Chapter 1

The Extended Family of Protein Tyrosine Phosphatases

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Abstract

In higher eukaryotes, the Tyr phosphorylation status of cellular proteins results from the coordinated action of Protein Tyrosine Kinases (PTKs) and Protein Tyrosine Phosphatases (PTPs). PTPs have emerged as highly regulated enzymes with diverse substrate specificity, and proteins with Tyr-dephosphorylation or Tyr-dephosphorylation-like properties can be clustered as the PTPome. This includes proteins from the PTP superfamily, which display a Cys-based catalytic mechanism, as well as enzymes from other gene families (Asp-based phosphatases, His-based phosphatases) that have converged in protein Tyr-dephosphorylation-related functions by using non-Cys-based catalytic mechanisms. Within the Cys-based members of the PTPome, classical PTPs dephosphorylate specific phosphoTyr (pTyr) residues from protein substrates, whereas VH1-like dual-specificity PTPs dephosphorylate pTyr, pSer, and pThr residues, as well as nonproteinaceous substrates, including phosphoinositides and phosphorylated carbohydrates. In addition, several PTPs have impaired catalytic activity as a result of amino acid substitutions at their active sites, but retain regulatory functions related with pTyr signaling. As a result of their relevant biological activity, many PTPs are linked to human disease, including cancer, neurodevelopmental, and metabolic diseases, making these proteins important drug targets and molecular markers in the clinic. Here, a brief overview on the biochemistry and physiology of the different groups of proteins that belong to the mammalian PTPome is presented.

Key words Tyrosine phosphatase, Lipid phosphatase, Asp-phosphatase, His-based phosphatase, Phosphorylation, Dephosphorylation

1 Tyr Phosphatases: Positive and Negative Protein Regulators of Cell Signaling

Tyr phosphorylation/dephosphorylation is a profuse regulatory mechanism of the responses of the cells to physiologic and pathologic changes in their environment, and it is exerted in holozoan organisms by the coordinated action of Protein Tyrosine Kinases (PTKs) and Protein Tyrosine Phosphatases (PTPs) [1, 2]. Unlike protein kinases, PTPs have evolved independently of the Ser/Thr Phosphatases, displaying a characteristic PTP domain, a CxxxxxR conserved catalytic loop (where C is the catalytic Cys, x is any amino acid, and R is an Arg), and a Cys-based catalysis [1, 3–7].

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Beyond that, the mammalian PTPome, considered as the cluster of proteins with Tyr-dephosphorylation or Tyr-dephosphorylation-like activity, includes proteins distributed in several families (Cys-based, His-based, Asp-based), among which the PTP family itself contributes with most of the members. In line with this, we have defined the concept of an open and extended PTPome whose members fulfill the following criteria: (a) harboring of a structurally defined PTP domain; *or* (b) presence of a CxxxxxR signature catalytic motif within a non-PTP phosphatase domain; *or* (c) displaying experimentally validated Tyr phosphatase activity; *or* (d) displaying high sequence similarity to members with demonstrated Tyr phosphatase activity. This updated human PTPome contains 125 genes, which encode both catalytically active and inactive (pseudophosphatases) proteins [8] (Fig. 1 and Table 1).

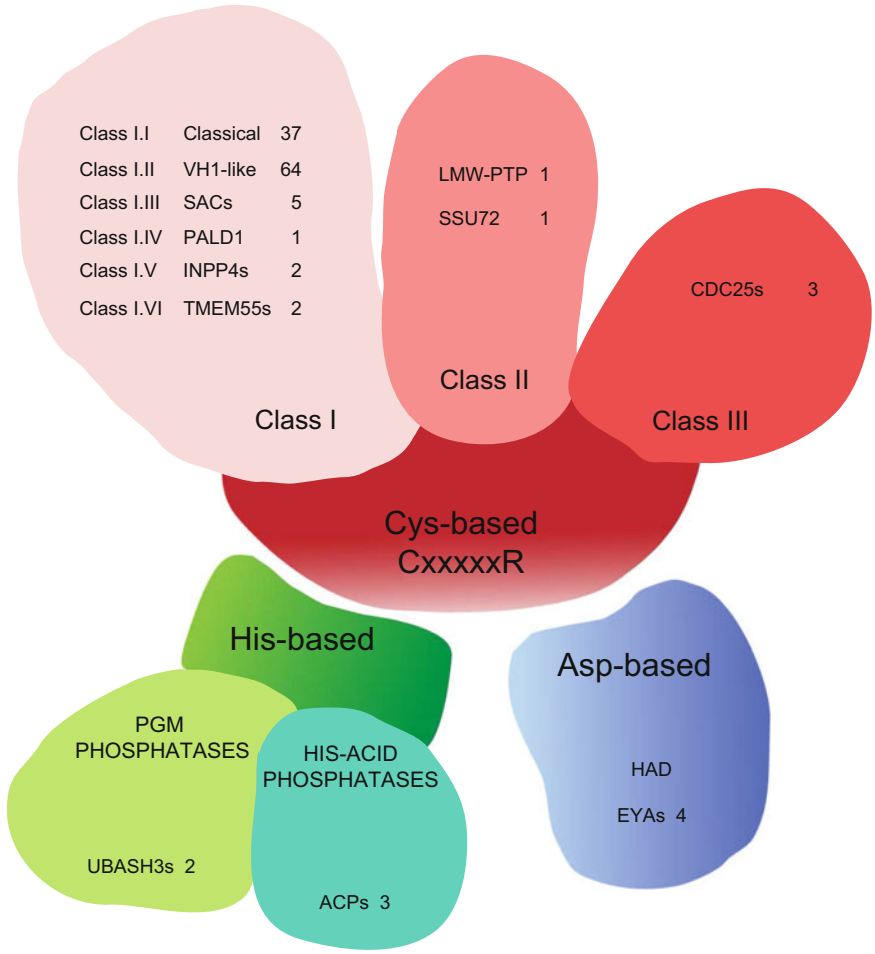

Fig. 1 Scheme of the extended family of Tyr phosphatases (extended PTPome). The classification is based on the nucleophilic catalytic residue (Cys, Asp, or His) and on protein topology. *Numbers* indicate the members included in each group. *See* Table 1 for a complete list of the members of the extended PTPome. *HAD* haloacid dehalogenase, *PGM* phosphoglyceromutase

