Expression of a Structurally Unique Osteoclastic Protein-tyrosine Phosphatase Is Driven by an Alternative Intrinsic, Cell Type-specific Promoter

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An osteoclastic protein-tyrosine phosphatase (PTP-oc), essential for osteoclast activity, shows sequence identity with the intracellular domain of GLEPP1, a renal receptor-like transmembrane PTP. PTP-oc has been assumed to be a truncated variant of GLEPP1, resulting from alternative splicing. However, the 5'-untranslated region sequence of PTP-oc mRNA contains 217 bp from an intron of GLEPP1. There are no splicing acceptor sites at the PTP-oc transcription site. The intronic sequence flanking the 5' end of the PTP-oc transcription start site contains potential promoter elements essential for transcriptional initiation. To test the hypothesis that the PTP-oc gene has an alternative, tissue-specific, intronic promoter, the promoter activity of a 1.3-kb PCR fragment covering the 5'-flanking region of the PTP-oc gene was measured. The putative PTP-oc promoter fragment showed strong promoter activity in U937 cells. Mutation of the putative TATA box within the PTP-oc promoter abolished 60–90% of its promoter activity. The PTP-oc promoter fragment showed strong promoter activity in cells that express PTP-oc (U937 cells and RAW264.7 cells) but not in cells that do not express the enzyme (skin fibroblasts, TE85 cells, and HEK2923 cells). These findings strongly support the conclusion that the 1.3-kb intronic fragment contains the tissue-specific, PTP-oc proximal promoter. Deletion and functional analyses indicate that the proximal 5' sequence flanking the TATA box of the PTP-oc contains potential repressor elements. The removal of the putative repressor elements led to the apparent loss of tissue specificity. In summary, we conclude that an intronic promoter within the GLEPP1 gene drives the expression of the PTP-oc in a cell type-specific manner. This GLEPP1/PTP-oc gene system is one of the very few systems in which two important tissue-specific enzymes are derived from the same gene by the use of alternative intronic promoters.

Studies with osteopetrotic mice deficient in the expression of c-Src (1), macrophage colony stimulating factor (2), or macrophage colony stimulating factor receptor (3) have clearly demonstrated that reversible protein-tyrosine phosphorylation, at least those mediated by c-src protein-tyrosine kinase (PTK) and c-Fms PTK, is essential for osteoclast activity and/or osteoclast formation. Protein-tyrosine phosphorylation is regulated by the two opposing dynamic activities of PTK and protein-tyrosine phosphatase (PTP). Although PTKs are key regulators of protein-tyrosine phosphorylation, recent work has documented that PTPs also have important regulatory functions in osteoclasts. Accordingly, motheaten mutant mice, which have a defective SHP1/PTP gene, showed elevated numbers of highly active osteoclasts and increased osteoclastic resorption (4). Inhibition of cellular PTP activities in osteoclasts (e.g. by bisphosphonates or PTP inhibitors) suppressed the bone resorption activity of osteoclasts in vitro (5).

We have cloned an osteoclastic transmembrane PTP, termed PTP-oc, from a rabbit osteoclast cDNA library (6), which has been shown to be an important regulator of osteoclast activity (7). PTP-oc is structurally unique in that, unlike most transmembrane PTPs, it lacks an extracellular domain, does not have a signal peptide proximal to the N terminus, contains only a single PTP catalytic domain, and is relatively small (405 amino acid residues). With the exception of an additional 28 amino acids (residues 66–93) at the cytoplasmic juxtamembrane region of PTP-oc, PTP-oc shows complete sequence identity with the intracellular domain of a large renal receptor-like transmembrane PTP, termed GLEPP1 and also known as PTP-U2, PTPRO, or PTP-ϕ (6, 8, 9). GLEPP1 and PTP-oc are each expressed in a tissue-specific manner (6, 9–13), and each has tissue-specific functions: GLEPP1 is expressed primarily in the kidney and the brain (9, 10) and has been shown to be essential for kidney functions (14). By contrast, PTP-oc is expressed predominantly in several hematopoietic cell types (including B lymphocytes and cells of monocyte-macrophage lineage, which are precursors of osteoclasts) and mature osteoclasts (6, 9, 11, 12) and, as indicated above, is important for osteoclast activity (7).

