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Cloning of hOST-PTP: the only example of a protein-tyrosine-phosphatase the function of which has been lost between rodent and human

W. Cousin [a], A. Courseaux [b], A. Ladoux [a], C. Dani [a], P. Peraldi [a,*]

[a] *Institute of Signaling Developmental Biology and Cancer-UMR 6543 CNRS, Faculté des Sciences, 06108 Nice Cedex 2, France*
[b] *INSERM U636, Centre de Biochimie, Parc Valrose, 06108 Nice Cedex 2, France*

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Abstract

Protein-tyrosine-phosphatases (PTP-ases), in concert with protein tyrosine kinases, control various biological activities such as cell growth and differentiation. In rodents, around 40 PTP-ases have been described. Functional orthologue for each of these PTP-ases have been identified in human, except for OST-PTP. OST-PTP is a transmembrane PTP-ase with a restricted tissue distribution. In silico analysis on public sequence databases reveals a human *OST-PTP* gene orthologue that encompasses 21 kb on chromosome 1q32.1. Using RT-PCR we isolated a 4 kb h*OST-PTP* transcript. h*OST-PTP* cDNA sequence exhibits numerous disablements indicating that it does not code for a PTP-ase but is rather a pseudogene with unique features. Indeed, (i) it has no "functional" parent in the human genome, (ii) it has retained an "intron–exon" structure, and (iii) it is transcribed in a regulated manner. Interestingly, we found two ESTs, from domesticated pig and from cow that exhibit ORF that would predict a functional *OST-PTP* orthologue in Artiodactyls. Taken together, these results indicate that *OST-PTP* is the only PTP-ase the function of which has been lost during the evolution process between rodents and human.

Keywords: Protein-tyrosine-phosphatase; OST-PTP; Esp; Evolution; Pseudogene; Gene loss

Protein tyrosine phosphorylation is a key mechanism for signal transduction leading to processes involved in the control of an extraordinary variety of cellular decisions affecting metabolism, proliferation, and differentiation [1–3]. The level of tyrosine phosphorylation is finely tuned by the balance between the activities of protein-tyrosine-kinases and protein-tyrosine-phosphatases (PTP-ases). These "antagonistic" proteins fall into two categories: transmembrane (receptor-like) and cytosolic enzymes. PTP-ases have been studied intensively in rodents where the use of transgenic animals has been a powerful tool to investigate their functions. So far, it is assumed that PTP-ases exert the same biological function in rodent and in human, a concept that supports the use of rodents as animal models of human biology and disease. For instance, the finding that PTP1B knockout animals have increased insulin sensitivity and are resistant to obesity [4,5] designates this PTP-ase as a promising therapeutic target for the treatment of type 2 diabetes and obesity in human [6].

So far, a human functional orthologue of all rodents' PTP-ases has been identified except for one: *OST-PTP*, also named *Esp* in the mouse [7]. *OST-PTP* encodes for a class III transmembrane PTP-ase [8,9]. It is composed of 10 fibronectin type III repeats in its extracellular part and two "catalytic" domains in its cytoplasmic region. As for most of the transmembrane tyrosine phosphatase the first catalytic domain is endowed with enzymatic activity while the second domain is catalytically inactive

* Corresponding author. Fax: +33-4-92076404.
E-mail address: peraldi@unice.fr (P. Peraldi).

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and thought to regulate the first domain [10]. Study of OST-PTP function has proven to be difficult because of its weak and restricted expression: gonads, bone, and embryonic stem cells. OST-PTP has been characterized as a marker of bone collar cells and therefore appears as a powerful tool to study osteoblast differentiation [11]. *OST-PTP* expression is increased during osteoblast differentiation [8,12] and inhibition of its expression interferes with differentiation of these cells in vitro [10] and apparently in skeletal formation in mouse [11]. Altogether, OST-PTP appears as an interesting candidate in the study of cell differentiation. We have therefore undertaken the cloning of *OST-PTP* orthologue in human.