Table 1
The extended human PTPome

Classical protein tyrosine phosphatases (PTPs) (Class I.I Cys-based)									
Classical receptor protein tyrosine phosphatases (RPTPs)									
1	PTPRA	5	PTPRE	9	PTPRJ	13	PTPRN2	17	PTPRS
2	PTPRB	6	PTPRF	10	PTPRK	14	PTPRO	18	PTPRT
3	PTPRC	7	PTPRG	11	PTPRM	15	PTPRQ	19	PTPRU
4	PTPRD	8	PTPRH	12	PTPRN[a]	16	PTPRR	20	PTPRZ1
Classical non-receptor protein tyrosine phosphatases (NRPTPs)									
21	PTPN1	25	PTPN5	29	PTPN11	33	PTPN18	37	PTPN23
22	PTPN2	26	PTPN6	30	PTPN12	34	PTPN20A/B		
23	PTPN3	27	PTPN7	31	PTPN13	35	PTPN21		
24	PTPN4	28	PTPN9	32	PTPN14	36	PTPN22		
Dual-specificity VH1-like PTPs (DUSPs) (Class I.II Cys-based)									
Dual-specificity MAPK phosphatases (MKPs)									
38	DUSP1	41	DUSP5	44	DUSP8	47	DUSP16		
39	DUSP2	42	DUSP6	45	DUSP9	48	STYXL1[a]		
40	DUSP4	43	DUSP7	46	DUSP10				
Dual-specificity atypical phosphatases (Atypical DUSPs)									
Small-size atypical DUSPs									
49	DUSP3	52	DUSP14	55	DUSP19	58	DUSP23	61	DUSP28
50	DUSP13	53	DUSP15	56	DUSP21	59	DUSP26	62	PTPMT1
51	DUSP13	54	DUSP18	57	DUSP22	60	DUPD1	63	STYX[a]
Other atypical DUSPs									
64	RNGTT	65	DUSP11	66	DUSP12	67	EPM2A	68	DUSP27[a]
Slingshots									
69	SSH1	70	SSH2	71	SSH3				
Phosphatases of regenerating liver (PRLs)									
72	PTP4A1	73	PTP4A2	74	PTP4A3				
CDC14s									
75	CDC14A	76	CDC14B	77	CDKN3	78	PTPDC1		
PTEN-like									
79	PTEN	81	TPTE2	83	TNS3[a]	85	DNAJC6[a]		
80	TPTE[a]	82	TNS1[a]	84	TENC1	86	GAK[a]		

(continued)

Table 1
(continued)

Myotubularins (MTMs)									
87	MTM1	90	MTMR3	93	MTMR6	96	MTMR9[a]	99	MTMR12[a]
88	MTMR1	91	MTMR4	94	MTMR7	97	MTMR10[a]	100	SBF2[a]
89	MTMR2	92	SBF1[a]	95	MTMR8	98	MTMR11[a]	101	MTMR14
SAC phosphoinositide phosphatases (Class I.III Cys-based)									
102	SACM1L	103	INPP5F	104	FIG4	105	SYNJ1	106	SYNJ2
Paladin (Class I.IV Cys-based)									
107	PALD1								
INPP4 inositol polyphosphate phosphatases 4′ (Class I.V Cys-based)									
108	INPP4A	109	INPP4B						
TMEM55 inositol polyphosphate phosphatases 4′ (Class I.VI Cys-based)									
110	TMEM55A	111	TMEM55B						
Low molecular weight PTP (LMW-PTP) (Class II Cys-based)									
112	ACP1								
SSU72 (Class II Cys-based)									
113	SSU72								
CDC25 (Class III Cys-based)									
114	CDC25A	115	CDC25B	116	CDC25C				
Eyes absent haloacid dehalogenase phosphatases (HAD-EYAs) (Asp-based)									
117	EYA1	118	EYA2	119	EYA3	120	EYA4		
UBASH3 HIS-PGM phosphatases (TULAs) (Branch 1 His-based)									
121	UBASH3A	122	UBASH3B						
ACP HIS-acid phosphatases (Branch 2 His-based)									
123	ACPP	124	ACP2	125	ACPT				

Official gene names are provided. PTPN20A and B are two duplicated identical genes located in the same locus. The two entries from DUSP13 correspond to DUSP13A and DUSP13B, two different genes located in the same locus
[a]Inactive phosphatases. PTPRN, TPTE, and TNS3, different to other inactive phosphatases which lack essential catalytic residues, contain all essential residues in their catalytic signature motif, making possible that these enzymes are active toward unknown substrates

Although Tyr phosphatases were initially considered cell signaling shutting-off enzymes, it is now widely known that Tyr phosphatases work both as positive and negative regulators of cell signaling, switching on and off with high specificity the biological activity of signal transduction molecules. Early after the first report of the amino acid sequence of a PTP in 1988 [9], findings of Tyr phosphatases working as positive signaling regulators followed and PTPRC/CD45 was shown to be essential for activation of the Src-family kinase (SFK)