Because GLEPP1 and PTP-oc are products of the same gene assigned to human chromosome 12p12-p13 (9, 10), it has generally been assumed that PTP-oc is a truncated variant of GLEPP1, resulting from an alternative splicing of the gene (11). Accordingly, PTP-oc has also been referred to as the PTP-U28, PTPROt, or truncated PTP-ϕ. On the other hand, there is no direct evidence that PTP-oc is derived from alternative splicing of the GLEPP1 gene. When we analyzed the 5'-UTR of rabbit PTP-oc sequence in the context of the human GLEPP1
gene, we noticed that, although most of the 5′-UTR sequence is derived from a GLEPP1 coding exon, a 217 bp of the PTP-oc 5′-UTR is derived from intrinsic sequences of GLEPP1. Alignment of both the human GLEPP1 gene and rabbit PTP-oc cDNA sequences showed 92% homology between the rabbit and human sequences in the exonic region and >70% homology in this short intronic region. Since the PTP-oc sequence is rabbit and the intronic sequence is human, one does not expect this high degree of homology to exist in this intron between the two species. If alternate splicing occurs to generate PTP-oc, one would also not expect intronic remnants of GLEPP1 in the 5′-UTR of the PTP-oc mRNA. Analysis of the sequence around the PTP-oc mRNA transcription start site reveals no sequence homology compatible with a splicing acceptor site. These preliminary analyses raise the interesting hypothesis that PTP-oc may not have resulted from alternate splicing and that there could be a functional promoter within the GLEPP1 intron that initiates the expression of PTP-oc. Consequently, the objective of this study was to test whether there is an intronic promoter that drives the expression of PTP-oc in a cell type-specific manner.

**Materials and Methods**

**Cell Cultures**—Human U937 promyelocytic leukemic cells (ATCC CRL-15932), obtained from American Type Culture Collection (ATCC, Manassas, VA), were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum. A murine RAW264.7 macrophage cell line (ATCC, Manassas, VA) was maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum. A murine macrophage cell line, generously provided by Dr. S. Khola of the Mayo Clinics, was maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. Human TE85 osteosarcoma cells, which exhibit osteoblastic characteristics (15), were originally obtained from Dr. J. Pogli at the New-York Presbyterian Hospital and were maintained in Dulbecco's modified Eagle's medium supplemented with 10% bovine calf serum. Human skin fibroblasts were isolated from a human foreskin sample according to the method of Wergedal and Baylink (16). Human Heka293 cells (ATCC CRL-1573), a permanent line of primary human embryonic kidney cells, were obtained from ATCC and were maintained in Dulbecco's modified Eagle's medium supplemented with 10% bovine calf serum. Cell media were changed every 2–3 days, and cells were passaged weekly.

**Promoter-Luciferase Reporter Plasmids Construction**—The luciferase reporter assay system purchased from Promega (Madison, WI) was used to monitor the promoter activity. A 1.2-kb human genomic BAC clone RPC11-3650B20 (BACPAC Resource Center, Oakland, CA) contains the putative intronic promoter of PTP-oc. Accordingly, to construct the 1.3-kb (−1037/+239) PTP-oc proximal promoter-luciferase (PTP-oc-Luc or p(−1037/+239)) reporter plasmid, the DNA fragment corresponding to the putative PTP-oc promoter was prepared by PCR using the high fidelity native Pfu DNA polymerase (Strategene, La Jolla, CA) with the genomic DNA from the RPC11-3650B20 BAC Clone as the template and the following primer set: 5′-CTGTTATGGATGAACTACATGACAG-3′ (forward primer) and 5′-GATTGCGGGTACGTTGCTT-3′ (reverse primer). Two deletions PTP-oc-Luc reporter plasmids were also prepared using the same reverse primer and various forward primers shown in Table I. The PCR reactions were subjected to a hot start for 3 min at 94°C prior to the addition of the polymerase followed by 35 amplification cycles: denaturing for 30 s at 94°C, annealing for 1 min at 59°C, and extending for 2 min at 72°C/cycle. The sequence of each PCR product of the promoter construct was confirmed by DNA sequencing and was cloned into the Xhol and HindIII sites of the plasmid DNA containing the luciferase gene driven by the powerful SV40 promoter, which was used as the template to prepare the putative promoter-Luciferase (PTP-oc-Luc) reporter plasmid. A 1.2-kb Xhol-HindIII PCR fragment covering the 5′-flanking proximal region (+1154/+118 of human GLEPP1 gene was inserted into the pGL3-control plasmid in which the luciferase gene driven by the powerful SV40 promoter was amplified using the following primer sets: 5′-CTGAGGCGGTCACCTTTGACAA-3′ (forward primer) and 5′-AAGCTTGCGTCAATTGGCAACTG-3′ (reverse primer). The restriction sites (underlined) were added to facilitate subsequent cloning into the pGL3-basic vector. The PCR amplification was subjected to a hot start for 3 min at 94°C prior to the addition of the polymerase followed by 35 amplification cycles: denaturing for 30 s at 94°C, annealing for 1 min at 62°C, and extending for 4 min at 72°C/cycle. The PCR product was purified after agarose gel electrophoresis. The sequence of the PCR product of the putative PTP-oc promoter was confirmed by DNA sequencing and was cloned into the Xhol and HindIII sites of the plasmid DNA containing the luciferase gene driven by the powerful SV40 promoter.