The sequencing of the human genome has been a powerful tool to identify in silico new genes and gene families. Using BLAST analysis, we predicted a potentially functional orthologue for *OST-PTP* on chromosome 1. This prediction was reinforced later on by different automated computational sequences of h*OST-PTP* published in Unigene/locuslink (www.ncbi.nlm.nih.gov/) and by a recent thorough analysis performed by Andersen et al. [7] on PTP-ases present on the human genome.

We describe herein the cloning of h*OST-PTP* and provide evidence that it is the only documented example of a PTP-ase which has lost its function between rodents and human.

Materials and methods

Cell culture. MCF7 cells were grown in Dulbecco's modified Eagle's medium (DMEM; Cambrex) and osteosarcoma cell lines (Saos-2, MG-63, and U-2Os) in α-MEM, supplemented with 10% FBS using standard tissue cell culture conditions. Human multipotent adipose derived stem cells (hMADS) were grown as described in [13].

In silico analysis. The genomic organization of the human h*OST-PTP* loci, orthologue to the rodent *OST-PTP* gene, was established through BLAST searches of GenBank against many databases in the Web site of the National Center for Biotechnology Information of the National Institutes of Health (www.ncbi.nlm.nih.gov/). The limit of the extent of the homology between the human and mouse orthologous genomic sequences was established by using the BLAST-2-Sequences program with modified parameters (word size, 7; match, 1; mismatch, −1; gap open, 1; and gap extension, 2). Repeated sequences in the human and rodent genomic regions were evidenced, analyzed and masked for further analysis (for database searches) by using RepeatMasker (Smit and Green, unpublished) (http://ftp.genome.washington.edu/RM/RepeatMasker.html). The translation of DNA sequences to protein sequences was conducted in the web site of NCBI of the NIH (www.ncbi.nlm.nih.gov/). Amino-acid alignment was performed using ClustalW at the infobiogen browser (http://www.infobiogen.fr/).

In addition to the human orthologous *OST-PTP* loci on chromosome 1q32.1 described below, another genomic sequence, located on chromosome 9, was detected (AL354751). However, its homology covered only a third of mouse *OST-PTP* cDNA suggesting that it is a pseudogene, as described by Andersen et al. [7].

Total RNA extraction and RT-PCR analysis. Total cellular RNA were extracted using TRI-REAGENT (Euromedex, France) according to the manufacturer's instructions. Two micrograms of total RNA, treated with DNase I (Promega), was subjected to RT-PCR analysis. First strand cDNA was generated with Superscript II reverse transcriptase (Invitrogen) in presence of 25 ng/μl oligo(dt)15 (Promega), following the manufacturer's instructions.

For h*OST-PTP* detection and probe synthesis, 30 cycles of PCR using GoTaq DNA polymerase (Promega) were performed. The following oligonucleotides were used (a) h*OST-PTP* detection; forward: CCTAGAATCCCAGACATTGGCA, reverse: GCTGGTTGTTGCT TGGAGGTT (amplicon: 139 bp) (b) h*OST-PTP* cDNA probe synthesis; forward: ATGAGGCTCCCAATCCTGTTCG, reverse: GGG TATGCAGTACCAGGTGATCT (amplicon: 815 bp).

To amplify (a) full-length h*OST-PTP* and (b) the "PTP-ase signature motif," 30 cycles of PCR were performed using a high fidelity DNA polymerase (Pfu, Promega). The primers used were: (a) forward: ATGA GGCTCCCAATCCTGTTCG, reverse: TCAGGAATGCTGTCCCC ATCCC (amplicon: 4006 bp), and (b) forward, GACCACTC CAGGG TCAGG, reverse: CAGGGTCTGGATCAT-3′ (amplicon: 501 bp).

