

A Brief Introduction to the Protein Phosphatase Families

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Summary

This chapter introduces the main families of protein phosphatases encoded by the human genome and discusses their classification, overall structure, regulation, and physiological functions in human health and diseases. The topics of redundancy, diversity, and dynamic expression in individual cell types are briefly introduced, and the importance of technological approaches to phosphatase research is emphasized.

Key Words: Protein phosphatases; PTPs, DSPs; HAD family; drug targets

1. Introduction

Protein phosphorylation is a fundamental mechanism for numerous important aspects of eukaryote physiology, as well as human health and disease (1–4). It has been estimated that at least one-third of cellular proteins contain covalently bound phosphate. Among the many phospho-acceptor amino acids, serine phosphorylation is the most prevalent, whereas tyrosine phosphorylation (5) stands out as a feature of higher eukaryotes, where it is used as a regulatory mechanism in cell-to-cell communication and functions that coordinate the behavior of cell populations within these multicellular organisms (1). However, tyrosine phosphorylation has recently also been found in bacteria and *Archaea* (6–8), the sequenced genomes of which usually contain several genes for PTPs. Thus, tyrosine phosphorylation might have flourished in more recent evolution, but its roots lay very far back. Bacterial genomes also contain Ser/Thr phosphatases, but usually lack eukaryote-type protein kinases. Instead, there are protein kinases of a different kind, which are not found in mammals. Nevertheless, it seems that protein phosphorylation is not nearly as central a regulatory mechanism in prokaryotes as it is in eukaryotes.

With some very rare possible exceptions, protein phosphorylation is a reversible posttranslational modification catalyzed by protein kinases and reversed by protein phosphatases. Thus, the state of phosphorylation of a protein, at a given moment in time, is the net result of the opposing activities of the relevant kinase(s) and phosphatase(s). A change in phosphorylation state can be the result of a change in the activity (or access) of either enzyme. Particularly in the realm of tyrosine phosphorylation, a general rule is that the balance is skewed very far toward the dephosphorylated state: Most tyrosine phosphorylated proteins are phosphorylated to a stoichiometry of only a few percent even under the most extreme inducing conditions and are often not phosphorylated at all under “resting” conditions. Thus, one could argue that phosphatases are more important than kinases in setting the levels of protein phosphorylation and that they should be much better drug targets. Indeed, phosphatases often play very specific, nonredundant, highly regulated, and very active roles in many cellular processes (9–17). Phosphatases are also often “positive” components of signaling events (18–20) and many phosphatase knockout mice have unique and complex phenotypes (21–30). Finally, the completion of the human genome has demonstrated that (1) there are more tyrosine phosphatases than tyrosine kinases (3,31), (2) the possible number of Ser/Thr phosphatase holoenzymes, generated by a combinatorial mechanism, far exceeds the number of all protein kinases (32), and (3) there are additional large families of protein phosphatases, such as the haloacid dehalogenase (HAD) family, and possibly others.

2. The Many Families of Protein Phosphatases

Based on structure, rather than function, the protein phosphatases can be classified into several completely separate families (*see Table 1*) that do not share any structural similarities and apparently evolved independently from different ancestral folds. Naturally, we cannot exclude the possibility that some of these folds may have evolved from one another at an ancient time beyond the abilities of bioinformatics tools to resolve. It is important to note that this newer structural classification overlaps, but does not coincide, with the older classification of protein phosphatases by substrate specificity into Ser/Thr-specific, Tyr-specific, and dual-specific phosphatases. Particularly, the so called dual-specific phosphatases (DSPs) (3) include many enzymes that are highly specific for Tyr, Ser, phosphoinositides, or mRNA. There are also examples of “Ser/Thr phosphatases” that dephosphorylate Tyr and enzymes that can dephosphorylate more than one type of substrate. Clearly, evolution cares little for our desires for simplicity and classification. In fact, the many solved crystal structures of protein phosphatases demonstrate that subtle alterations in structure can drastically alter substrate specificity (e.g., from