Lck in T lymphocytes, by virtue of dephosphorylation of its inactivating C-terminal Tyr residue [10, 11]. Later on, PTPRA/RPTPα overexpression was reported to cause cell transformation of rat embryo fibroblasts, in association with dephosphorylation and activation of Src [12]. In fact, several PTPs are bona fide positive regulators of SFKs by specific Tyr dephosphorylation [13]. A classic example of targeted negative regulation of cell signaling by Tyr phosphatases is that of the kinase interaction motif (KIM)-containing MAPK-PTPs (PTPRR, PTPN5, and PTPN7), which specifically dephosphorylate the activating Tyr residue in the catalytic loop of MAPKs upon KIM-mediated binding [14, 15]. Thus, Tyr phosphatases may display exquisite substrate specificity and drive distinct signal outputs in coordination with specific TKs, as it has been recently illustrated in the case of PTPN1/PTP1B [16]. Some other examples of the positive and negative role of Tyr phosphatases in cell signaling, using EGFR-mediated signaling as a paradigm, are schematically depicted in Fig. 2.

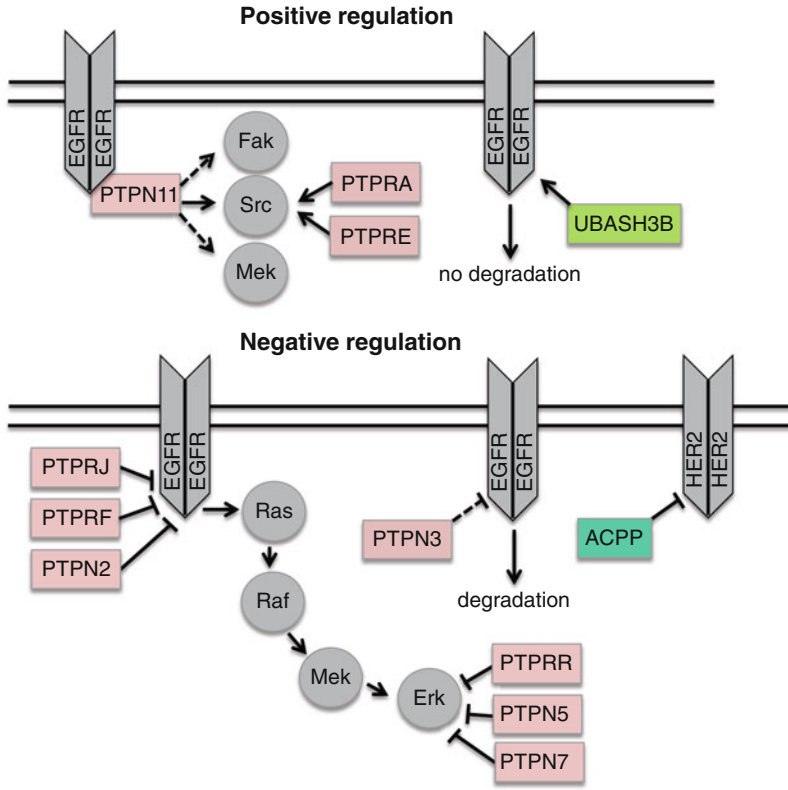

Fig. 2 Examples of positive and negative regulation of cell signaling exerted by different Tyr phosphatases. Examples were chosen using EGF receptor-mediated signaling as paradigm, and Tyr phosphatases acting on kinases upstream or downstream in the pathway. Direct dephosphorylation of regulatory pTyr on kinases is indicated by *solid lines*. Indirect effect on the Tyr phosphorylation status of kinases is indicated by *broken lines*. In most of the examples shown, Tyr dephosphorylation directly affects the catalytic activity of the kinase targeted. In the case of UBASH3B, both direct and indirect effects on EGFR have been documented to block the degradation of the receptor

Next, a brief account of the properties of Tyr phosphatases, as exemplified by the mammalian PTPome members, is presented.

2 Classification of Tyr Phosphatases

Here, we will follow the classification of PTPs by Alonso et al. [3], updated according to Alonso et al. [8] (Fig. 1 and Table 1). As shown, most of the PTPome members (116 genes) are Cys-based Tyr phosphatases, although many of those do not have pTyr as their physiologic substrate but rather phosphoinositides (PIPs). The rest includes the EYAs Asp-based (four genes) and the His-based (five genes) Tyr phosphatases.

3 Class I Cys-Based Phosphatases

Class I of Tyr phosphatases includes most of the Tyr phosphatases identified so far in the human genome. Over 100 phosphatases form part of this group of enzymes, characterized by a similar topology, the presence of common essential catalytic residues and a similar catalytic mechanism [7]. In our classification, six major groups can be differentiated in this class: classical PTPs (subclass I.I), VH1-like/DUSPs (subclass I.II), SACs (subclass I.III), Paladin (subclass I.IV), INPP4s (subclass I.V), and TMEM55s (subclass I.VI) phosphatases.