Human OST-PTP cloning. After full-length h*OST-PTP* RT-PCR, fragments were analyzed on a 0.8% agarose gel and fragments ranging from 2.5 to 8 kb were purified using Wizard SV Gel and PCR Clean-Up System (Promega). Blunt-end fragments were submitted to an A-tailing procedure, ligated in pGEM-T Easy Vector (Promega), transformed in *Escherichia coli* DH5-α (Invitrogen), and plated. Bacterial colonies were transferred on Neutral Membrane (Qbiogene) and screened with a radio-labelled probe obtained by PCR (as described above). It corresponds to the 5′ 815 nt h*OST-PTP* and was labelled with [^{32}P]dCTP using Prime-a-Gene labeling system (Promega). Positive clones were analyzed by enzymatic restriction, verified by PCR and sequenced using the ABI PRISM BigDye Terminators v3.0 Cycle Sequencing Kit (Applied Biosystems) and an ABI Prism 310 genetic analyzer. Identity between clones was established by sequencing. One clone was sequenced entirely on both strands. The sequence was submitted to the "Nucleotide Sequence Database" at the EMBL, its Accession No. is: AJ629456.

Results

Cloning of human OST-PTP

In silico analysis undertaken to detect a human *OST-PTP* orthologue was conducted as described in Material and methods. Two partially overlapping human genomic DNA fragments were retrieved from clones RP11-572A16 (AL356953) and RP11-294K24 (AL592300). The human orthologous *OST-PTP* locus was shown to encompass a region of 21 kb located on chromosome 1q32.1, a region which displays a strong conservation of synteny with mouse *OST-PTP* locus on chromosome 1E4 [14]. The predicted h*OST-PTP* transcript exhibits 77% of sequence identity with mouse *OST-PTP* and exhibits a similar intron/exon structure (Fig. 1). In order to isolate a h*OST-PTP* transcript by RT-PCR, we designed several oligo-nucleotide primers from the genomic DNA sequence. In rodent, *OST-PTP* expression is restricted to gonads and differentiating osteosarcoma. We were unable to detect h*OST-PTP* mRNA in classical models of differentiating human osteosarcoma cell lines (Saos-2, U-2OS, and MG-63), or in hMADS, a human cell line isolated from adipose tissue with a normal karyotype [13], even after 45 RT-PCR cycles. During our study, p53 was identified as an inducer of mouse *OST-PTP* mRNA expression (J.C. Marine, personal communication). MCF-7 cells (a p53-competent cell line) were treated