Table 1
Phosphatase Families

Phosphatase families	Examples of members
1. PPM family	PP2C
2. FCP family	FCP
3. PPP family	PP1, PP2A, calcineurin, PP5
4. HAD family (Asp-based)	Eya, CTD, cronophin
5. Class I Cys-based PTPs	
5.1. Classical PTPs	
5.1.1. Transmembrane PTPs	PTP α , CD45, CD148, IA-2, GLEPP1
5.1.2. NRPTPs	PTP1B, TCPTP, SHP1, LYP, MEG2
5.2. DSPs or VH1-like PTPs	
5.2.1. MKPs	MKP1–5, MKP7, PAC1
5.2.2. Atypical DSPs	VHR, PIR, Laforin, VHZ, STYX
5.2.3. Slingshots	SSH1, SSH2, SSH3
5.2.4. PRLs	PRL-1, PRL-2, PRL-3
5.2.5. CDC14s	CDC14A, KAP, PTP9Q22
5.2.6. PTENs	PTEN, TPIP, tensin, C1ten
5.2.7. Myotubularins	MTM1, MTMR1–15
6. Class II Cys-based PTPs	CDC25A, CDC25B, CDC25C
7. Class III Cys-based PTPs	LMPTP

phosphoamino acid to phospholipid; *see* **refs. 33** and **34**) and that the same substrate specificity can be achieved in many different ways.

The Ser/Thr phosphatases have been classified into three structurally distinct families: (1) the PPM family of Mg²⁺-dependent phosphatases, including PP2C, (2) the Mg²⁺-dependent FCP family, and (3) the PPP family, which is the largest and contains the well-known enzymes PP1, PP2A, PP2B (calcineurin), PP5, and many others (**32**). Altogether there are 25–30 genes for catalytic subunits of these enzymes in the human genome. In addition, there are numerous regulatory subunits, which participate in the formation of heterodimeric or trimeric phosphatase holoenzymes with unique substrate specificities, regulatory mechanisms, subcellular locations, and physiological functions (**32**).

We defined PTPs as the proteins with structural homology to the catalytic domains of any of the known enzymes with PTP activity, regardless of their specificity (**3**). There are four evolutionary distinct families of such genes: the class I, II, and III Cys-based PTPs, and the Asp-based phosphatases, exemplified by the Eya (eyes absent) tyrosine phosphatases (**14**). These Asp-based PTPs are part of the HAD family, which is now emerging as a very large protein family with representatives in plants (**35**), prokaryotes (**36**), and mammals

and includes numerous enzymes with other than Tyr-specificity—indeed, with a broader substrate spectrum than hydroxyl-containing amino acid residues in proteins, such as phospholipids (37), sugars (38), nucleotides (39), and epoxides (40). The protein phosphatases of the HAD family can be specific for tyrosine (14) or serine, as in the case of the cofilin phosphatase chronophin (41) and RNA polymerase C-terminal phosphatase (42). Several crystal structures of HAD family phosphatases have already been reported (43–46) and a more definitive picture of this family is now emerging.

Class I Cys-based PTPs are structurally related to the first PTP PTP1B, whose amino acid sequence was determined (47). There are at least 99 members of this family in the human genome (3) and they can be further subclassified into the classical PTPs (receptorlike and nonreceptor), and the VH1-like phosphatases, which contain the mitogen-activated protein (MAP) kinase phosphatase (MKPs), the atypical DSPs, the slingshots, the PRLs, the CDC14s, the PTEN group, and the myotubularins. The two latter dephosphorylate inositol phospholipids (48,49). Within all of these homologous phosphatases, it appears that the atypical DSPs represent the evolutionary most ancient members of the family. Genes with a high degree of similarity can be found across all kingdoms of life, including *Archaea* and plants (50). In contrast, the classical PTPs, particularly the receptorlike group, seem to be more recent groups that have flourished and diversified in multicellular organisms.

The class II Cys-based protein phosphatases comprise a small group of cell-cycle regulators known as the CDC25 phosphatases. Although their catalytic machinery is very similar to the class I enzymes, they are structurally unrelated and, instead, bear considerable resemblance to bacterial rhodanese enzymes (51), and are thought to have evolved relatively late in eukaryote evolution. Interestingly, the MAP kinase phosphatases, which belong to the class I family, have incorporated a catalytically inactive rhodaneselike domain, which functions as a MAP kinase docking module (52). This creates a region of homology between the CDC25s and the MAP kinases, which, however, is not indicative of a common ancestry of their catalytic domains.

