Identification of a Hormonally Regulated Protein Tyrosine Phosphatase Associated with Bone and Testicular Differentiation*

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Absence of the tyrosine kinase activity of c-src and c-fms results in impairment of bone remodeling. Such dysfunction underscores the importance of tyrosine phosphorylation, yet the role of protein tyrosine phosphatases in bone metabolism remains unexamined. We have isolated the cDNA for a novel receptor-like tyrosine phosphatase expressed in bone and testis named osteotesticular protein tyrosine phosphatase (OST-PTP). The deduced 1711-residue protein possesses an extracellular domain with 10 fibronectin type III repeats and a cytoplasmic region with two catalytic domains. In primary rat osteoblasts, the 8.8-kilobase OST-PTP transcript is up-regulated in differentiating cultures and down-regulated in late stage mineralizing cultures. In addition, a presumed alternate transcript of 4.8-5.0 kilobases, which may lack PTP domains, is present in proliferating osteoblasts, but not detectable at other stages. Parathyroid hormone, a modulator of bone function, as well as cyclic AMP analogues, increase OST-PTP mRNA 5-8-fold in UMR 106 cells. In situ hybridization of adult rat testis revealed stage-specific expression of OST-PTP. OST-PTP may function in signaling pathways during bone remodeling, as well as serve a broader role in cell interactions associated with differentiation in bone and testis.

The development and remodeling of bone tissue entails complex, stringently regulated cell growth and differentiation, with the formation of the mineralized bone matrix by osteoblasts coupled to the resorption of that matrix by osteoclasts. Recent studies have shown that regulation of tyrosine phosphorylation in bone cells is crucial for maintenance of bone tissue. The absence of specific protein tyrosine kinase activity, such as that encoded by the proto-oncogenes c-src (2) and c-fms (3), leads to osteopetrosis, a disease characterized by the absence of appropriate bone resorption due to impaired osteoclastic function. In the c-src-deficient mouse, the osteoclast is capable of responding to modulatory factors with increased proliferation and formation of mature osteoclasts, yet is incapable of resorbing bone, failing to exhibit the ruffled membrane borders and lacunar pits associated with resorption (4). In the op/op mouse, signaling through the c-fms receptor is impaired due to a point mutation in the gene encoding its ligand, macrophage colony-stimulating factor, resulting in the lack of biologically active macrophage colony-stimulating factor necessary for osteoclast maturation (5). The osteoblast, which secretes macrophage colony-stimulating factor, is also involved in the phosphorylation of cellular proteins for bone matrix formation and coupled modulation of osteoclast function. Osteoblast proliferation and differentiation requires cell signaling through a number of receptor protein tyrosine kinases such as epidermal growth factor (6) and insulin-like growth factors (7).

To gain a better understanding of the specific roles of tyrosine phosphorylation in bone metabolism, the activity of the protein tyrosine phosphatases (PTPs), as well as these protein tyrosine kinases, must be considered. The PTPs represent a diverse family of enzymes that serve as critical signal transduction proteins in cell division, proliferation and differentiation (8). These enzymes are multidomain proteins whose structural features separate them into two main groups: the transmembrane or receptor-like PTPs and the intracellular PTPs. Each protein possesses at least one 230-residue catalytic domain with the consensus motif, (IV)HCXAGXXR/S/PTG (X is any amino acid), which bears no resemblance to that of the serine/threonine or the alkaline or acid phosphatases (8). The receptor-like PTPs are characterized by their highly divergent extracellular domains which include glycosylated serine/threonine (human PTPα), tandem repeats of immunoglobulin or fibronectin type III domains similar to cell adhesion molecules (PTPα), or alternatively spliced lengths of sequence containing N- and O-linked carbohydrates (CD45). The activity of these receptor-like PTPs may be regulated by ligand binding to these extracellular domains. The intracellular PTPs possess a diversity of sequences outside the catalytic domain which are thought to target these proteins to cellular membranes, to the cytoskeleton and the nucleus (9).