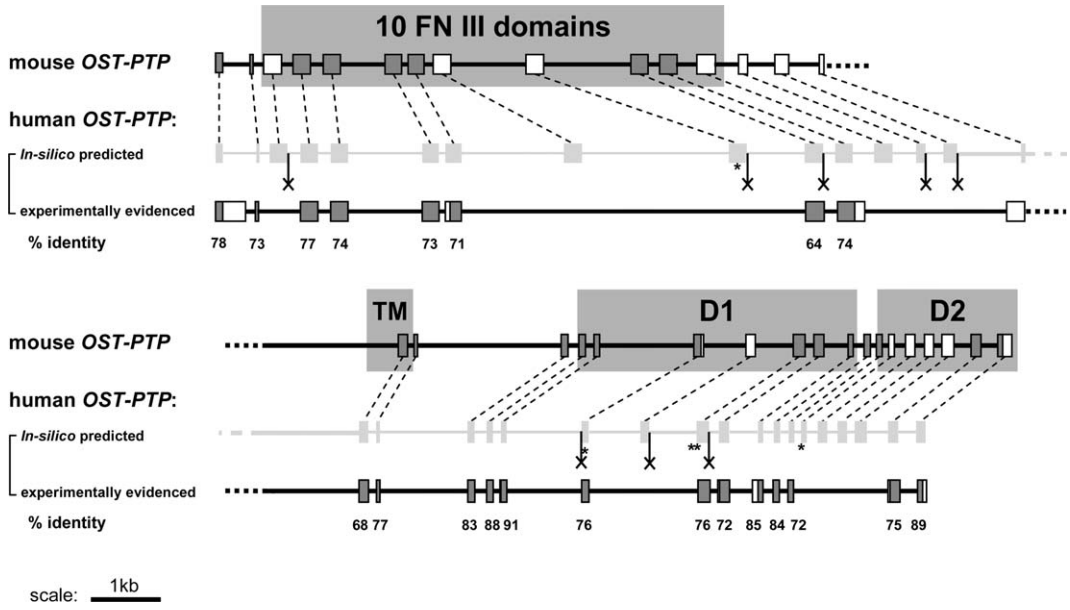

Fig. 1. Comparison of the genomic structural organisation of mouse *OST-PTP*, and human in silico-predicted and experimentally evidenced *OST-PTP*. Exons (represented by boxes) and introns (represented by lines) are drawn to scale. The upper part of the figure represents the region of the mouse gene that code for the extracellular domain of *OST-PTP*, the lower part for the transmembrane and intracellular domain. Conservation between mouse *OST-PTP* and in silico-predicted h*OST-PTP* exons is indicated by a dotted line. Conserved exons between the two species are visualized as shaded boxes, distinct exons by open boxes. The percent of sequence identity between mouse and human exons is indicated below h*OST-PTP*. The position of the exons coding for specific protein domain is indicated by large shaded boxes (FN, fibronectin; TM, transmembrane domain, and D1 and D2, PTP-ase domain 1 and 2). A (*) represents a stop codon in the in silico predicted human h*OST-PTP* ORF. An (×) at the extremity of an exon represents a non-consensus splicing signal in the in silico predicted transcript.

with a p53 inducer, etoposide (10 μM), for 24 h. In these cells, the expression of h*OST-PTP* was detected through the amplification of a 139 bp fragment located in the 5′ region of the transcript. Cloning of h*OST-PTP* was then undertaken as described in Materials and methods.

hOST-PTP sequence and sequence alignments

The isolated h*OST-PTP* cDNA is 4006 bp long (Accession No.: AJ629456) and presents 58% of sequence identity with mouse *OST-PTP* cDNA. As expected, BLAST analysis of this fragment against the human genome shows unambiguously that this sequence originated from 1q32. h*OST-PTP* consists in 22 exons spanning 21 kb in the genomic DNA. The intron–exon junction sequences conform to the GT/AG consensus for splicing signals (Fig. 2). Strikingly, h*OST-PTP* transcript is shorter than mouse *OST-PTP* and exhibits a distinct intron–exon structure than the in silico prediction (Fig. 1). This different splicing affects 11 out of 33 exons, and is located in the regions corresponding to the extracellular domain of *OST-PTP* as well as in the two phosphatase domains.

Human OST-PTP mRNA does not encode for a functional PTP-ase

Analysis of h*OST-PTP* cDNA sequence revealed numerous stop codons. Only two putative ORFs, which utilize a reading frame consistent with that of rodent *OST-PTP*, were detected. One, from nucleotide 470 (in the second exon) to 2134 (in the eighth exons) would encode for a protein of 554 amino-acids. This putative protein is homologous to a portion of the extracellular domain of rodent OST-PTP and is predicted to have three type III fibronectin domains by SMART analysis http://smart.embl-heidelberg.de/. The second putative protein, from nucleotide 2876 (in the 13th exon) to 3550 (in the 18th exon), would encode a 225 amino-acid protein. This region maps to the first catalytic domain of mouse OST-PTP. However, several disablements indicate that this protein should lack tyrosine phosphatase activity. Indeed, when compared to the consensus sequence alignment of PTP-ases [15], several critical residues necessary for tyrosine phosphatase activity are mutated or missing (Fig. 3). The putative protein described here misses the first 30 amino-acids of the phosphatase domain and lacks several crucial amino-acids necessary for enzymatic activity such as those present in the so-called PTP-ase signature motif "(I/V)HCSxGxGR(S/T)." To make certain that this h*OST-PTP* transcript was not specific to MCF-7 cells, we amplified and sequenced a 487 bp fragment covering the PTP-ase "signature motif" from hMADS cells treated for 24 h with etoposide. These are non-transformed cells with a normal karyotype [13]. Only one amplicon was detected, and its DNA sequence