The class III Cys-based protein phosphatases are widely distributed in all kingdoms of life and most bacteria have the genes for one or two such enzymes in their genomes. In *Escherichia coli*, one such phosphatase regulates the tyrosine phosphorylation of a transmembrane tyrosine autokinase, which regulates synthesis of polysaccharides of the bacterial capsule (53). In the Gram-negative *Bacillus subtilis*, the two class III phosphatases YfkJ and YwIE have clearly distinct properties and bacterial knockout strains have distinct phenotypes (54). The human genome contains a single gene for a class III PTP, the low-*Mr* PTP (LMPTP), which undergoes alternative splicing to yield two active and one inactive isoforms. Although a polymorphism in this gene correlates with

numerous common human diseases (4), the function of LMPTP has remained obscure.

3. Single-Chain Multidomain Versus Single-Domain Multi-Subunit Organization

As mentioned above, many PTPs are larger proteins with multiple modular domains, whereas Ser/Thr phosphatases consist of catalytic polypeptides that can associate with several different regulatory or targeting subunits, resulting in numerous different holoenzymes. Thus, the end result is similar, but one cannot help wondering why the strategies are so different. What are the advantages of each strategy? Are single-chain multidomain enzymes more strictly regulated? Does a combinatorial mechanism allow for more flexibility in cellular responses? The completion of many genomic sequencing projects has revealed that the number of PTP genes has increased during evolution, whereas the number of Ser/Thr phosphatases has remained nearly constant. Instead, the number of regulator/targeting subunits has increased sharply in eukaryote evolution (17). Perhaps the large increase in protein phosphorylation during early evolution outpaced the diversification of Ser/Thr phosphatase domains and, instead paradoxically, led to a situation in which a few phosphatase catalytic domains with broad substrate specificity fulfill the need better, as long as their regulation and targeting is taken into account.

4. Regulation of Phosphatases

Both Ser/Thr phosphatases and PTPs seem to be regulated to a large extent by similar mechanisms, which are also shared by protein kinases, namely by protein–protein and protein–phospholipid interactions. Both targeting to substrate-containing locations or complexes and direct allosteric modulation of the catalytic domain/subunit seem to play important roles. Catalytic activation is often accomplished by the removal of pseudosubstrate motifs or blocking regulatory subunits/domains from the active site of the phosphatase as a result of interaction of the holoenzyme with ligands or phospholipids. Another interesting aspect of regulation is the abundance of catalytically inactive PTP domains (approx 10% of all PTPs), which often partner with active PTPs and perform crucial regulatory or targeting functions. Good examples of this is provided by many receptorlike PTPs, which have two tandem catalytic domains in their intracellular C-terminus. In most cases, the second domain has less than 1% as much activity as the first (membrane-proximal) domain; in some PTPs, the second domain does not even have the catalytic cysteine. Nevertheless, in many cases, the second domain is still crucial for the physiological function of the phosphatase (55). Another striking example can be found within the myotubularins: whereas many patients with the inherited nerve myelina-

tion disease Charcot–Marie–Tooth syndrome type 4B have a debilitating mutation in the class I Cys-based phosphatase myotubularin-related protein 2 (*MTMR2*) (56), a subset of patients were found to have a mutation in the catalytically inactive phosphatase *MTMR13* (57). In both cases, the disease is the same, raising the question of how the loss of an inactive phosphatase can lead to the same disease as the loss of an active phosphatase. The answer to this puzzle was provided by the discovery that the two proteins form a heterodimer, in which the catalytically inactive *MTMR13* provides critical aid to the function of the active *MTMR2*.