Our knowledge of the relevance of protein tyrosine phosphatases in bone metabolism is very limited. In osteoblast cultures, inhibition of PTP activity using orthovanadate appears to enhance osteoblast proliferation and matrix formation (10). PTP activity has been previously described in osteoblasts and enzymes have been partially purified that exhibit activity toward phosphorytropic substrates (11-13). However, neither the identity of these PTPs nor their function is known. In this report, we describe the isolation of a novel receptor-like PTP, designated as osteotesticular PTP (OST-PTP), whose expression appears to be restricted to bone and testis. Interestingly, the abbreviations used are: PTP, protein tyrosine phosphatase; OST, osteotesticular; PCR, polymerase chain reaction; PTH, parathyroid hormone; GST, glutathione S-transferase; CAM, cell adhesion molecule; pNPP, p-nitrophenylphosphate; DPBS, Dulbecco's phosphate-buffered saline; FN, fibronectin; PKA, cyclic AMP-dependent protein kinase; bp, base pairs; kb, kilobase pairs; RACE, rapid amplification of cDNA ends.
the expression of OST-PTP is regulated during osteoblast differentiation and following parathyroid hormone stimulation. In addition, this PTP shows stage-specific expression during spermatogenesis in rat seminiferous tubules. Our studies suggest that OST-PTP is a unique member of the PTP family which may serve a critical function in cell signaling during bone remodeling.

**EXPERIMENTAL PROCEDURES**

**Isolation of Putative PTP Clones from Bone**—Three sets of degenerate oligonucleotide primers were designed to map conserved regions within the catalytic domain of PTPs that were freshly translated in vitro. These primers were used in reverse transcription PCR to amplify novel sequences from bone cells. Three different 5' primers corresponding to the conserved amino acids, DYINA (5'-CACTGGAGTACCA/TGATC/TATACA/TATCA/TCCGC-3'; 72-fold degeneracy), YIATGPQ (5'-CACTGGAGTACCATCAG/CTTTC/CAAGT/CAC/A/GCG-3'; 96-fold degeneracy) and KDCQYWF (5'-CACTGGAGTACCATCAG/CTTTC/CAAGT/CAC/A/GCG-3'; 512-fold degeneracy) were paired with a common 3' primer corresponding to the active site, HCSAGVGR (5'-CTAGTC-TAGACCATA/TCTAG/CNCGGCA/AC/GCATG-3'; 788-fold degeneracy). The 5' and 3' primers included sites for restriction enzymes, BamHI and XbaI, respectively. The template for PCR reactions was first-strand cDNA synthesized using poly(A') RNA isolated from the rat osteosarcoma cell line UMR 106 and fetal rat calvaria and random hexamer primers. The cDNA synthesis reactions were performed with the Invitrogen cDNA cycle kit, using recommended manufacturer's instructions. The PCR reactions included 0.2 to 0.1 volume of the original CDNAsynthesis reaction and 500 ng of oligo-dT primmer, along with the recommended reagent concentrations in the GeneAmp kit (Perkin-Elmer). The PCR conditions were: 94 °C, 1 min; 55 °C, 1 min; 72 °C, 1 min; for 35 cycles. For PCR from cDNA, resamplification of original PCR reactions was necessary to see products. Products of the expected size, using the DYINAPF (485-bp) and the YIATGPQF (474-bp) primers, were isolated by gel electrophoresis and ligated into the XbaI and cloned into the pBluescript II vector (Stratagene). Despite attempts to vary PCR conditions, no products were seen using the KC-DQYWF primer. Approximately 200 independent clones were sequenced by the dideoxy chain termination method, using the Sequenase kit (U.S. Biochemical Corp.).

**Isolation of cDNA Clone**—Two rat UMR 106 cDNA libraries were screened to isolate the full-length clone of OST-PTP. One of these libraries was constructed from poly(A') RNA of normal confluent UMR 106 cells using the ZAP II library construction kits (Stratagene). The second library was a custom ZAP Stratagene library constructed with poly(A') RNA from PTH-stimulated UMR 106 cells (100 mM PTH for 18 h), using both oligo-d(T) and random hexamer primers. The original PCR fragment, corresponding to OST-PTP (i.e., DYINAPF), was freshly transformed into the BL21 strain of E. coli and screened into the custom Stratagene library. Several clones, ranging in size from 1.5 to 2.0 kb, were isolated and found to span the collective sequence of the RACE products. The final sequence of the full-length clone was verified by sequencing of both strands using an automated fluorescent DNA sequencer (Applied Biosystems, Inc.).