Exon-intron boundaries			Exon size	Intron size
	ATGAGGCTCC…TCAGGAAGAG	gtgggcttgt	461	110
cttcctgcag	GAGGAAGCAT…GAGAGTGCAG	gtaggtgtct	49	643
gctgatgcag	CCCCGTCAAC…CAGTGGACAG	gtaaggtggg	269	198
cttcctccag	CCCCTGTGTC…GAGTGGACCT	gtgagtgcct	264	1140
ttctccacag	ATACCTCTGC…TGGCTGGACG	gtgagtcccc	255	101
tcgcctccag	ATTCCGCAGC…GGCCACAAAA	gtgagtgcca	246	5262
ctcctcgcag	GCCCGCTTGC…TGCAGGAGAG	gtgagcaagg	285	186
ccttctgtag	CCCTGCACAC…GCTTCTCCAG	gtgggcagtc	419	2172
tttgttttag	GCTTTTAGCA…CCTTTACCAG	gtacagccct	279	1268
ctctccacag	AGCCCTGGGC…AGGGGCAGAG	gtgagtggga	127	104
cccttgcag	GGCAGGGAAG…ACAACCTGCG	gtgagtgcat	48	1191
ctcccaccag	GTAGACCCAC…GCAATTCGAG	gtaaggcctt	94	162
atccctgcag	GAGCTGAAGG…GTGCTTCCCT	gtgagtgctg	91	92
gaatgcacag	CTTTCACTCT…CTTCATCCCA	gtaagggcca	93	1022
tacaccccag	GGCTACACCC…GGAGTAGCAG	gtccgcatca	98	1481
cccttctcag	GTTGTCCAGC…AGGAGGGCAG	gtgagtgcat	169	103
acccattcag	TTCCTCAGGG…CCAGACCCTG	gtgagggcct	159	311
ctccctgcag	GCCCCCCTCG…ACATCTCTGA	gtaagtccca	144	129
cccccaccag	GTCTTGGCCC…GGAGTACAAG	gtacacttca	88	106
tccttcccag	CTCTTGCTGC…CCCATCTCCT	gtgagtctca	79	1285
ccccctttag	GTACCTCATC…CCCAACGCTG	gtgagaggcg	166	238
ccatctccag	AAGCAGTATA…GCATTCCTGA		123	

Fig. 2. Intron–exon junction sequences and sizes of introns and exons of h*OST-PTP*. Exon sequences are shown in capital and introns in lowercase. The intron sequences and sizes were obtained from the human genome sequence after completion of the Human Genome Project (presenting supposedly less than one error for 10^4 nucleotides) at ncbi human genome resource (http://www.ncbi.nlm.nih.gov/genome/guide/human/).