Phosphatases are often phosphorylated themselves, suggesting that they are also substrates for protein kinases and phosphatases and can be part of kinase cascades, phosphatase cascades, or mixed cascades. Dephosphorylation of phosphatases can occur by autocatalysis, but in some cases (e.g., VHR), the phosphorylated residue is not accessible to the catalytic pocket of the same molecule and therefore dephosphorylation must occur either *in trans* or by another PTP. As with many other signaling molecules, tyrosine phosphorylation of phosphatases is typically of very low stoichiometry and difficult to study. Nevertheless, there are a few examples where tyrosine phosphorylation of a PTP is of regulatory importance, such as the cases of *RPTP α* (58), *LMPTP* (59), *VHR* (60). Phosphotyrosine can also be found in dozens of other phosphatases, but is still in most cases of unknown physiological relevance.

5. How Many Phosphatase Are There in a Cell?

Although it has become increasingly apparent that phosphatases often have a high degree of specificity and that there are so many phosphatase genes in the human genome, the question of functional redundancy is still largely unresolved. It is probably prudent to assume that closely related phosphatases have at least partly overlapping sets of substrates. There is some evidence for this, for example, from mouse knockouts where the phenotype has been milder than expected [e.g., *MKP1* (61) or *PEP* (62)]. A more systematic analysis of redundancy is complicated by issues of tissue expression profile, relative expression levels, and differential regulation of expression during embryogenesis and development. An alternative approach to study the question of redundancy is to take a given cell and first ask how many of the existing phosphatase genes are expressed in it and then study this set (e.g., by RNA interference). From preliminary analysis carried out in our laboratory, it seems that each cell type expresses a surprisingly large portion of the 107 PTP genes (the “PTPome”); for example, monocytes and T-lymphocytes express at least 68 PTPs each, whereas B-cells contain over 70. The set expressed in each cell type is unique (albeit overlapping) both in terms of which PTPs are expressed and in their relative expression levels. The entire set responds with both qualitative and

quantitative changes to many external stimuli, cell activation, differentiation, and so forth. Interestingly, each stimulus elicits a unique response and identical stimuli elicit somewhat different responses in different cells, even when they are closely related. Finally, the PTP expression profile is somewhat different in identical cell types from different individuals. All of these levels of complexity will need to be considered when studying the extent of possible redundancy of phosphatases.

6. Protein Phosphatases and Human Disease

Whether phosphatases exhibit some overlaps in function or not, it is clear that even subtle alterations in many of them can cause human disease (2,3). As might be expected, loss of phosphatases has been reported in cancer: The list contains over 30 different PTPs and loss can occur by genetic (e.g., chromosomal abnormalities, frame-shift mutations, or point mutations) or epigenetic (e.g., promoter methylation or changes in transcription) mechanisms. More interestingly, several phosphatases have been found to be overexpressed in human malignancies, such as PRL3 in metastatic colon cancer (63).

Phosphatases are also implicated in inherited genetic diseases, including Noonan syndrome (SHP2; 64), Lafora's epilepsy (laforin; 65), muscle dystrophies (myotubularin; 66), and immunodeficiencies (CD45; 67,68), as well as in autoimmune diabetes (LYP; 69–71, and PTPRN; 72) and other major autoimmune diseases (LYP; 73–75), and myelodysplastic syndrome and acute myeloblastic leukemia (SHP1; 76, and HePTP; 77,78). Also, PP2A has been implicated in a monogenic disorder, Opitz BBB/G syndrome (79), which is characterized by malformations of the ventral midline, as well as in tumorigenesis (80,81). Given the broad significance of protein phosphorylation and the very limited studies performed so far, I predict that a very large number of human health concerns will be found to involve a central role for protein phosphatases. I also predict that the pharmaceutical industry will become increasingly interested in phosphatases as drug targets. In fact, this trend is already evident (82–91).

7. Concluding Remarks

The mission of my laboratory is to make the scientific community more familiar with the PTPs and their importance in human health and disease. We strive to elucidate the molecular mechanisms of PTP function in normal as well as pathological cellular processes and to explore the value of individual PTPs as drug targets. Over the years, I have come to value the inclusion of multiple phosphatases in each experiment and a more unbiased comparison of enzymes, rather than a strict focus on a single one at a time. We often ask “Which phosphatase does this?” rather than “What does this phosphatase do?”