**Construction of Mutated PTP-oc Promoter-Luciferase Plasmids**—A site-specific mutation of the promoter was introduced to the putative TATA box (sequence position −78) by site-directed mutagenesis using the QuikChange site-directed mutagenesis kit (Stratagene) using the 1.3-kb PTP-oc promoter fragment as the template and a specific oligonucleotide primer containing the site-specific mutation of the TATA box was mutated to TCGCGA. The sequence of the TATA-mutated PTP-oc promoter fragment was confirmed by DNA sequencing, cut with Xhol and HindIII restriction enzymes, and cloned into the Xhol and HindIII sites of the plasmid DNA containing the luciferase gene driven by the powerful SV40 promoter.

**DNA Transfection and Luciferase Assay**—Briefly, U937 cells (or the test cells) were plated for 24 h at 5 × 10^5^ cells/well in 24-well plates. One μg (for U937 cells) or 0.2 μg (for other test cell types) each of the PTP-oc-Luc, the GLEPP1-Luc, or the promoterless pGL3-basic plasmid was transiently transfected into the test cells with the Effectene transfection reagent (Qiagen, Valencia, CA). Two days after the transfection, cell extracts were prepared, and the luciferase activity in the extract was determined using a kit purchased from Promega with the Analytical Scientific Instruments model 3010 luminometer (Richmond, CA) and was expressed as arbitrary light units. As an internal control for normalization of transfection efficiency, cells in each transfection experiment were also transfected with the pGL3-control plasmid, which contains the luciferase gene driven by the powerful SV40 promoter. The PTP-oc or GLEPP1 promoter activity was normalized against the SV40 promoter activity and was reported as the percentage of the promoter activity of pGL3-control. Each experiment was repeated at least once. In some experiments, cells were co-transfected with a β-galactosidase expression plasmid vector, and the transfection efficiency was also normalized against β-galactosidase expression in these experiments. Identical results were obtained regardless of whether normalization was against the promoter activity of pGL3-control or against β-galactosidase expression.

**Functional Analysis of the Putative Repressor Elements**—To confirm that segments of PTP-oc promoter indeed contain repressor elements, the DNA fragments containing putative repressor elements were cloned upstream to the SV40 promoter of the pGL3-control plasmid vector, in which the luciferase reporter gene expression is driven by the powerful SV40 promoter and the SV40 enhancer. If the inserted DNA fragment contains repressor elements, the SV40 promoter-driven luciferase expression should be abolished or markedly suppressed. Accordingly, we have studied the effect of the inserted DNA fragment on the SV40 promoter-driven luciferase expression. Briefly, three DNA fragments (i.e., −1037/−738; −738/−388; −388/−98) corresponding to three regions of the PTP-oc intronic promoter (identified from deletion analysis) that contain putative repressor elements were prepared and amplified by PCR using the 1.3-kb (−1037/−239) PTP-oc promoter-luciferase (PTP-oc-Luc) reporter plasmid as the template. The following three sets of primers: a) for p(−1037/−738), 5′-CTGTCGCTGAACTACATGACAG-3′ (forward primer) and 5′-AGATCTGAGAAGCTTGTTGCTT-3′ (reverse primer); b) for p(−738/−388), 5′-CTGTCGCTGAACTACATGACAG-3′ (forward primer) and 5′-AGATCTGAGAAGCTTGTTGCTT-3′ (reverse primer); and c) for p(−388/−98), 5′-CTGTCGCTGAACTACATGACAG-3′ (forward primer) and 5′-AGATCTGAGAAGCTTGTTGCTT-3′ (reverse primer). The NheI (forward primers) and BglII (reverse primers) restriction sites, respectively (underlined), were added to facilitate subsequent cloning into the NheI/BglII cloning sites immediately upstream to the SV40 promoter of