3.1 Classical PTPs

The most homogeneous group is that of classical PTPs. Alignments of their primary sequence and determination of the structures of the PTP domains (about 250 amino acids in length) from most of the classical Tyr phosphatases have allowed the identification of structural motifs conserved in this family and their implication in catalysis and physiological function [17, 18]. Classical PTPs are pTyr specific enzymes. This pTyr specificity is due to a loop present in classical PTPs structure and named pTyr loop that deepens the active site and impedes access of pSer/Thr to the catalytic Cys. In spite of this, the classical PTPs PTPRN2 and PTPRQ dephosphorylate phosphoinositides. This group is further divided into receptor and non-receptor phosphatases. Apart from the presence of a transmembrane domain that localizes the receptor enzymes in the plasma membrane, 13 out of 20 of these phosphatases contain two PTP domains. The domain close to the membrane, named D1, harbors the catalytic activity, while the second domain, D2, is mostly inactive and its function seems to be regulatory. D2 domains in RPTPs show a high degree of sequence conservation, indicating a conserved function through evolution [19]. Despite presenting receptor-like extracellular domains, ligands have only been

identified in some cases. For instance, PTPRK/RPTPκ, PTPRM/RPTPμ, PTPRU, and PTPRT interact by homotypic interactions, which seem to be involved in cell-cell adhesion processes that limit cell growth. On the other hand, the proteolysis of these PTPs generates extracellular and intracellular independent domains, which have been proposed as important mediators of oncogenicity [20, 21]. Other RPTPs, such as PTPRD/RPTPδ, PTPRF/LAR, and PTPRS/RPTPσ, bind membrane-bound ligands on adjacent cells and orchestrate cell signaling at specific cell compartments, like the synaptic junctions [22, 23]. In this regard, heparan sulfate proteoglycans (HSPGs) bind to PTPRF increasing its phosphatase activity, while another ligand, the protein Dallylike, inhibits its activity, regulating the function of this phosphatase in the formation of synapses [24]. Sugar components from PTPRC/CD45 bind to the endogenous lectin Galectin-1, which reduces PTPRC phosphatase activity and facilitates T cell death during thymic maturation [25, 26]. Remarkably, binding of Galectin-3 to PTPRC from B cells also diminishes PTPRC phosphatase activity, but conferring resistance to apoptosis-inducing agents [27], illustrating the cell type-dependent tuned specificity of the biological activity of PTPs. Another interesting example is that of PTPRZ1/RPTPζ, which binds to pleiotrophin and contactins. Binding to the cytokine pleiotrophin leads to a decrease in its phosphatase activity, and to an increase in Tyr phosphorylation of β-catenin and the ALK TK receptor [28, 29].

Non-receptor classical PTPs are 17 phosphatases which in addition to the PTP domain present additional regulatory and targeting domains and motifs. Some of these domains (FERM, BRO1, FYVE, or C2) are involved in binding to lipids in cellular membranes. Other domains, like the SH2 domain present in PTPN6/SHP1 and PTPN11/SHP2, regulate the activity of the phosphatase, whereas Pro-rich motifs permit the interaction with SH3 or F-BAR domains [30, 31]. As mentioned above, by virtue of these protein-interaction motifs and domains, as well as by intrinsic specificity for substrate recognition in the catalytic domains, PTPs show a physiologically well-tuned substrate specificity that exerts both positive and negative inputs in cell signaling pathways (Fig. 2).

3.2 VH1-Like/Dual-Specificity Phosphatases (DUSPs)

VH1-like/DUSP PTPs are more diverse than classical PTPs and present a phosphatase domain much smaller, usually 100 amino acids shorter. The first phosphatase of this group was identified in the vaccinia poxvirus and was named VH1 [32]. Then many others were identified up to the 64 genes that are included in this group in the human genome. The phosphatases in this group show a great diversity of substrates, from RNA to lipids, and include pTyr, pSer, and pThr phosphatases. Several groups can be distinguished among VH1-like phosphatases [3, 8]. Among them, the ten active MAP kinase (MAPK) phosphatases (MKPs), which target with

great specificity MAPKs thanks to the presence of specific binding domains, are major players in the regulation of cell growth, survival, and differentiation [33, 34]. Myotubularins (MTMs) dephosphorylate PI(3)P and PI(3,5)P2 to produce PtdIns(5)P, and constitute an important VH1-like/DUSP subfamily, with nine phosphatase-active and six phosphatase-inactive members (Table 1). They regulate endocytosis and membrane and vesicle trafficking, and have been genetically linked with human myopathies and neuropathies [35, 36]. A major human disease-related enzyme which belongs to the VH1-like/DUSP phosphatases is the PTEN tumor suppressor, which targets as the major substrate the PI(3,4,5)P3 product of the oncogenic PI3K. PTEN is a physiologic homeostatic regulator whose involvement in human disease goes beyond cancer [36]. Interestingly, some VH1-like/DUSP phosphatases are the lowest expression of a phosphatase, being almost exclusively a PTP domain of around 150 amino acids, like DUSP23/VHZ [37]. Some of these small phosphatases also dephosphorylate MAPKs, like DUSP3/VHR [38, 39].

3.3 SAC Phosphatases

The SAC phosphoinositide phosphatases have in common the presence of a SAC catalytic domain, whose core is topologically similar to that of the PTP catalytic domain of some VH1-like/DUSPs and contains a CxxxxxR catalytic motif [40]. The SAC enzymes included in the human PTPome display substrate specificity towards mono- and multi-phosphorylated PIPs, and form part of two subgroups: SACML1/SAC1, INPP5F/SAC2, and FIG4/SAC3, which possess the SAC domain as the only catalytic domain; and SYNJ1/Synaptojanin 1 and SYNJ2/Synaptojanin 2, which possess a non-Cys-based Mg^{2+}-dependent 5-phosphatase domain in addition to the SAC domain [41–44].