This approach not only gives a better insight into questions of specificity vs redundancy, but often reveals unexpected or novel functions. I believe that the time has come to consider and analyze entire families of phosphatases.

This volume of *Methods in Molecular Biology* addresses a perceived obstacle in phosphatase research: the notion that phosphatases are technically difficult to study. Not so, there are numerous well-established techniques and protocols, as well as an increasingly complete coverage of antibodies and plasmids, plus many new avenues, such as small-molecule inhibitors, activity-based probes (92), and technologies for RNA interference and “substrate-trapping” mutants. I hope that this volume of *Methods in Molecular Biology* will entice more researchers to enter the field of protein phosphatases and will stimulate work with the numerous enzymes that so far have received little attention.

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References

1. Hunter, T. (1998) The role of tyrosine phosphorylation in cell growth and disease. *Harvey Lect.* **94**, 81–119.
2. Andersen, J. N., Jansen, P. G., Echwald, S. M., et al. (2004) A genomic perspective on PTPs: gene structure, pseudogenes, and genetic disease linkage. *FASEB J.* **18**, 8–13.
3. Alonso, A., Sasin, J., Osterman, A., et al. (2004) The PTPs in the human genome. *Cell* **117**, 699–711.
4. Bottini, N., Bottini, E., Gloria-Bottini, F., and Mustelin, T. (2002) LMPTP and human disease: in search of biochemical mechanisms. *Arch. Immunol. Ther. Exp. (AITE)* **50**, 95–104.
5. Hunter, T. and Sefton, B. M. (1980) Transforming gene product of Rous sarcoma virus phosphorylates tyrosine. *Proc. Natl. Acad. Sci. USA* **77**, 1311–1315.
6. Chow, K., Ng, D., Stokes, R., and Johnson, P. (1994) Protein tyrosine phosphorylation in *Mycobacterium tuberculosis*. *FEMS Microbiol. Lett.* **124**, 203–207.
7. Kennelly, P. J. (2003) Archaeal protein kinases and protein phosphatases: Insights from genomics and biochemistry. *Biochem. J.* **370**, 373–389.
8. Cozzone, A. J., Grangeasse, C., Doublet, P., and Duclos, B. (2004) Protein phosphorylation on tyrosine in bacteria. *Arch. Microbiol.* **181**, 171–181.