| Table I | Forward oligonucleotide primers used in the PCR amplification of various PTP-oc promoter deletion constructs | PTP-oc promoter deletion construct | Forward oligonucleotide primer sequence |
|---------|-------------------------------------------------|-------------------------------|--------------------------------------|
| p(−738/−239) PTP-oc-Luc | GGACAGACAAACACGCTCCTC | p(−337/−239) PTP-oc-Luc | GGACAGACAAACACGCTCCTC |
| p(−556/−239) PTP-oc-Luc | CAGGGCAATGCTAACTTCAG |
| p(−98/−239) PTP-oc-Luc | CTGTAAGCTAAGAACAGAAG |
| p(9/+239) PTP-oc-Luc | AATGGAGGGGGAAGAGCGCA |
the pGL3-control vector. The PCR amplification was performed with an initial hot start for 5 min at 94 °C followed by 35 amplification cycles, each consisting of 94 °C for 30 s (denaturing), 57 °C for 1 min (annealing), and 72 °C for 2 min (extension). Each PCR product was gel-purified, digested with NheI and BglII, and cloned into the pGL3-control plasmid vector at the NheI and BglII sites. Each of the resulting repressor-pGL3-control plasmid vectors was used to transiently transf ect U937 cells or RAW264.7 cells, and the luciferase activity was determined as described above.

Statistical Analysis—Results are shown as mean ± S.D. of 4–8 replicates. Statistical significance was determined with two-tailed Student's t test, and the difference was significant when p < 0.05.

RESULTS

Genomic Structure of the GLEPP1/PTP-oc Gene—The genomic structure of GLEPP1 or that of the PTP-oc gene has not been determined previously. Thus, we first performed a BLAST search of the rabbit PTP-oc cDNA sequence (acccession number U32587) against the GenBank™ human genomic data base and identified a 162-kb human BAC clone RPCI11-326J20 (accession number AC007542), which contains the entire PTP-oc gene and most of the GLEPP1 genomic sequences (missing exon 1 and part of intron 1). Further BLAST searches of the human GLEPP1 cDNA sequence (accession number U20489) against human genomic data base reveal that a 122-kb human BAC clone RPCI11-365O10 (accession number AC022334) contains exon 1 and the major part of the intron 1 of GLEPP1. The intron 1 of GLEPP1 turns out to be very large and is 160 kb in length, and the majority of the intron 1 sequences have been identified within the reverse sequence of a 160-kb human BAC clone RPCI116K23 (accession number AC092183). Comparison and analysis of the sequences of the three human BAC clones with the cDNA sequences of GLEPP1 and PTP-oc allow us to determine the genomic structure of both GLEPP1 and PTP-oc (Fig. 1). Accordingly, the GLEPP1/PTP-oc gene contains 27 exons spanning over 223 kb. (The boxes represent coding exons, and the lines between the boxes are the introns.) Exon 17, which corresponds to the unique region of residues 66–93 of PTP-oc (6), is not expressed in the kidney GLEPP1 mRNA (10) but is expressed in the mRNA of brain GLEPP1 isoform (17). By contrast, the PTP-oc gene spans ~56 kb encoded by 15 exons (corresponding to exon 13–27 of the GLEPP1 gene). Intriguingly, the PTP-oc mRNA transcription start site is located at the last 217 bases of intron 12 of GLEPP1. Accordingly, 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. The stop codon of both genes is located near the end of exon 26.