**RNA Isolation and Northern Analysis**—Total RNA was isolated from cells and tissues by centrifugation through a cesium chloride cushion (16) or using the RNAzol B reagent (Tel-Test). Polyadenylated RNA was isolated using the PolyATtract kit (Promega). Polyadenylated RNA from spleen, smooth muscle, pancreas, and retina was purchased from Clontech. Samples (2–10 μg) were resolved in a formaldehyde-agarose gel (14) and transferred to a Nitran membrane (Schleicher & Schuell). Prehybridization (at least 5 h) and hybridizations (overnight) were conducted at 42 °C in 5 x SSC, 5 x Denhardt's solution, 0.1% SDS, 50% formamide with 250 μg/ml of H4. Stringent washes were performed at 65 °C, 0.1 x SSC. Probes corresponding to the OST-PTP cDNA were: PCR fragment (PTP domain I, 1 bp 3811–4272; cytoplasmic domain (PTP domain I and II, 1 bp 3539–4545) and extracellular domain (bp 819–2889). The cDNA probes used as molecular markers of osteoblast differentiation were: histone H4, alkaline phosphatase, osteocalcin, type I collagen, ribophores (m-32P-CTP-labeled; Promega protocol) or random primed labeled (Boehringer Mannheim biochemicals kit) DNA probes corresponding to these sequences were used, as indicated for each figure. Controls probes for RNA integrity and concentration were human ribosomal 18S rRNA and rat cytochrome c. Quantitative determinations of RNA were determined using a Betagen scanner or a PhosphorImager. Relative cpm's for each band were calculated and normalized for RNA concentration using cyclophilin units.

**Expression and Characterization of Recombinant OST-PTP Protein**—The recombinant OST-PTP protein was produced as a glutathione S-transferase (GST) fusion protein using the pGEX-KG Echerichia coli expression vector (17). A blunt Nol-XbaI fragment corresponding to the entire cytoplasmic domain of OST-PTP was subcloned into Smal-XbaI digested pGEX-KG vector. For protein expression, this construct, pGEX-OST, was expressed in the E. coli strain BL21 (DE3) and the GST-OST PTP fusion protein produced and purified as described previously (17). The recombinant fusion protein was approximately 70% pure as determined by SDS-polyacrylamide gel electrophoresis and Coomassie Blue staining.

Basic kinetic analysis of the recombinant protein was performed using the hydrolysis of the artificial substrate, p-nitrophenylphosphate (pNPP), as the measure of phosphatase activity. Assay for hydrolyzed pNPP were conducted as described previously (18). The extinction coefficient of 1.8 x 10^4 M^(-1) cm^(-1) was used to determine the molar concentration of hydrolyzed pNPP. Subsequent Michaelis-Menton analysis of data was performed using KinetAsyst software (IntelliKinetics, State College, PA).

Substrate specificity was determined using tyrosine-phosphorylated Raytide (Oncogene Sciences) and serine-phosphorylated Kemptide (Sigma). Raytide was phosphorylated on tyrosine by incubating 10 μg of Raytide in a 30-μl reaction which included assay buffer (50 mM Hapes (pH 7.5), 0.1 mM EDTA, 0.1 mg/ml bovine serum albumin, 10 mM MgCl2, 0.1 mM ATP, 0.2% mercaptoethanol), 1.2 μl of [γ-32P]ATP (6000 Ci/mmol), and 0.5 μg of GST-Tyr-e at 30 °C for 30 min. Kemptide was phosphorylated on serine by incubating 10 μg of the peptide in a 50-μl reaction which included assay buffer (40 mM Tris-HCl (pH 7.5), 10 mM MgCl2, 0.1 mM ATP), 5 μl of [γ-32P]ATP (6000 Ci/mmol), and 5 μg of the cytoplasmic subunit of bovine protein kinase A (Fluka) at 30 °C for 30 min. The reactions were terminated by addition of 120 μl of 10% phosphoric acid and then applied to P81 filters. Free ATP was removed by 3 x 100-ml washes with 0.5% phosphoric acid, and the peptide was eluted with 2 x 1-ml aliquots of 0.5 M ammonium bicarbonate. Aliquots were lyophilized and reconstituted in distilled water, and radioactivity was determined. Phosphatase activity was determined in a 50-μl reaction containing assay buffer (0.1 mM imidazole (pH 5.6–7.0), 5 mM EDTA, 0.2% β-mercaptoethanol), 100,000 cpm of phosphorylated substrate, and 100 ng of enzyme. The reaction was terminated by addition of 750 μl of a charcoal mix (0.9 m HCl, 90 m sodium pyrophosphate, 2 m sodium phosphate dibasic and 4% (w/v) Norit). The resulting supernatant after centrifugation was counted to determine the amount of 32P (counts/min) released.