9. Walton, K. M. and Dixon, J. E. (1993) Protein tyrosine phosphatases. *Annu. Rev. Biochem.* **62**, 101–120.
10. Tonks, N. K. and Neel, B. G. (1996) From form to function: signaling by PTPs. *Cell* **87**, 365–368.
11. Mustelin, T., Vang, T., and Bottini, N. (2005) Protein tyrosine phosphatases and the immune response. *Nat. Rev. Immunol.* **5**, 43–57.
12. Stoker, A. W. (2005) Protein tyrosine phosphatases and signaling. *J. Endocrinol.* **185**, 19–33.
13. Kappert, K., Peters, K. G., Bohmer, F. D., and Ostman, A. (2005) Tyrosine phosphatases in vessel wall signaling. *Cardiovasc. Res.* **65**, 587–598.
14. Rebay, I., Silver, S. J., and Tootle, T. L. (2005) New vision from Eyes absent: transcription factors as enzymes. *Trends Genet.* **21**, 163–171.
15. Wong, W. and Scott, J. D. (2004) AKAP signalling complexes: focal points in space and time. *Nat. Rev. Mol. Cell. Biol.* **5**, 959–970.
16. Cohen, P. T. (2002) Protein phosphatase 1: targeted in many directions. *J. Cell. Sci.* **115**, 241–256.
17. Ceulemans, H. and Bollen, M. (2004) Functional diversity of protein phosphatase-1, a cellular economizer and reset button. *Physiol. Rev.* **84**, 1–39.
18. Feng, G. S. (1999) Shp-2 tyrosine phosphatase: Signaling one cell or many. *Exp. Cell Res.* **253**, 47–54.
19. Mustelin, T., Coggeshall, K. M., and Altman, A. (1989) Rapid activation of the T cell tyrosine protein kinase pp56lck by the CD45 phosphotyrosine phosphatase. *Proc. Natl. Acad. Sci. USA.* **86**, 6302–6306.
20. Bottini, N., Stefanini, L., Williams, S., et al. (2002) Activation of ZAP-70 through specific dephosphorylation at the inhibitory Tyr-292 by the low molecular weight phosphotyrosine phosphatase (LMPTP). *J. Biol. Chem.* **277**, 24,220–24,224.
21. Cote, J. F., Charest, A., Wagner, J., and Tremblay, M. L. (1998) Combination of gene targeting and substrate trapping to identify substrates of PTPs using PTP-PEST as a model. *Biochemistry* **37**, 13,128–13,137.
22. Saxton, T. M., Henkemeyer, M., Gasca, S., et al. (1997) Abnormal mesoderm patterning in mouse embryos mutant for the SH2 tyrosine phosphatase Shp-2. *EMBO J.* **16**, 2352–2364.
23. Gronda, M., Arab, S., Iafrate, B., Suzuki, H., and Zanke, B. (2001) Hematopoietic PTP suppresses extracellular stimulus-regulated kinase activation. *Mol. Cell. Biol.* **21**, 6851–6858.
24. Elchebly, M., Payette, P., Michaliszyn, E., et al. (1999) Increased insulin sensitivity and obesity resistance in mice lacking the PTP-1B gene. *Science* **283**, 1544–1548.
25. You-Ten, K. E., Muise, E. S., Itie, A., et al. (1997) Impaired bone marrow microenvironment and immune function in T cell PTP-deficient mice. *J. Exp. Med.* **186**, 683–693.
26. Byth, K. F., Conroy, L. A., Howlett, S., et al. (1996) CD45-null transgenic mice reveal a positive regulatory role for CD45 in early thymocyte development in the selection of CD4⁺CD8⁺ thymocytes and B-cell maturation. *J. Exp. Med.* **183**, 1707–1718.

27. Wharram, B. L., Goyal, M., Gillespie, P. J., et al. (2000) Altered podocyte structure in GLEPP1 (Ptpro)-deficient mice associated with hypertension and low glomerular filtration rate. *Clin. Invest.* **106**, 1281–1290.
28. Uetani, N., Kato, K., Ogura, H., et al. (2000) Impaired learning with enhanced hippocampal long-term potentiation in PTPdelta-deficient mice. *EMBO J.* **19**, 2775–2785.
29. Di Cristofano, A., Pesce, B., Cordon-Cardo, C., and Pandolfi, P. P. (1998) Pten is essential for embryonic development and tumour suppression. *Nat. Genet.* **19**, 348–355.
30. Koop, E. A., Gebbink, M. F. B. G., Sweeney, T. E., et al. (2005) Impaired flow-induced dilation in mesenteric resistance arteries from receptor protein tyrosine phosphatase- μ -deficient mice. *Am. J. Physiol. (Heart Circ. Physiol.)* **288**, H1218–H1223.
31. Manning, G., Whyte, D. B., Martinez, R., Hunter, T. and Sudarsanam, S. (2002) The protein kinase complement of the human genome. *Science* **298**, 1912–1934.
32. Schonthal, A. H. (1998) Role of PP2A in intracellular signal transduction pathways. *Front. Biosci.* **3**, D1262–1273.
33. Lee, J. O. Yang, H., Georgescu, M. M., et al. (1999) Crystal structure of the PTEN tumor suppressor: implications for its phosphoinositide phosphatase activity and membrane association. *Cell* **99**, 323–334.
34. Begley, M. J. Taylor, G. S., Kim, S. A., Veine, D. M., Dixon, J. E., and Stuckey, J. A. (2003) Crystal structure of a phosphoinositide phosphatase, MTMR2: insights into myotubular myopathy and Charcot-Marie-Tooth Syndrome. *Mol. Cell* **12**, 1391–1402.
35. Rayapureddi, J. P., Kattamuri, C., Chan, F. H., and Hegde, R. S. (2005) Characterization of a plant, tyrosine-specific phosphatase of the aspartyl class. *Biochemistry* **44**, 751–8.
36. Roberts, A., Lee, S. Y., McCullagh, E., Silversmith, R. E., and Wemmer, D. E. (2005) Ybiv from *Escherichia coli* K12 is a HAD phosphatase. *Proteins* **58**, 790–801.
37. Roberts, S. J., Stewart, A. J., Sadler, P. J., and Farquharson, C. (2004) Human PHOSPHO1 exhibits high specific phosphoethanolamine and phosphocholine phosphatase activities. *Biochem. J.* **382**, 59–65.
38. Allegrini, S., Scaloni, A., Careddu, M. G., et al. (2004) Mechanistic studies on bovine cytosolic 5'-nucleotidase II, an enzyme belonging to the HAD superfamily. *Eur. J. Biochem.* **271**, 4881–4891.
39. Lunn, J. E., Ashton, A. R., Hatch, M. D., and Heldt, H. W. (2000) Purification, molecular cloning, and sequence analysis of sucrose-6F-phosphate phosphohydrolase from plants. *Proc. Natl. Acad. Sci. USA* **97**, 12,914–12,919.
40. Cronin, A., Mowbray, S., Durk, H., et al. (2003) The N-terminal domain of mammalian soluble epoxide hydrolase is a phosphatase. *Proc. Natl. Acad. Sci. USA* **100**, 1552–1557.
41. Gohla, A., Birkenfeld, J., and Bokoch, G. M. (2005) Chronophin, a novel HAD-type serine protein phosphatase, regulates cofilin-dependent actin dynamics. *Nat. Cell Biol.* **7**, 21–29.