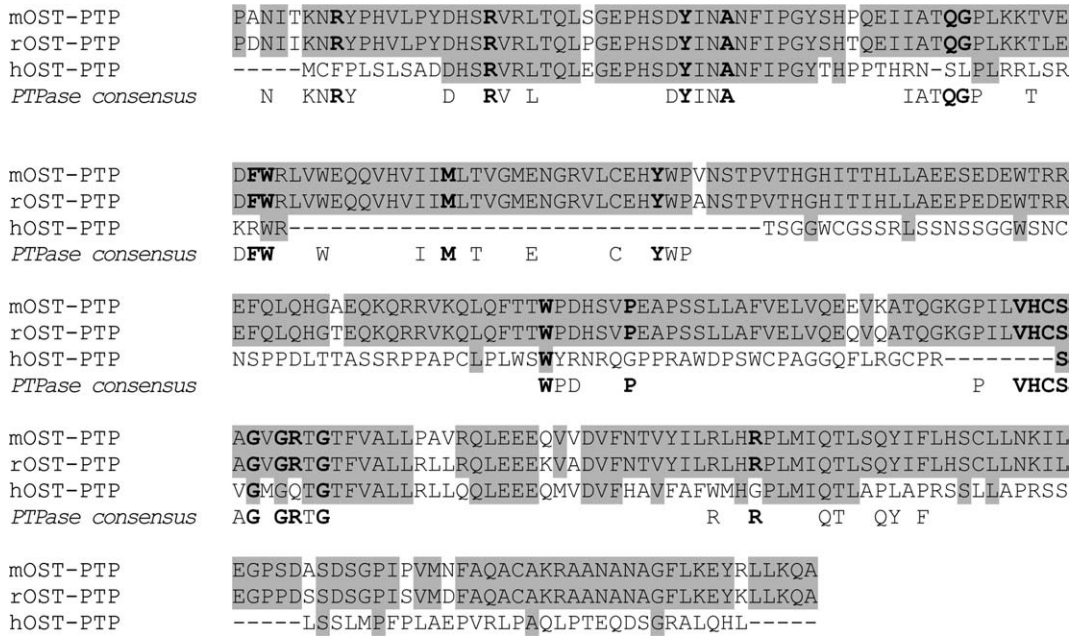

Fig. 3. Amino-acid alignment of mouse and rat OST-PTP (aa 1172–1453) with a putative hOST-PTP peptide (aa 1–225 corresponding to an ORF at nt 2876–3550). Conserved amino-acids are indicated by shaded boxes. Highly conserved PTP-ase amino-acids [15] are indicated below the sequence, amino-acids necessary for PTP-ase activity are indicated in bold.

was identical to the one obtained in MCF-7 cells. These data indicate that, in two human cell lines, h*OST-PTP* gene does not encode for a functional tyrosine phosphatase.

Potential functional OST-PTP in Artiodactyls

We have identified two ESTs, one in *Sus scrofa* (domesticated pig) and one in *Bos taurus* (cow). These