**Isolation of Primary Rat Osteoblasts**—Primary rat osteoblasts were isolated using a modification of the procedure of Aronow et al. (19). Briefly, calvaria from 19- to 21-day fetal rats were dissected, and the cut surfaces were removed. Approximately six to eight pregnant rats (average of 10–15 pups per litter) were used for each experiment. Initial digestion of calvaria was performed for 20 min at 37 °C in Dulbecco's phosphate-buffered saline (DPBS, no calcium/magnesium) containing collagenase (2 mg/ml; Boehringer Mannheim), penicillin (200 units/ml), streptomycin (200 μg/ml), and amphotericin B (0.5 μg/ml) (2 x penicillin, streptomycin, and amphotericin; Life Technologies, Inc.). Supernatant was discarded after DPBS washes and two subsequent dips at 30 °C. Samples were conducted in a water-jacketed, humidified CO2 (5–7%) incubator. At 5 days post-plating, the medium was replaced with BGG medium containing 10% fetal bovine serum with the additives, ascorbic acid (50 μg/ml) and β-glycerol phosphate (10 mM), necessary to promote "obligate" differentiation and mineralization of cultures. Cultures were monitored for expression of RNA encoding phenotypic markers of differentiation and mineralization over a period of 33–36 days. The cDNA probes used for these markers (histone H4, alkaline phosphatase, osteocalcin, and type I collagen) were kindly provided by Dr. Gary Stein. Multiple experiments showed that timing of maximal

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expression of markers did not vary more than 48–72 h.

**Culturing of Osteosarcoma Cells**—The rat osteosarcoma cells (UMR 106) were obtained from American Type Culture Collection. Cells were maintained in Dulbecco’s modified Eagle’s medium (high glucose) containing 10% fetal bovine serum at 37 °C in a water-jacketed, humidified CO2 (5-7%) incubator. Cells were subcultured at confluence (approximately every 3 days) into multiple 75-cm² tissue culture flasks at a density of 5 x 10⁶ cells/flask. At least 24 h prior to hormonal treatment, cells at 60–70% confluency were rinsed and treatment medium (Dulbecco’s modified Eagle’s medium, 0.3% fetal bovine serum), without hormone/drug, was added to the flasks. Treatments with rat PTH-1-34 (Peninsula Labs) or chlorophenylthio-CAMP (Boehringer Mannheim) were conducted at times and concentrations indicated. Total RNA was isolated by centrifugation through a cesium chloride cushion (16) and polyadenylated mRNA using the PolyATtract system (Promega). The cDNA probe encoding rat collagenase, kindly provided by Dr. Nicola Partridge, was used to verify biological activity of substances and consistency of culturing methods.