3.4 Paladin

Paladin/PALD1 is a protein that presents two putative PTP domains that contain the signature motif CxxxxxR. However, no phosphatase activity has been demonstrated yet for Paladin [45]. The phosphatase domains of Paladin belong to the PTP-like phytase (PTP-LP) type. Phytases are phosphatases that hydrolyze phosphate from myo-inositol hexakisphosphate, also called phytate [46], and they are found in different families of enzymes: histidine acid phosphatases, β-propeller phytases, purple acid phosphatases, and PTP-like phytases [46, 47]. PTP-LPs are found in anaerobic bacteria in ruminants [48], and in some human pathogens, such as *Clostridium botulinum*. The phytase domain from *Selenomonas ruminantium* (PhyAsr) has been crystallized, showing a similar topology to VH1-like phosphatases [49], with a PTP-loop that contains the catalytic Cys. Paladin expression is regulated during embryonic development [50–52] and it has been implicated in vascular biology [53]. A mouse knockout has been generated, but no phenotype has been reported for the loss of expression of this gene [53]. Paladin has also been involved in the regulation of insulin signaling [45].

3.5 INPP4 and TMEM55 Phosphatases

These two groups of Cys-based phosphatases have been recently added to the PTPome based on the presence on their four members of a conserved CxxxxxR motif (Tables 1 and 2) and phosphatase activity towards inositol polyphosphates and phosphoinositides [8].

INPP4A and INPP4B are two related enzymes that dephosphorylate the D4 position from PI(3,4)P2, Ins(3,4)P3, and Ins(1,3,4)P2. By virtue of their relative specificity towards PI(3,4)P2, INPP4A and INPP4B are involved in the negative regulation of PI3K-mediated signaling and AKT activation [54–56]. INPP4A has been related with Huntington's disease because of its involvement in the control of excitotoxic cerebellar- and striatum-neuronal cell death [57, 58], as well as with the regulation of platelet aggregation and asthma-related inflammation [59–61]. INPP4B exerts tumor suppressor activities in different human cancers by both PI3K/AKT-dependent and -independent mechanisms [56, 62–65]. In addition, INPP4B has been related with osteoporosis by its negative regulatory role on osteoclast differentiation [66].

TMEM55A and TMEM55B are two small phosphoinositide phosphatases that dephosphorylate the D4 position of PI(4,5)P2 [67]. TMEM55A and TMEM55B regulate EGFR lysosomal degradation [67], and a role for TMEM55B in p53 stabilization of nuclear p53 has also been reported [68].

4 Class II Cys-Based Phosphatases

The Class II of Cys-based PTPs now includes two phosphatases, the former member LMW-PTP/ACP1, and the new addition Ssu72 (suppressor of Sua72), which has been added to this family due to its structural homology with LMW-PTP [69]. LMW-PTP has been studied for many years and it has been linked with diseases related with the immune response, inflammation, and cancer [70, 71]. However, the physiological role of this phosphatase is still poorly defined. LMW-PTP and Ssu72 present the typical PTP CxxxxxR signature motif at the N-terminus of the PTP domain, and the Asp acid involved in catalysis is C-terminal, more than 100 amino acids away in the primary sequence, in contrast with Class I PTPs, where precedes the signature motif. LMW-PTP and Ssu72 are evolutionarily related to bacterial arsenate-reductases coupled to thioredoxin, which present the PTP CxxxxxR signature motif and display the same topology [72, 73]. Unlike LMW-PTP, which is specific for pTyr, Ssu72 dephosphorylates specifically pSer5 and pSer7 in the C-terminal domain (CTD) of RNA polymerase II [74–76], and thus Ssu72 is involved in mRNA processing. Ssu72 has also been recently involved in sister chromatid segregation during cell division [77] through the regulation of the cohesin protein complex [78].

Table 2
Catalytic motifs and substrate specificity from some PTPome members

Gene/protein	Catalytic motif	Specificity	Consensus catalytic motif
(Classical; Class I.I Cys-based) (37)			
PTPRA/RPTPα(D1)	**HCS**AG**V**G**R**	pTyr	**HCS**x**G**x**GR**
PTPRQ	**HCS**AG**V**G**R**	PIPs	
PTPN1/PTP1B	**HCS**AG**I**G**R**	pTyr	
(VH1-like/DUSPs; Class I.II Cys-based) (64)			
DUSP1/MKP1	**HC**QAG**I**S**R**	pSer/Thr/Tyr	**HC**xx**G**xx**R**
DUSP3/VHR	**HC**REG**Y**S**R**	pSer/Thr/Tyr	
PTPMT1/PLIP	**HC**KAG**R**S**R**	pGP,PIPs	
EPM2A/Laforin	**HC**NAG**V**G**R**	pGlycogen,pTyr	
SSH1	**HC**KMG**V**S**R**	pSer	
PTP4A3/PRL-3	**HC**VAG**L**G**R**	pSer/Thr/Tyr,PIPs	
CDC14A	**HC**KAG**L**G**R**	pSer/Thr	
PTEN	**HC**KAG**K**G**R**	PIPs/pSer/Thr/Tyr	
MTM1	**HC**SDG**W**D**R**	PIPs	
MTMR4	**HC**SDG**W**D**R**	PIPs,pSer	
(SACs; Class I.III Cys-based) (5)			**C**xxxxx**R**
SACML1/SAC1	N**C**MDCLD**R**	PIPs	
SYNJ1	S**C**ERAGT**R**	PIPs	
(Paladin; Class I.IV Cys-based) (1)			
PALD1(D1)	S**C**QMGVG**R**	?	
PALD1(D2)	S**C**LSGQG**R**	?	
(INPP4s; Class I.V Cys-based) (2)			
INPP4A	S**C**KSAKD**R**	PIPs	
(*TMEM55s; Class I.VI Cys-based) (2)*			
TMEM55A	I**C**KDTSR**R**	PIPs	
(LMW-PTP; Class II Cys-based) (1)			
ACP1/LMW-PTP	V**C**LGNIC**R**	pTyr	
(SSU72; Class II Cys-based) (1)			
SSU72	V**C**SSNQN**R**	pSer	
(CDC25s; Class III Cys-based) (3)			
CDC25A	H**C**EFSSE**R**	pThr/Tyr	
(EYAs; Asp-based) (4)			
EYA1	WDLDET	pSer/Tyr	
(UBASH3s; His-based) (2)			
UBASH3A/TULA/Sts-2	RHGE	pTyr	
(ACPs; His-based) (3)			
ACPP	RHGD	Nonproteinaceous,pSer/Thr/Tyr	

