-K [Chellaiah et al., 2001; Gupta et al., 2003]. Co-immunoprecipitation of PTP-PEST with the actin-binding protein, gelosin, substantiates this association. Treatment of osteoclasts with osteopontin to promote podosome assembly results in transient serine phosphorylation of PTP-PEST. In other cell models, phosphorylation of PTP-PEST has been shown to lower its substrate affinity and decrease its phosphatase activity [Garton and Tonks, 1994]. Therefore, it is possible that the osteopontin-induced phosphorylation of PTP-PEST inhibits its catalytic activity, resulting in enhancement of tyrosine phosphorylation of associated proteins in the podosome and stabilizaton of the assembled protein complex. When

PTP-PEST is maximally active, it would be presumed to promote podosome disassembly by dephosphorylating proteins within the complex and disrupting protein–protein interactions. This would be consistent with the proposed role of this PTP in focal adhesion formation where it promotes the turnover of these podosomal structures which is necessary for cell migration [Garton and Tonks, 1999]. Further studies are needed to explore the regulation of PTP-PEST activity, identify its substrate within the podosome complex, and determine the effects of loss of PTP-PEST function on podosome formation.

In addition to PTP-PEST, the PTP epsilon (*PTP*ε) gene also appears to contribute to podosome function (Fig. 1, Table I). The unique expression of this gene results in the production of two major isoenzymes, the membrane-associated receptor (PTPε) and the intracellular (cyt-PTPε) proteins. Each of these forms appears to have distinct physiological functions and cell-specific expression. Of these, the cyt-PTPε isoform is most highly expressed in osteoclasts. Mice lacking expression of *PTP*ε gene (*Ptpre* −/− mice) exhibit increased trabecular bone mass and possess osteoclasts with reduced capacity to mature and resorb bone in in vitro resorption assays [Chiusaroli et al., 2004]. Closer examination of podosomes from the PTPε-deficient mice revealed a disorganization of podosome structure with poorly distributed actin and vinculin proteins. This phenotype is restricted to younger (<12 weeks old), female mice and does not appear to affect src activity or Pyk2 phosphorylation. These intriguing observations warrant further research to determine the mechanism of this age- and gender-specific phenotype. Also, it is necessary to verify that the intracellular isoform, cyt-PTPε, is indeed the PTP epsilon gene product that is important, along with PTP-PEST, for proper podosome organization.

SHP-1 and RANKL Signaling

Growing evidence supports the role of the hematopoietic cell-enriched SHP-1 in bone resorption. Like SHP-2, it also possess SH2 domains and a single PTP domain but, in contrast, its expression is restricted to cells of hematopoietic origin (Fig. 1; Table I). The "motheaten" and "viable motheaten" mutant mice have been a fortuitous model in the study of SHP-1 function [Shultz et al., 1984; Neel et al., 2003]. This mouse phenotype, named for the patchy hair loss or "motheaten" appearance, is characterized by severe immunodeficiency, systemic autoimmunity, and premature death. Spontaneous point mutations in the hematopoietic cell phosphatase (*Hcph*) gene which encodes SHP-1 results in little to no SHP-1 protein expression or catalytic activity in these mutant mice [Shultz et al., 1993]. Several researchers have shown that the loss of SHP-1 activity in these animals results in enhanced osteoclast function and increased bone resorption. Umeda et al. [1999] observed that mice homozygous for the viable motheaten mutation (me^v/me^v) exhibit osteopenia with decreased bone mineral density and mineral content as well as lower trabecular volume and cortical thickness as compared to wild-type littermates. These deficiencies appear to be due to increased osteoclastogenesis (i.e., osteoclast differentiation from hematopoietic precursors) as a greater number of TRAP positive cells (i.e., mature osteoclasts) are observed in vivo in me^v/me^v mice and in in vitro osteoblast/bone marrow coculture assays. These observations have been substantiated by other researchers [Aoki et al., 1999].

The mechanism by which SHP-1 regulates bone resorption is beginning to be elucidated. SHP-1 appears to be an important modulator of the RANKL/RANK signaling pathway which is critical for osteoclastogenesis, osteoclast function, and survival. Binding of RANKL to its receptor RANK leads to association of TRAFs and subsequent activation of multiple signaling cascades. Studies have shown that overexpression of a dominant negative SHP-1 protein ('phosphatase dead' C453S mutant) results in increased RANKL-induced osteoclast formation in vitro and enhancement of downstream RANKL-stimulated NF-κB/IκB signaling such as increased IκB-α phosphorylation and degradation [Zhang et al., 2003]. Following RANKL stimulation, SHP-1 is recruited to a complex containing TRAF6; this association is thought to involve the C terminus of SHP-1. These studies suggest that SHP-1 acts as a negative regulator of RANKL/RANK signaling in osteoclasts, which is in agreement with its role in immune cells. The identity of the substrate that SHP-1 dephosphorylates downstream in this cascade is not known. RANKL stimulation can also lead to the association of TRAF6 with the tyrosine kinase, c-src, resulting in enhanced c-src activity and downstream activa-

tion of the Akt/PKB pathway; these signaling events are important in osteoclast survival [Wong et al., 1999]. Additional studies to identify SHP-1 substrates and determine the interactions of SHP-1 in these multiple pathways activated by RANKL will further establish the role of this phosphatase in RANKL/RANK signaling.

CONCLUSIONS

The evidence presented in this review strongly supports the view that PTPs are critical regulators of tyrosine phosphorylation at multiple stages of bone development and metabolism. Many questions and challenges remain to be addressed to help us better understand the identity and functions of specific PTPs. The most crucial is that the physiological substrates of these PTPs and the specific signaling pathways they regulate in bone cells need to be clarified. This is not a trivial task considering we are still unsure of the relevant substrates for the tyrosine kinase, c-src, after over 20 years of research. Nevertheless, the use of methodologies such as combinatorial libraries and 'substrate trapping' mutant screens combined with proteomics analyses should prove valuable in future studies.

In addition, the utilization of established transgenic PTP mouse models and the development of new models will be an important step. Such models will provide alternative experimental approaches which will help establish functional relevance of these molecules as well as provide a valuable resource. Researchers should be aware that, to date, many PTP 'knock-in' or 'knock-out' models have not exhibited an overt phenotype. Often a 'challenge' to the homeostasis of a physiological system has been necessary to reveal the function of the PTP molecule. For example, since the genetic ablation of PTP1B expression resulted in healthy mice it could have been concluded that this molecule was not essential. Additional analysis of glucose metabolism via glucose and insulin tolerance tests [Elchebly et al., 1999] as well as subsequent studies helped establish PTP1B as a negative regulator of insulin signaling and a viable therapeutic target for the treatment of diabetes. Therefore, when evaluating any such models, it may be necessary to manipulate bone metabolism through hormonal stimulation or the experimental induction of a trauma (i.e., fracture repair, arthritic injury) or disease state (i.e., ovariectomy-induced osteoporosis) to reveal the functional relevance of specific PTPs in the skeleton. Finally, with over 107 human PTP genes identified, we need to determine if other PTP molecules, besides those discussed in this review, have a role in skeletal development and metabolism.

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