To test whether PTP-oc is an alternative spliced variant, we searched the sequence at and around the transcription start site of PTP-oc for potential splicing acceptor sites by the Splice Site Prediction software program by Neural Network of the Berkeley Drosophila Genome Project (www.fruitfly.org/seq_tools/splice.html). Although the program accurately predicted the location of a splice acceptor site at the intron 12/exon 13 junction of GLEPP1 (Fig. 2), no potential alternative splicing acceptor and/or donor sites were found at or around the PTP-oc transcription start site, a finding that is incompatible with the premise that PTP-oc is a product of an alternative splicing. Analysis of the sequence of the 5′-flanking region of the PTP-oc gene within the GLEPP1 intron 12 for transcriptional elements with the Transcription Element Search Software on the World Wide Web (TESS, Computational Biology and Informatics Laboratory, University of Pennsylvania, www.cbil.upenn.edu/tess) using stringent sequence requirements reveals a TATA box near the PTP-oc transcription start site (consistent with the notion being a promoter) and at least 11 potential promoter elements, many of which (e.g. TATA box, CAT box) are essential for transcriptional initiation (Fig. 2). These findings support the interpretation that intron 12 of GLEPP1 could contain an intronic promoter for PTP-oc. Consistent with the possibility that the intron sequence contains the proximal promoter for PTP-oc, analysis of the intronic sequence for eukaryotic transcription promoter with the Neural Network Promoter Prediction software program of the Berkeley Drosophila Genome Project (www.fruitfly.org/seq_tools/promoter.html) accurately predicted the transcription start site of PTP-oc within the intronic fragment with a score of 0.94 out of a maximum of 1.0.

Basal Promoter Activity of Putative PTP-oc Proximal Promoter Fragment—To determine whether intron 12 of the GLEPP1 gene contains a proximal promoter for PTP-oc and to compare the promoter activity of the putative PTP-oc proximal promoter with that of the putative GLEPP1 proximal promoter, we prepared, by PCR, a 1.3-kb DNA fragment (−1037/+239) corresponding to the intronic sequence of the 5′-flanking region.

![Fig. 1. A schematic comparison between the renal GLEPP1 gene structure and that of osteoclastic PTP-oc. Open boxes are the exons of GLEPP1, and filled boxes are exons of PTP-oc. With the exception of intron 1, which is 120 kb in length, the length of the boxes and lines is proportional to the number of nucleotide bases, respectively. Exon 17 (indicated by the *) is expressed in PTP-oc and brain GLEPP1 but not in renal GLEPP1. The first exon of PTP-oc is made up with a part (217 bp) of the intron 12 and exon 13 of GLEPP1. ATG and TAG indicate the location of the start and stop codons, respectively, of PTP-oc. P1, putative GLEPP1 proximal promoter; P2, putative PTP-oc proximal promoter.](image-url)
of the human **PTP-oc** transcription start site and a 1.2-kb DNA fragment corresponding to the 5'/H11032-1154/5' of the **GLEPP1** transcription start site and cloned it into pGL-3 basic plasmid to generate the PTP-oc-Luc and GLEPP1-Luc reporter plasmid, respectively. The two promoter reporter constructs were then used in transient transfections of human promyelomonocytic U937 cells for monitoring the promoter activity of respective reporter plasmids. U937 cells were used because these cells express both GLEPP1 and PTP-oc proteins (18).

**Fig. 3** shows that both the PTP-oc-Luc and the GLEPP1-Luc reporter plasmids expressed relatively strong basal promoter activity in human U937 cells. Interestingly, the promoter activity of both PTP-oc-Luc and GLEPP1-Luc reporter plasmids was similar and was each −15−25% of the promoter activity of the SV40 promoter-driven pGL3-control (SV40 promoter driven plasmid). When the putative TATA box (sequence −78) of the PTP-oc-Luc promoter construct was mutated, the TATA-mutated reporter construct lost 60−90% of the basal promoter activity as compared with the wild type PTP-oc-Luc construct (Fig. 3B), suggesting that this putative TATA box was probably a functional regulatory element and supporting the contention that this 1.3-kb intronic fragment might contain a proximal promoter that drives the expression of PTP-oc in U937 cells.

**Deletion Analysis of the Putative PTP-oc Proximal Promoter Fragment**—To further characterize the putative intronic **PTP-oc** promoter fragment, we initiated deletion analysis in which several deletion promoter reporter constructs, e.g. p(-1037/+239), p(-378/+239), p(-98/+239), p(-33/+239), and p(+9/+239), were prepared. The basal promoter activity of the