**In Situ Hybridizations of Adult Testis**—Sprague-Dawley male rats (200-250 g) were anesthetized with sodium pentobarbital and perfused with 4% paraformaldehyde, and rinsed in 0.1 M phosphate buffer, pH 7.4, then dehydrated in a series of ethanol washes and air dried. Sections were exposed to Hypefilm (Amersham) for 5–7 days to determine quality. Appropriate slides were dipped in Kodak photographic emulsion (NBT-2) and exposed for 14 days. Sections were dehydrated in a series of ethanol washes and air dried. Hybridizations were rinsed in 0.1 M sodium phosphate (pH 7.4), 50 mM sodium phosphate (pH 7.4), 1 x Denhardt’s solution, 0.1 mg/ml yeast tRNA, 10 mg/ml dithiothreitol containing 1-2 x 10⁶ cpm of riboprobe slide. Sections were washed in 2 x SSC, RNase treated as above and final stringent washes conducted at 65 °C in 0.1 x SSC, 0.1% SDS for 1 h. Following this incubation, slides were rinsed in 0.1 x SSC, dehydrated with serial ethanol washes and air dried. Slides were exposed to Hyperfilm (Amersham) for 5–7 days to determine quality. Appropriate slides were dipped in Kodak photographic emulsion (NBT-2) and exposed for 14 days. Sections were developed and counterstained with hematoxylin-eosin (Richard Allan, Inc). Slides were viewed and photographed on a Zeiss Axioskop with a MicroVideo darkfield illumination system.
PTP possessing an extracellular domain (1068 amino acids in length), a hydrophobic membrane spanning region (33 amino acids) and a cytoplasmic region (610 amino acids) containing two PTP domains (Figs. 2 and 3A). The extracellular domain contains a signal peptide (17 amino acids) and 10 fibronectin type III-like (FN-III) domains which share 21% sequence identity with fibronectin (27) and human PTPα (20) (Fig. 2). These FN-III domains possess the conserved residues characteristic of FN-III repeats found in numerous cell adhesion molecules (CAMs) and extracellular matrix proteins (i.e. tenascin) as well as other receptor-like PTPs (8, 28). Homophilic binding of such structural domains in PTPα and PTPe can mediate cell-cell aggregation (29, 30). In addition to the FN-III domains, there are 16 potential N-glycosylation sites scattered throughout the extracellular portion of OST-PTP, as indicated in Fig. 2.

The cytoplasmic region of OST-PTP has two tandemly repeated phosphatase domains designated domain I and II (Fig. 2). Domain I is a typical 250 residue catalytic domain, containing the characteristic 11 conserved residues at the active site of the enzyme, with 45% sequence identity to human PTPα (Fig. 2). In contrast, domain II has a highly divergent active site but possesses many of the conserved motifs surrounding this site,
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A

B

TM

10 fibronectin III-like domains

2 PTPase domains

1711 aa

VHCSAGVGRTG

SHSSKNTNOLG

PTP A,
schematic representation of the predicted OST-PTP protein

FIG. 3. Schematic diagram and sequence alignment of rat OST-PTP. A, schematic representation of the predicted OST-PTP protein showing the signal peptide (SP), the 10 fibronectin type III-like domains (hatched boxes), the transmembrane region (TM), and the two phosphatase domains I and II. Domain I has the typical conserved residues at the active site as shown. In contrast, domain II contains only the conserved histidine and glycine residues. B, sequence alignment of the cytoplasmic domain of OST-PTP (OS27 and that of human PTP ζ (DEL) showing the conserved motifs present throughout both PTP domains. Conserved residues within the active site are indicated with asterisks.

as shown in the sequence alignment in Fig. 3B. The active site of domain II lacks the invariant cysteine necessary for catalysis, retaining only the histidine and glycine residues and sharing 42% identity with domain II of CD45 (31) (Figs. 2 and 3B). Such divergent PTP domains are presumed to be inactive and are found in a limited number of transmembrane PTPs (8).

A schematic diagram showing the overall structural features of the predicted OST-PTP protein is shown in Fig. 3A. Overall, OST-PTP has the highest degree of similarity to the human PTPζ, sharing 45% sequence identity within the entire coding region. This low homology to other PTPs as well as the presence of multiple FN-III repeats in the extracellular domain of this phosphatase indicate that OST-PTP is a new member of the type III class of receptor tyrosine phosphatases.

Kinetic Analysis of Recombinant OST-PTP-To verify the phosphotyrosine specificity of OST-PTP and determine kinetic parameters, the cytoplasmic region of OST-PTP (OST-PTP) was expressed as a GST fusion protein (GST-OST) in E. coli. A, pH optimum curve of GST-OST toward the artificial substrate, pNPP. Phosphatase activity is expressed as micromoles of hydrolyzed pNPP/μg of recombinant protein and represented as log(activity). Negative log values are bracketed. B, tyrosine-specific dephosphorylation of the Raytide peptide. The amount of counts/min (×10⁶) released following dephosphorylation of the tyrosine phosphorylated Raytide peptide (white boxes) versus the serine-phosphorylated Kemptide peptide (black dots) by the GST-OST protein (μg). C, inhibition of Raytide dephosphorylation by sodium vanadate. Amount of counts/min released following dephosphorylation of Raytide in the presence (black dots) or absence (white boxes) of 5 mM sodium vanadate.