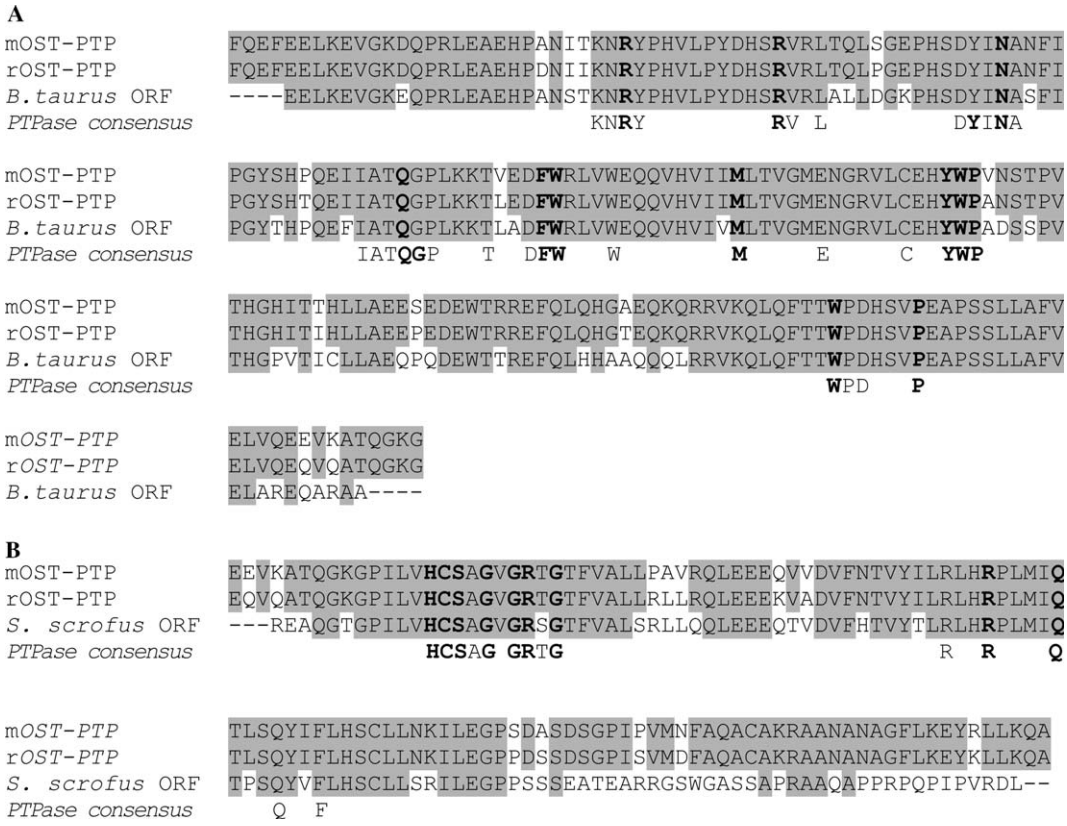

Fig. 4. Amino-acid alignment of mouse and rat OST-PTP (aa 1151–1342 in (A) and aa 1335–1453 in (B)) with putative ORF encoded by *B. taurus* (5A) (Accession No.: BE722693) and *S. scrofa* (5B) (Accession No.: BM483890) ESTs. Conserved amino-acids are indicated by shaded boxes. Highly conserved PTP-ase amino-acids [15] are indicated below the sequence, amino-acids necessary for PTP-ase activity are indicated in bold.

ESTs display ORF that would code for putative *OST-PTP* orthologues. Both are located within the first tyrosine phosphatase domain of *OST-PTP*. Amino-acid sequences deduced from *Sus scrofa* and *Bos taurus* ORFs are respectively, 67% and 82% identical to those of mouse OST-PTP. Interestingly, the encoded proteins would possess all critical amino-acids necessary for PTP-ase activity (Figs. 4A and B). Although the cloning of a full-length *OST-PTP* cDNA orthologue in these species would be necessary to draw definitive conclusions, these ESTs suggest that OST-PTP orthologue in Artiodactyls possesses functional phosphatase domain.

Discussion

Rodent PTP-ases are a family of about 40 members. In concert with protein-tyrosine-kinases they exert various biological functions ranging from cell growth and differentiation to metabolism. So far, a functional orthologue for each of these proteins has been detected in human, OST-PTP being the only exception [7]. Using computational approach we have detected *OST-PTP* human orthologue on chromosome 1q32.1, a region which displays a strong conservation of synteny with mouse *OST-PTP* genomic locus on chromosome 1E4 [14]. We have been unable to detect this in silico predicted transcript in various cell lines. However, several evidences argue against its existence, and even if transcribed, it would not code for an active PTP-ase. Indeed, (i) 8 out of 64 intron/exon junctions of the in silico predicted human *OST-PTP* do not conform to the GT/AG consensus for splicing signals (indicated by × on Fig. 1), three of them being located on crucial exons of the first catalytic domain, (ii) the theoretical ORF is interrupted by five in-frame stop mutations (indicated by * on Fig. 1), and (iii) four, out of the 20, amino-acids which are crucial for the catalytic activity of the phosphatase [15] are mutated.

Taken together, these evidences argue against the existence of a functional *OST-PTP* in human and suggest that the tyrosine phosphatase function of h*OST-PTP* has been lost during the evolution process. This information was unexpected since a functional orthologue for each of the known rodent's PTP-ases has been identified in human. *OST-PTP* is expressed in few cell type, i.e., gonads, osteoblast, and embryonic stem cells [8,9]. Due to its specific expression *OST-PTP* appeared as a useful tool to elucidate the role of immature bone collar

cells in the transition of skeletal elements from cartilage template to bone [11]. OST-PTP has also been involved in osteoblast differentiation, in vitro [10] and possibly in vivo [11]. Because the human *OST-PTP* does not code for a functional tyrosine phosphatase, findings about *OST-PTP* function in rodent could not be extended to human. It is likely that other PTP-ases compensate for the lack of OST-PTP tyrosine phosphatase activity during human osteoblast differentiation.