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\text{FIG. 3.} \text{The basal promoter activity of the putative PTP-oc proximal promoter in human U937 cells. In A, U937 cells were transiently transfected with the 1.3-kb PTP-oc-Luc and the 1.2-kb GLEPP1-Luc reporter construct using Effectene. The promoterless pGL3-basic and the SV40 promoter-driven pGL3-control plasmids were included as negative and positive control, respectively. The luciferase activity was measured in cell extracts 48 h after transfection. Cells transfected with the pGL3-control plasmid showed luciferase activity of 308,056 ± 27,103 light units. Results are shown in mean ± S.D. as percent of pGL3-control, n = 4 per group. In B, PTP-oc promoter reporter construct with the TATA box mutated (TATA-mutated) by site-directed mutagenesis was prepared and was used to transiently transfect U937 cells along with the unmodified PTP-oc-Luc plasmid.}
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deletion constructs were assessed and compared with that of the full-length PTP-oc-Luc construct (i.e. p(1037/1037)). The deletion analysis was performed in two cell types that are known to express PTP-oc, namely U937 cells (Fig. 4, top panel) and RAW264.7 cells, an osteoclast precursor cell line (Fig. 4, bottom panel). In both cell lines, the deletion of the distal 300 bp of the 5′ promoter sequences (i.e. p(1037/738)) slightly reduced the basal promoter activity. However, further deletion of 5′ promoter sequences distal to the TATA box (i.e. p(738/98)) markedly enhanced (by 4–5-fold) the promoter activity of the putative PTP-oc intronic promoter in both cell types. This raises the possibility that there are repressor sequences for binding of repressive transcription factors within 738 and 98 of the promoter to suppress the basal PTP-oc promoter activity. Deletion of the TATA box and the removal of the PTP-oc transcription start site greatly or completely abolished the promoter activity, further supporting the conclusion that the putative TATA box is functional in PTP-oc gene transcriptional regulation.

Functional Analysis of the Putative Repressor Sequences of the PTP-oc Intronic Promoter—To further assess whether the PTP-oc promoter sequence −738 to −98 indeed contains repressor sequences, we cloned each of the three pieces of DNA sequences corresponding to three regions of the PTP-oc intronic promoter that may contain repressor sequences (identified from deletion analysis) immediately upstream to the SV40 promoter of the pGL3-control plasmid to produce three repressor-Luc reporter constructs (i.e. p(1037/−738), p(−738/−388), and p(−388/−98)). The U937 cells (Fig. 5, top panel) or RAW264.7 cells (Fig. 5, bottom panel) were then transiently transduced with these repressor-reporter constructs, along with the pGL3-control (positive control) and pGL3-basic (negative control), and the effect of each repressor sequence on the SV40 promoter activity was determined. Fig. 5 shows that the insertion of the DNA sequence −738/−388 almost completely abolished the SV40 promoter activity in U937 cells and RAW264.7 cells (by 97 and 94%, respectively). Similarly, the sequence −388 to −98 also greatly suppressed the SV40 promoter activity (by 90 and 86%, respectively) in U937 cells and RAW264.7 cells. In contrast, the distal sequence (from −1037 to −738) could only partially suppress the SV40 promoter activity (by 61 and 64% in U937 cells and RAW264.7 cells, respectively). These findings support our conclusion of the presence of repressor sequences for binding of repressive transcription factors within −738 and −98 of the promoter to suppress the basal PTP-oc promoter activity.

Cell Type Specificity of PTP-oc Intronic Promoter—The expression of the PTP-oc gene (and GLEPP1 expression) is cell type-specific (6, 9–13). Thus, we investigated whether the putative intronic PTP-oc promoter is regulated in a cell type-specific manner by first assessing the basal promoter activity of the 1.3-kb putative PTP-oc promoter reporter plasmid.
(p(-1037/-239)) in U937 cells (cells that express PTP-oc) and in human skin fibroblasts (cells that do not express PTP-oc). Fig. 6 shows that the 1.3-kb putative PTP-oc promoter was highly active in U937 cells but not at all active in human skin fibroblasts. To further evaluate the tissue specificity of the putative PTP-oc intronic promoter, we compared the promoter activity of the 1.3-kb PTP-oc promoter construct to that of the putative GLEPP1 promoter (GLEPP1-Luc) in other cells that express PTP-oc (U937 cells and RAW264.7 cells), that do not express PTP-oc (human TE85 osteosarcoma cells), and that express GLEPP1 primarily (HEK293 cells) (Fig. 7). Because we postulate that the putative repressor elements within the PTP-oc promoter play an important role in the cell type specificity of PTP-oc expression, we also measured the relative promoter activity of the p(-98/+239) deletion construct, which appears to be devoid of repressive elements, in these cell types. As shown previously, the 1.3-kb PTP-oc promoter (p(-1037/+239)) also showed strong promoter activity in RAW264.7 cells, which express PTP-oc. The 1.3-kb PTP-oc-Luc construct had no appreciable promoter activity in cell types that do not express PTP-oc (i.e. TE85 cells and HEK293 cells). Conversely, the truncated p(-98/+239) promoter construct showed relatively strong basal promoter activity in each test cell type. Interestingly and unexpectedly, the p(-1154/+118) GLEPP1-Luc plasmid did not exhibit the anticipated cell type specificity and showed appreciable promoter activity in each of the test cell types.