reported to exhibit optimal activity at 5.6 (32) and recombinant rat LAR has a pH optimum of 5.0 (18). Michaelis-Menton analysis of GST-OST activity toward pNPP showed a K_m of 0.52 mM. Using an estimate of 70% purity for the fusion protein (based on SDS-polyacrylamide gel electrophoresis and Coomassie Blue staining), a k_cat value of 41 s⁻¹ was obtained. These K_m and k_cat values are similar to those reported for other PTPs, using pNPP as a substrate (33).

Substrate specificity was determined using tyrosine-phosphorylated Raytide and serine phosphorylated Kemptide as substrates and monitoring dephosphorylation as a function of ³²P released. The GST-OST protein readily dephosphorylated Raytide at concentrations as low as 50 ng, yet could not dephosphorylate Kemptide at any protein concentration (Fig. 4B). This dephosphorylation of Raytide was virtually elimi-
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Expression of OST-PTP mRNA during Osteoblast Differentiation—Bone remodeling involves complex cell-cell and cell-matrix interactions which dictate the differentiation and function of the osteoblast and osteoclast. Because of the structural similarity of OST-PTP to the CAMs, we hypothesized that this protein might function in signaling pathways important in osteoblast differentiation. Established methods for culturing fetal osteoblasts provide an excellent experimental paradigm to study the differentiation of the osteoblast phenotype and the associated extracellular matrix (34). In these primary osteoblasts, the regulated expression of genes important for cell growth, osteoblast function, and matrix formation can be monitored during progression through the stages of proliferation, differentiation, and mineralization of the bone matrix. This system was employed to examine the expression of OST-PTP mRNA in relation to other regulated genes. Primary osteoblasts from the calvaria of 21-day fetal rats were isolated and maintained in culture. Polyadenylated RNA was harvested from proliferating (day 4), differentiated (day 18), and mineralizing (day 35) cultures. Northern analysis was conducted using riboprobes corresponding to the PTP domain I (DY11), the entire cytoplasmic region or a portion of the extracellular domain (OST-PTP<sub>ex</sub>). A representative experiment is pictured in Fig. 5. The transition from proliferation (P) to differentiation (D) is marked by a 3.7-fold increase in the 5.8-kb OST-PTP transcript at day 18. In independent experiments, this increase in OST-PTP mRNA varied between 4–6-fold in differentiating cultures at days 15–18. Levels of alkaline phosphatase mRNA, a marker of the mature osteoblast, paralleled the changes in OST-PTP mRNA, increasing 8-fold above the expression of proliferating cultures. The further progression from differentiation to matrix mineralization (M) was also marked by a dramatic change in OST-PTP expression (Fig. 5). Mineralizing cultures at day 35 showed no detectable expression of the 5.8-kb OST-PTP transcript. A 50% decrease in alkaline phosphatase mRNA to a level only 4-fold of the proliferating cultures was seen.

This increase and decline of alkaline phosphatase is the expected pattern of expression in cultures spanning the stages of proliferation to late, heavy mineralization (34). If cultures are harvested earlier in the mineralization stage, the expression of both OST-PTP and alkaline phosphatase mRNA remains elevated, similar to that observed in differentiating cultures. Therefore, OST-PTP and alkaline phosphatase consistently exhibited parallel trends in expression during the development of the osteoblast phenotype. In addition, the other markers of osteoblast function (histone H4, type I collagen, and osteocalcin) exhibited the expected fluctuations during each stage (data not shown). These results indicate that the expression of OST-PTP in primary osteoblasts is tightly regulated in a manner similar to genes known to be critical in osteoblast differentiation.