42. Yeo, M., Lin, P. S., Dahmus, M. E., and Gill, G. N. (2003) A novel RNA polymerase II C-terminal domain phosphatase that preferentially dephosphorylates serine 5. *J. Biol. Chem.* **278**, 26,078–26,085.
43. Peisach, E., Selengut, J. D., Dunaway-Mariano, D., and Allen, K. N. (2004) X-ray crystal structure of the hypothetical phosphotyrosine phosphatase MDP-1 of the haloacid dehalogenase superfamily. *Biochemistry* **43**, 12,770–12,779.
44. Stewart, A. J., Schmid, R., Blindauer, C. A., Paisey, S. J., and Farquharson, C. (2003) Comparative modelling of human PHOSPHO1 reveals a new group of phosphatases within the haloacid dehalogenase superfamily. *Protein Eng.* **16**, 889–895.
45. Allen, K. N. and Dunaway-Mariano, D. (2004) Phosphoryl group transfer: evolution of a catalytic scaffold. *Trends Biochem. Sci.* **29**, 495–503.
46. Lahiri, S. D., Zhang, G., Dai, J., Dunaway-Mariano, D., and Allen, K. N. (2004) Analysis of the substrate specificity loop of the HAD superfamily cap domain. *Biochemistry* **43**, 2812–2820.
47. Charbonneau, H., Tonks, N. K., Kumar, S., et al. (1989) Human placenta protein-tyrosine phosphatase: amino-acid sequence and relationship to a family of receptor-like proteins. *Proc. Natl. Acad. Sci. USA* **86**, 5252–5256.
48. Maehama, T. and Dixon, J. E. (1998) The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. *J. Biol. Chem.* **273**, 13,375–13,378.
49. Wishart, M. J. and Dixon, J. E. (2002) PTEN and myotubularins phosphatases: from 3-phosphoinositide dephosphorylation to disease. *Trends Cell Biol.* **12**, 579–585.
50. Alonso, A., Sasin, J., Burkhalter, S., et al. (2004) The minimal essential core of a cysteine-based PTP revealed by a novel 16-kDa VH1-like phosphatase, VH2. *J. Biol. Chem.* **279**, 35,768–35,774.
51. Bordo, D. and Bork, P. (2002) The rhodanese/Cdc25 phosphatase superfamily. Sequence–structure–function relations. *EMBO Rep.* **3**, 741–746.
52. Alonso, A., Rojas, A., Godzik, A., and Mustelin, T. (2003) The dual-specific PTP family. *Top. Curr. Genet.* **5**, 333–358.
53. Vincent, C., Duclos, B., Grangeasse, C., et al. (2000) Relationship between exopolysaccharide production and protein-tyrosine phosphorylation in Gram-negative bacteria. *J. Mol. Biol.* **304**, 311–321.
54. Musumeci, L., Tautz, L., Perego, M., Mustelin, T., and Bottini, N. (2005) Characterization of the YfkJ protein tyrosine phosphatase of *Bacillus subtilis* and *Bacillus anthracis*. *J. Bacteriol.*, in press.
55. Kashio, N., Matsumoto, W., Parker, S., and Rothstein, D.M. (1998). The second domain of the CD45 protein tyrosine phosphatase is critical for interleukin-2 secretion and substrate recruitment of TCR ζ in vivo. *J. Biol. Chem.* **273**, 33856–33863.
56. Bolino, A., Muglia, M., Conforti, F. L., et al. (2000) Charcot-Marie-Tooth type 4B is caused by mutations in the gene encoding myotubularin-related protein-2. *Nat. Genet.* **25**, 17–19.