Only some members from the distinct PTPome subfamilies are shown. For a complete list of PTPome members, see Table 1. The consensus catalytic motifs are shown for the Cys -based Tyr phosphatases. For PTPRA, only the catalytic motif from the active PTP D1 domain is shown. For PALD1, the catalytic motifs from the two PTP domains are shown. Note that VH1-like/DUSP subfamily constitutes a heterogeneous group of enzymes. The numbers in brackets indicate the number of genes in each category. PIPs, phosphoinositides; pGP, phosphatidylglycerophosphate.

5 Class III Cys-Based Phosphatases

This class contains three members, cell division cycle (CDC) 25A, B, and C (CDC25A, CDC25B, and CDC25C), which activate CDKs by dephosphorylating Thr14 and Tyr15 in the ATP binding loop of CDKs [79]. Thus, CDC25 phosphatases are involved in cell-cycle progression and in the checkpoint pathways that control DNA damage response [80]. This family has expanded through evolution from a single gene in yeast to three genes in mammals. The catalytic domain of CDC25 is a Rhodanese domain [81, 82], which was first found in a sulfurtransferase called rhodanese [83]. This domain presents an ample distribution in living organisms, being present in Eukarya, Archaea, and Eubacteria. An inactive rhodanese domain, named CDC25 homology domain (CH2), in which the catalytic Cys is replaced by another amino acid, is present in MKPs [84]. This CH2 domain includes a kinase interaction motif (KIM) involved in binding to MKPs [14, 85]. CDC25s present an extended catalytic loop. Whereas other rhodanese enzymes present four amino acids between the catalytic Cys and Arg, CDC25s contain five amino acids by insertion of one extra residue, to generate the signature motif of Cys-based PTPs. The addition of this extra amino acid seems to change the enzyme activity from a sulfur transfer reaction to phosphate hydrolysis [86]. This family lacks a WPD loop containing the general acid/base that works in the second step of the catalysis in Classes I and II. In this sense, it has been proposed that, in CDC25 phosphatases, the initial proton donor is the monoprotonated phosphate that acts as its substrate in lieu of the bisanionic phosphate used by Class I PTPs. An invariant Glu, placed in the CDC25s PTP-loop contiguous to the catalytic Cys, has been proposed to be involved in the catalysis [87, 88].

6 Asp-Based Phosphatases

Among the members of the large family of Asp-based phosphatases, aka Haloacid Dehalogenase (HAD) phosphatases, there are a few that possess Tyr phosphatase activity [89]. We refer to the four members of the Eyes absent (**EYA**) family of transcription factors, which are involved in the formation of many tissues and organs [90]. They contain a poorly conserved N-terminal domain, responsible for its transactivation activity [91], and a highly conserved C-terminal domain, called EYA domain, that participates in protein interactions, mainly with the Six family proteins, and through these interactions, in DNA binding [92]. EYA domain shares the active core of the HAD phosphatases and presents Tyr phosphatase activity [93–95]. EYA proteins also have Thr phosphatase activity, but this activity is catalyzed by other active sites located in the

N-terminal domain and not related to HAD phosphatase activity [96, 97]. Hence, EYA proteins have a dual specificity that is based on two separated catalytic domains that probably act on different substrates. The only avowed substrate for the Tyr phosphatase activity of EYA proteins is the histone H2AX [98, 99], whereas no substrate for the Thr phosphatase activity has been discovered.

7 His-Based Phosphatases

The His phosphatase (HP) superfamily includes numerous enzymes that dephosphorylate a great variety of substrates, from proteins to small molecules involved in metabolism [100]. Two branches are distinguished in this family. Branch one is called PGM (phosphoglycerate mutase) group, because the enzyme Diphosphoglycerate mutase (dPGM) is here included. The second branch is termed AP (acid phosphatases). The PGM subfamily is better represented in prokaryotes, while the AP subfamily is more abundant in eukaryotic organisms. Tyr phosphatases have been identified in both subfamilies, UBASH3 phosphatases in the PGM branch and some acid phosphatases in the second branch [8].

The **UBASH3** (Ubiquitin-associated and SH3 domain-containing protein) group of phosphatases includes UBASH3A and UBASH3B [101–104]. UBASH3 proteins contain an N-terminal UBA (ubiquitin-associated) domain, an SH3 (Src homology 3) domain, and a phosphatase domain similar to the PGM branch of the His phosphatases. The UBA domain interacts with ubiquitin and ubiquitylated proteins, including UBASH3 phosphatases [101, 103, 105]. The PGM domain, in addition to phosphatase activity, allows dimerization of these phosphatases. Expression of UBASH3B is ubiquitous, while UBASH3A expression is restricted to lymphocytes [103, 106]. The phosphatase activity of UBASH3B is much higher than UBASH3A [107–109].