In addition to the increased expression of the 5.8-kb transcript during differentiation, our analysis revealed the existence of an additional RNA transcript which appears to be down-regulated during the developmental progression (Fig. 5). Hybridization with the OST-PTP<sub>ex</sub> probe reveals two transcripts in primary rat osteoblasts, the 5.8-kb transcript as well as a smaller 4.8–5.0-kb transcript. This smaller transcript (lower arrow in Fig. 5) is abundant in proliferating cells, yet is essentially nondetectable in differentiating and mineralizing cultures. Probes to a portion (DY11, upper panel in Fig. 5) or the entire cytoplasmic domain (data not shown) hybridize only to the larger transcript. In addition, the smaller transcript does not appear to be expressed in UMR 106 osteosarcoma cells or in whole adult testis (data not shown) regardless of the hybridization probe. These results suggest that the smaller transcript may encode an isoform which lacks one or both of the PTPase domains. Evidence of such PTP isoforms has been reported for the neuronal specific, receptor-like RPTP<sub>β</sub> which appears to have an extracellular splice variant encoding a soluble protein lacking the PTP domains (35, 36). This protein, named phosphacan, is thought to be a brain proteoglycan which interacts with neural CAMs and modulates neurite outgrowth. Definitive proof that this smaller RNA is an alternate transcript encoding an non-signaling isoform of OST-PTP awaits the isolation and characterization of this RNA species. This work is currently in progress.

Modulation of OST-PTP Expression by Parathyroid Hormone—Because OST-PTP is regulated during osteoblast differentiation, its expression could also be modulated by factors known to influence osteoblast function. PTH, a major calcitrophic hormone, is a potent modulator of bone remodeling that exhibits both catabolic effects to enhance bone resorption (37) and anabolic effects to augment bone formation (38). PTH modifies the gene expression of critical enzymes such as alkaline phosphatase (39) and collagenase (40), along with matrix and matrix-associated proteins such as osteocalcin (41). Experiments with the osteoblast-like cell
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Expression of OST-PTP during Spermatogenesis—Although our tissue distribution analysis revealed a seemingly incongruous restriction in expression to bone and testis, both these tissues possess highly regulated temporal and spatial organization which is necessary for the continuous differentiation and function of specific cell populations. Within the seminiferous tubule, coupled paracrine and cell surface interactions among the Sertoli cell, germ cell, and the extracellular matrix (base ment membrane) are essential for spermatogenesis (43). In addition to its role in bone, OST-PTP might also function in the regulation of germ cell differentiation in the testis. Numerous proteins important in signaling pathways show differential expression during germ cell differentiation including protein kinases such as c-kit (44), protein phosphatases such as the calmodulin-dependent phosphatase (45) and nuclear transcription factors such as cAMP-responsive element modulator (46). In situ hybridizations were performed with adult rat testis to determine the pattern of expression of OST-PTP during spermatogenesis. OST-PTP transcripts were found to be spatially restricted to the basal portion of the seminiferous tubule (Fig. 7). The specific clustering of silver grains appeared to be over cells possessing lightly stained, irregularly shaped nuclei suggesting localization to the Sertoli cell and/or primary spermatagonia (Fig. 7, C and D). Interestingly, this expression is stage-specific (Fig. 7, A and B). Abundance of OST-PTP transcripts appears greatest between stages I and VII when maturing spermatids remain buried within the Sertoli epithelium. Those tubules with low or nondetectable signal possess mature spermatids at the luminal surface of the Sertoli epithelium (stage VIII-IX), or immature spermatids with heads lacking the densely staining chromatin or the strong “bent rod” appearance (stage X-XI) (Fig. 7, C and D). Preliminary analysis of OST-PTP expression in neonates (day 10–18) has revealed both temporal and spatial regulation of its expression within the developing seminiferous tubule.2

1. L. J. Mauro, E. A. Olmsted, A. R. Davis, and J. E. Dixon, unpublished observations.
We have reported the isolation and characterization of a novel type III receptor-like PTP, named OST-PTP, whose expression is restricted to bone and testis. The cellular studies presented here highlight numerous properties of this new molecule which make it a very unique member of the PTP family. First, OST-PTP is one of the few tyrosine phosphatases whose expression has been shown to be tightly regulated during the differentiation of a specific cell type. The OST-PTP mRNA is up-regulated following differentiation and matrix formation of primary osteoblasts and subsequently down-regulated in the osteoblasts which are actively mineralizing bone in culture. The expression of this phosphatase also appears to be tightly regulated during the differentiation of the germ cell to mature sperm during the process of spermatogenesis. Of course, it remains to be proven that these fluctuations in mRNA are associated with increases in protein and phosphatase activity and that OST-PTP is necessary for these processes. Yet, these results strongly suggest that this PTP could be a relevant signaling molecule in the differentiation of these cells. Two of the PTPs that have been proven to be essential for cell differentiation are CD45, expressed in T cells, and PTP1C, expressed in cells of hematopoietic origin. Gene knockouts and mutation linkage studies have shown that CD45 is necessary for proper T cell maturation (47) and PTP1C is required for hematopoietic cell proliferation and differentiation (48, 49).