**DISCUSSION**

The presumption that PTP-oc is an alternative spliced truncated variant of GLEPP1 has primarily been based on the fact that PTP-oc and GLEPP1 share the same gene in human chromosome 12p12-p13 (9, 10). However, this study presented several pieces of compelling evidence that the PTP-oc gene is probably not a product of alternative splicing and has its own promoter. First, the 5'UTR sequence of PTP-oc contains a 217-bp sequence of intron 12 of GLEPP1 (Fig. 7). Because we postulate that the putative repressor elements within the PTP-oc promoter play an important role in the cell type specificity of PTP-oc expression, we also measured the relative promoter activity of the p(-98/+239) deletion construct, which appears to be devoid of repressive elements, in these cell types. As shown previously, the 1.3-kb PTP-oc promoter (p(-1037/+239)) also showed strong promoter activity in RAW264.7 cells, which express PTP-oc. The 1.3-kb PTP-oc-Luc construct had no appreciable promoter activity in cell types that do not express PTP-oc (i.e. TE85 cells and HEK293 cells). Conversely, the truncated p(-98/+239) promoter construct showed relatively strong basal promoter activity in each test cell type. Interestingly and unexpectedly, the p(-1154/+118) GLEPP1-Luc plasmid did not exhibit the anticipated cell type specificity and showed appreciable promoter activity in each of the test cell types.
fragment for a number of potential transcription promoter elements, including those essential for transcription (i.e., TATA box and CAT boxes), is supportive of the possibility that PTP-oc gene expression is driven by its own proximal promoter. Moreover, the transcription start site of PTP-oc mRNA is the predicted transcription start site of a promoter, based on computer analysis of putative promoters. However, two additional important observations of this study afford the strongest pieces of evidence for the existence of the intronic promoter for PTP-oc: 1) The 1.3-kb intronic DNA fragment of GLEPP1 gene proximal to 5’ end of the transcription start site of PTP-oc exhibited basal promoter activity in cell types that are known to express PTP-oc, e.g., U937 cells and RAW264.7 cells. The basal promoter activity of this putative promoter fragment appeared to be relatively strong in that it was greater than 30% of that of the powerful viral SV40 promoter. 2) More importantly, mutation of the putative TATA box of this promoter fragment drastically reduced the basal promoter activity, indicating that the TATA box of the putative PTP-oc intronic promoter is functional. Consequently, these findings led us to conclude that PTP-oc is probably not an alternative spliced product of the GLEPP1 gene, but rather, it has its own intronic promoter to drive its gene expression.

Expression of PTP-oc and GLEPP1 is tissue- and cell type-specific in that PTP-oc expression appears to be restricted to cells of osteoclast lineage whereas GLEPP1 is expressed predominantly in the kidney and brain (6, 9–13). Our findings that the 1.3-kb intronic PTP-oc promoter fragment exhibited strong basal promoter activity only in cell types that express PTP-oc (i.e., U-937 cells and RAW264.7 cells) but not in those cell types that do not express the enzyme (i.e., human skin fibroblasts, TE85 osteosarcoma cells, and HEK293 cells) provide strong evidence for the conclusion that the putative intronic PTP-oc promoter indeed functions in a cell type-specific manner. In addition, the promoter deletion and functional analyses of this study strongly suggest that the putative PTP-oc intronic promoter could be controlled by repressors, since deletion of 5’ sequences distal to the TATA box greatly enhanced the basal promoter activity and since insertion of DNA sequences corresponding to the putative repressor sequences into the pGL3-control plasmid vector almost completely blocked the SV40 promoter activity in U937 and RAW264.7 cells. Tissue-specific genes are frequently silenced in non-expressing cells by binding of the repressors to repressive elements within their promoters and are activated in expressing cells by the removal of the repressive effects of the repressors through enhancers (20, 21). The fact that the removal of sequences containing the putative repressor elements (i.e., 5’ sequences distal to the...