UBASH3A and UBASH3B Tyr phosphatases dephosphorylate ZAP70 and Syk Tyr kinases [106, 109–111] with exquisite specificity, as they just dephosphorylate a few Tyr in these kinases [107, 109, 111, 112]. UBASH3B has also been implicated in dephosphorylation of the EGFR, where it also targets specific Tyr [112].

UBASH3 phosphatases can work both as negative or positive regulators. In the immune system, they seem to function as negative regulators, mainly through the regulation of immunoreceptor tyrosine-based activation motif (ITAM) associated receptors, such as TCR and FcεR. Identification of several SNPs associated with autoimmune diseases in these phosphatases further supports their relevance in the immune system [113]. On the other hand, it has been found that UBASH3B works as positive regulator of EGFR. Upon EGFR stimulation, UBASH3B is recruited to the EGFR

through the interaction with the E3 ubiquitin ligase CBL [101, 103]. UBASH3 SH3 domain binds to the central Pro-rich region of CBL [101, 103], and this complex dephosphorylates EGFR and inhibits its subsequent degradation, which is dependent on EGFR ubiquitination by CBL. In this sense, it has been found that UBASH3 is overexpressed in triple negative breast cancer (TNBC), as well as in prostate cancer cells [114]. UBASH3B is involved in tumor growth and metastasis mainly by inhibiting EGFR degradation, and for this reason it seems that UBASH3B could behave as an oncogenic phosphatase in TNBC.

The group of His-based **acid phosphatases** related with Tyr dephosphorylation includes ACPP/PAP, ACP2/LAP, and ACPT (Table 1). They dephosphorylate small organic nonproteinaceous moieties, as well as pTyr from peptides and proteins [115]. In particular, ACPP and ACPT have been associated with Tyr dephosphorylation and inactivation of distinct members of the EGFR family [116–118]. ACPP, which is abundant in prostate tissue, has been proposed as a tumor suppressor for prostate cancer [119, 120].

8 Catalytic Mechanism of Tyr Phosphatases

Hydrolysis of phosphate from Tyr-phosphorylated proteins is initiated by a nucleophilic attack. The nucleophile that starts the reaction is different in each family of PTPs: Cys in Cys-based phosphatases, Asp in Eya HAD, and His in HPs [3, 115, 121]. The catalytic mechanism for Cys, Asp, and His phosphatases has been well established and involves two steps. The reaction is initiated by a nucleophilic attack carried out by the catalytic amino acids, Cys, Asp, or His. A phospho-enzyme intermediate is formed and the dephosphorylated substrate is released. In addition to the catalytic residue that starts the nucleophilic attack, in the first step of the reaction, an Asp participates donating a proton to the tyrosyl leaving group of the substrate. Next, in the second step of the reaction, the phosphate is released and the phosphatase is regenerated. In this step, a water molecule acting as nucleophile breaks the phospho-enzyme intermediate. This molecule of water is deprotonated by an Asp, which is the same that intervened as a general acid in the first step of the reaction, and now works as a general base (Fig. 3). Representative catalytic signature motifs from the distinct groups of Cys-based Tyr phosphatases are shown in Table 2. Note that these groups can be classified, at least in part, based on the sequence of this motif.

The correct orientation of the phosphate for catalysis as well as the stabilization of the transition state during the reaction is mediated by the Arg present in the catalytic pocket at the end of the P-loop, which is part of the signature motif (CxxxxxR) in the Cys-based PTPs. In HPs, the function of this Arg in Cys-based