Therefore, the human *OST-PTP* gene product seems to have undergone adaptative selection. h*OST-PTP* is still transcribed in a regulated manner. Compared to rodent *OST-PTP*, h*OST-PTP* lacks 12 exons (Fig. 1) and does not code for a functional PTP-ase. Does this messenger perform a specific biological task? h*OST-PTP* possesses two ORFs that would code for proteins in the same reading frame as rodent *OST-PTP*. At this point it is difficult to predict if these proteins are synthesized in intact cells. None of these ORFs possesses the minimal consensus context for a correct translational start site (RNNatgG, where R is a purine, or RNNatgY or YNNatgG where Y is a pyrimidine) [16]. In agreement with these predictions, we have been unable to detect any protein in an in vitro transcription/translation assay in a reticulocyte lysate (data not shown). Finally, h*OST-PTP* does not exhibit any significant additional ORF that would encode for a protein with homology to a known protein. Altogether, these data suggest that h*OST-PTP* does not code for a protein.

h*OST-PTP* has the characteristics of a pseudogene, i.e., a gene that does not produce a functional, full-length protein. Pseudogenes are not uncommon. Indeed, the human genome is estimated to contain up to 20,000 pseudogenes [17]. They are usually classified as (i) processed pseudogenes that result from the process of retrotransposition (reverse transcription of mRNA transcript followed by integration into the genomic DNA), usually easily recognizable due to their lack of intron–exon structure and a polyA tail and (ii) non-processed pseudogenes, which arise from gene duplication or gene extinction [18]. In their study of the PTP-ases present in the human genome Andersen et al. [7] have identified nine processed pseudogenes, mainly SHP-2 pseudogenes, and three non-processed pseudogenes. h*OST-PTP* emerges as an "atypical" pseudogene since it seems to be the consequence of a gene loss, it does not have a functional parent in the genome, it is still transcribed and its transcription has kept some of its regulation.

Although pseudogenes are usually considered as non-functional, some of them have been found to display a biological function. For instance, expression of the makorin mRNA pseudogene is required for high expression level of the makorin transcript [19]. In this example, the pseudogene regulates the expression of its "parent" gene and both sequences exhibit a high identity level. h*OST-PTP* does not have a functional parent and does not share significant identity with any other PTP-ase in particular or any human gene in general. Therefore, its potential ability to regulate the expression of other gene is unlikely.

The 75% of sequence identity between mouse *OST-PTP* cDNA and human genomic sequence, together with the detection of a potentially expressed and functional OST-PTP orthologue in Artiodactyls' suggests that the function of this gene has been modified recently, after the divergence between Artiodactyls and Primates. Interestingly, h*OST-PTP* is located on 1q32.1, a region which has been the site of segmental duplications during evolution [20,21]. These events are associated with rapid gene innovation and chromosomal rearrangement in the genomes of human and the great apes [22]. As shown here, these regions, sites of new gene arising, could also be associated with gene extinction. On the other hand, h*OST-PTP* could also be considered as a potogene, i.e., a DNA sequence with a potentiality for becoming a new gene [23]. At this point, a better understanding of the biological meaning, if any, of the transcribed pseudogenes, is required to assign a putative function for h*OST-PTP*.

In summary, we describe here that h*OST-PTP*, the human orthologue of rodent *OST-PTP*, does not code for a functional PTP-ase and is likely to be a pseudogene. This is the only example of a PTP-ase whose function has been lost during the evolution process between rodent and human and indicated that findings on OST-PTP function in rodents could not be extended to human.

Acknowledgments

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Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2004.06.137.

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