The existence of a second, regulated transcript is another intriguing property of OST-PTP. It is possible that this phosphatase may be expressed as a transmembrane protein possessing phosphatase activity or as an inactive transmembrane/soluble protein lacking phosphatase domains. Such isoforms in the PTP family may prove to be an interesting means of modulating phosphatase activity and subsequent intracellular signaling events. Finally, OST-PTP is one of the first tissue-specific PTPs whose expression is modulated through a G protein-coupled receptor via the stimulation of the protein kinase A pathway. Stimulation of the G protein-coupled somatostatin (50) and dopamine D2 (51) receptors has been shown to increase PTP activity, but the identity of these activated PTP(s) involved in the anti-proliferative effects of these hormones is unknown. Parathyroid hormone is known to have both proliferative and anti-proliferative effects on bone, activating both the protein kinase A as well as Ca++/protein kinase C pathways (52). Studies on the role of OST-PTP in the biological effects of PTH could provide an interesting model for investigating the involvement of PTPs in G protein-activated signaling pathways.

At present, we can only speculate as to the role of this phosphatase in osteoblast differentiation or spermatogenesis. The changes in expression observed during osteoblast differentiation and following PTH stimulation suggest that OST-PTP may function as a critical modulator of tyrosine phosphorylation status during bone metabolism, as has been shown for the protein tyrosine kinases, c-src and c-fms (2, 3). Early studies on phosho-tyrosine phosphatase activity in calvarial osteoblast cultures revealed PTP activity, which was correlated with alkaline phosphatase activity, was inhibited by vanadate and was enhanced by treatment with PTH and 1,25-(OH)$_2$D$_3$ (vitamin D$_3$) (14, 15). It was proposed that this PTP activity could be attributed to alkaline phosphatase itself or to an unidentified osteoblastic PTP. In light of our studies, OST-PTP could potentially be one of the PTPs responsible for this activity. As a receptor-like molecule similar to CAMs, this PTP could serve to transduce osteoblast-osteoblast and osteoblast-matrix interactions into intracellular signals, modifying osteoblast function and thereby influencing bone remodeling. Extrapolating from osteoblasts in culture to bone tissue, for example, the preosteoblast or immature osteoblast situated away from the bone surface may express little active OST-PTP. Instead, this cell may express an inactive OST-PTP isoform, a soluble or cell surface protein, which may mediate cell proliferation or cell-cell interactions. When such a cell migrates to the surface of the bone, this differentiating osteoblast may require the cell surface expression of OST-PTP to mediate interactions with matrix proteins and neighboring osteoblasts, all necessary for maturation.
of the osteoblast and optimal bone formation. Following new matrix formation and mineralization, the detached or bone-encased osteoblast ceases to require OST-PTP.

The regulated expression of this phosphatase in the testis suggests that it may also be relevant in the establishment and disruption of cell basement and Sertoli cell-cell interactions that occur during spermatogenesis. A conspicuous absence of OST-PTP RNA transcripts was observed between stages VIII and X in the basal portion of the seminiferous tubule. During these stages, there is a movement of differentiating spermatocytes from the basal to the adluminal compartment entailing the dissolution and subsequent formation of junctions between a Sertoli cell and a neighboring Sertoli cells, a neighboring germ cell or the basal lamina. Such reconstitution of cell junctions is necessary to maintain the blood-testis barrier. One could speculate that OST-PTP may be important in the maintenance of such interactions and expression during these stages might be undesirable.

Future research is necessary to address these speculative roles of OST-PTP in the signaling pathways important during osteoblast differentiation and spermatogenesis. Considering our limited understanding of tyrosine phosphorylation in bone, such studies should provide insights into the relevance of this phosphatase and tyrosine phosphorylation during normal bone metabolism and, ultimately, in the etiology and treatment of common bone diseases.

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