Fig. 6. Comparison of basal promoter activity of the 1.3-kb putative PTP-oc intronic promoter (p(−1037/+239)) in U937 cells (which express PTP-oc) and in human skin fibroblasts (which do not express PTP-oc). Results are shown in mean ± S.D., n = 4 per group as percent of pGL3-control. The 100% of the pGL3-control in U937 cells was 35,854 ± 5,513 light units, and that in human skin fibroblasts was 29,219 ± 4,912 light units. N.S., not significant.

Fig. 7. Cell type specificity of the 1.3-kb putative PTP-oc intronic promoter (p(−1037/+239)), the truncated PTP-oc promoter (p(−98/+239)), and the 1.2-kb putative GLEPP1 promoter (GLEPP1-Luc) in U937 cells (top left panel), RAW264.7 cells (top right panel), TE85 cells (bottom left panel), and HEK293 cells (bottom right panel). Results are shown in mean ± S.D., n = 4 per group as percent of pGL3-control. The 100% of the pGL3-control in U937 cells was 91,726 ± 8,678 light units, that in RAW264.7 cells was 60,567 ± 3,917 light units, that in TE85 cells was 70,107 ± 6,791 light units, and that in HEK293 cells was 81,570 ± 6,770 light units. *, p < 0.001. N.S., not significant.
TATA box) led to the complete loss of cell type specificity supports strongly the concept that repressor elements within the PTP-oc promoter could have a major role in allowing the promoter to exhibit cell type specificity. Consequently, the possibility of the repressive elements within the promoter provides strong, albeit circumstantial, evidence for the notion that this putative PTP-oc intronic promoter is a tissue-specific promoter. Two observations of the putative proximal promoter of GLEPP1 (i.e. the 1.2-kb DNA fragment covering the 5’-flanking region of GLEPP1 transcription start site) are noteworthy. First, the putative GLEPP1 promoter, unlike the PTP-oc promoter, does not contain a TATA box near the transcription start site. Thus, GLEPP1 promoter belongs to the family of promoters that do not involve the binding of TFIIID to TATA box for enhancement of gene transcription. Second, although the 1.2-kb putative GLEPP1 promoter fragment showed relatively strong basal promoter activity, it did not exhibit the anticipated tissue specificity for renal cells (i.e. HEK293 cells) since this promoter fragment was active for all test cell types. However, we cannot rule out the likely possibility that this 1.2-kb fragment does not contain the entire GLEPP1 proximal promoter and that upstream transcriptional elements are required for the tissue specificity of the promoter.

The concept that PTP-oc gene transcription is controlled by an osteoclast-specific, intronic promoter is intriguing and noteworthy. Although there are some examples that certain genes, such as acetyl-CoA carboxylase α (22) or p45 NF-E2 transcription factor (23), use intronic alternative promoters to regulate tissue- or cell type-restricted gene expression, the GLEPP1/PTP-oc gene system, to our knowledge, is one of the very few, if not the only, systems in which two distinct gene products, each of which has important function in different tissues (i.e. kidney and bone, respectively), are derived from the same gene through the use of intronic promoters rather than alternative splicing.

In summary, we have demonstrated for the first time that the expression of a structurally unique osteoclast protein-tyrosine phosphatase is driven by an intronic, cell type-specific promoter. These findings, in general, support our view that PTP-oc has important cell type-specific functions in cells of osteoclast lineage. If the hypothesis that a cell type-specific, intronic promoter drives the expression of PTP-oc is confirmed, targeted disruption of this PTP-oc promoter should block basal, as well as stimulated, PTP-oc transcription without affecting the GLEPP1 gene transcription. This possibility is exciting because the most direct approach to confirm the physiological function of PTP-oc is to evaluate the effect of knocking out PTP-oc gene expression on osteoclast differentiation and resorption. Because GLEPP1 and PTP-oc share parts of the same gene, the commonly used approaches for producing knockout animals (e.g. interruption of exons coding for important portions of PTP-oc) would also knock out GLEPP1 gene expression. Consequently, we believe that it would be feasible to create PTP-oc knockout mice that maintain normal GLEPP1 expression by targeted disruption of this intronic promoter.

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