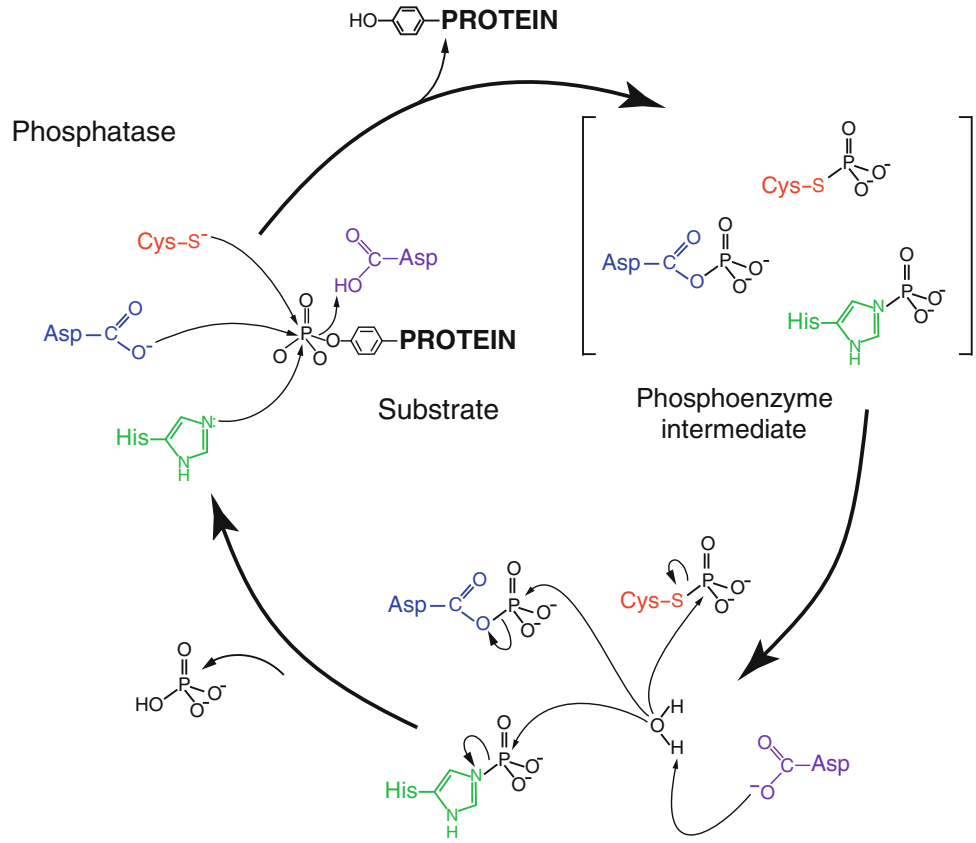

Fig. 3 Scheme of the two-step catalytic mechanism used by Tyr phosphatases. The scheme is shown with a pTyr-protein as the substrate. In the first step, the Cys-, His-, or Asp-catalytic residue from each family of phosphatases initiates a nucleophilic attack on the phosphate group of the substrate forming a transient phospho-enzyme intermediate and releasing the substrate dephosphorylated. In many cases, an Asp works as general acid in this step of the reaction, donating a proton to the tyrosyl group. In the second step of the reaction, the enzyme is restored after hydrolysis of the phospho-enzyme intermediate. In general, the catalytic Asp, working as a general base, deprotonates a water molecule, which now acts as nucleophile over the phospho-enzyme intermediate to release the phosphate group from the enzyme

phosphatases seems to be mediated by two Arg and a His within the catalytic pocket, which together with the catalytic His form the quartet of residues conserved in all HPs. On the other hand, HAD phosphatases use an Asp as the catalytic residue and Mg^{2+} as a cofactor to stabilize the transition state (Table 2) [122].

In contrast to the diversity found in the initial nucleophile in these families of phosphatases, they have adopted an Asp as the general acid/base. Nevertheless, in UBASH3 phosphatases, HPs of the PGM branch, it seems that a Glu develops this function [107, 123]. In most of the Cys-based PTPs, the general acid/base Asp is usually located in an additional loop in the active site. In Class I PTPs this Asp precedes by 30–40 amino acids the P-loop that contains the Cys. This loop contains a conserved stretch of amino

acids, WPD, in classical PTPs that is used to name this loop as WPD loop. However, these residues are not conserved outside this group of phosphatases. On the other hand, in Class II the Asp-loop is 100 amino acids C-terminal to the catalytic Cys. Interestingly, it has been suggested than in Class III phosphatases a Glu in the P-loop develops this function [87, 88], as well as in Myotubularins [36]. Similarly, in the EYAs HADs, the Asp is found in the same loop that the catalytic Asp at a +2 position (Table 2) [121].

9 The PTPome in Model Organisms

PTPome human and mouse genes are well conserved, with the exception of *PTPRV/Ptprv*, which is a transcribed pseudogene in human and a normal gene in mouse [124]. In addition, the two human *TPTE* and *TPTE2* genes are represented with one single gene in mouse [125]. *Dario rerio* (zebrafish) and *Drosophila melanogaster* (fruit fly), two widely used metazoan model organisms, contain members from all Tyr phosphatase families. Within the group of classical PTPs, *D. rerio* ortholog genes to all the mammalian PTPs have been found, with the exception of PTPN7, PTPN12, and PTPN14. In addition, at least 14 classical PTP genes are duplicated in *D. rerio* [126, 127]. In the case of *D. melanogaster*, the representation of Cys-based phosphatases covers about half of the human Cys-based phosphatase genes, with all the *D. melanogaster* Tyr phosphatases having orthologs in humans [127, 128]. The PTPome of the yeast *Saccharomyces cerevisiae* (budding yeast), the more used experimental model from Fungi kingdom, is mostly composed of Tyr-specific and dual-specificity Cys-based phosphatases dedicated to MAPKs dephosphorylation, as well as of dual-specificity phosphatases of unknown function [127, 129, 130]. Two SAC members are present in *S. cerevisiae* [131], as well as Ssu72 [75].

10 Conclusion and Future Perspectives

A variety of mammalian phosphatases from different gene families (PTPome) dephosphorylate pTyr residues or show topological similarity in their catalytic domains with the canonical PTP domain. This has been used to establish classifications and evolutionary relationships between these enzymes [3–6, 132, 133]. We have summarized here our concept of an open and extended PTPome which includes proteins that: (a) harbor a structurally defined PTP domain; *or* (b) contain a CxxxxxR signature catalytic motif within a non-PTP phosphatase domain; *or* (c) display experimentally validated Tyr phosphatase activity; *or* (d) display high sequence similarity to members with demonstrated Tyr phosphatase activity.

The relative conservation of Tyr phosphatase functions in distant Phyla, and the evolutionary convergence of several PTPome members towards Tyr- or PIP-dephosphorylation, outlines the physiologic importance of such functions. In this regard, bacteria and protozoan parasite Tyr phosphatases have a pathogenic role in several infectious diseases, which make them direct drug targets for therapeutic intervention [134–136]. Moreover, Tyr phosphatases have direct roles in the etiology of many hereditary and nonhereditary human diseases, including cancer, neurodegenerative, metabolic, immune, and heart diseases [33, 136–152]. The dual role of many of the Tyr phosphatases in relation with human disease constitutes both a challenge and an open scenario for the implementation of therapies based on these enzymes. For instance, many classical PTPs display pro-oncogenic or anti-oncogenic roles depending on the tissue and the physiologic context [134, 135, 140, 144]. A clinically relevant example is the lipid phosphatase PTEN, whose reconstitution, activation, or delivery could be beneficial in cancer therapy, whereas its inhibition could be beneficial in the treatment of neuroregeneration-related diseases [153–156]. The feasibility of Tyr phosphatases as both potential targets for inhibition and active drugs in human disease therapy will be under dedicated scrutiny in the upcoming future.

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