57. Azzedine, H., Bolino, A., Taieb, T., et al. (2003) Mutations in MTMR13, a new pseudophosphatase homologue of MTMR2 and Sbf1, in two families with an autosomal recessive demyelinating form of Charcot-Marie-Tooth disease associated with early-onset glaucoma. *Am. J. Hum. Genet.* **72**, 1141–1153.
58. Mustelin, T. and Hunter, T. (2002) Meeting at mitosis: cell cycle-specific regulation of c-Src by RPTP α . *Science's STKE*. http://stke.sciencemag.org/cgi/content/full/OC_sigtrans;2002/115/pe3.
59. Tailor, P., Williams, S., Gilman, J., Couture, C., and Mustelin, T. (1997) Regulation of the low molecular weight phosphotyrosine phosphatase (LMPTP) by phosphorylation at tyrosines 131 and 132. *J. Biol. Chem.* **272**, 5371–5376.
60. Alonso, A., Rahmouni, S., Williams, S., et al. (2003) Tyrosine phosphorylation of VHR phosphatase by ZAP-70. *Nat. Immunol.* **4**, 44–48.
61. Dorfman, K., Carrasco, D., Gruda, M., Ryan, C., Lira, S. A., and Bravo, R. (1996) Disruption of the *erp/mkp-1* gene does not affect mouse development: normal MAP kinase activity in ERP/MKP-1-deficient fibroblasts. *Oncogene* **13**, 925–931.
62. Hasegawa, K., Martin, F., Huang, G., Tumas, D., Diehl, L., and Chan, A. C. (2004) PEST domain-enriched tyrosine phosphatase (PEP) regulation of effector/memory T cells. *Science* **303**, 685–689.
63. Saha, S., Bardelli, A., Buckhaults, P., et al. (2001) A phosphatase associated with metastasis of colorectal cancer. *Science* **294**, 1343–1346.
64. Tartaglia, M., Mehler, E. L., Goldberg, R., et al. (2001) Mutations in *PTPN11*, encoding the PTP SHP-2, cause Noonan syndrome. *Nat. Genet.* **29**, 465–468.
65. Minassian, B. A., Lee, J. R., Herbrick, J. A., et al. (1998) Mutations in a gene encoding a novel PTP cause progressive myoclonus epilepsy. *Nat. Genet.* **20**, 171–174.
66. Laporte, J., Hu, L. J., Kretz, C., et al. (1996) A gene mutated in X-linked myotubular myopathy defines a new putative tyrosine phosphatase family conserved in yeast. *Nat. Genet.* **13**, 175–182.
67. Tchilian, E. Z., Wallace, D. L., Wells, R. S., Flower, D. R., Morgan, G., and Beverley, P. C. L. (2001) A deletion in the gene encoding the CD45 antigen in a patient with SCID. *J. Immunol.* **166**, 1308–1313.
68. Kung, C., Pingel, J. T., Heikinheimo, M., et al. (2000) Mutations in the tyrosine phosphatase CD45 gene in a child with severe combined immunodeficiency disease. *Nat. Med.* **6**, 343–345.
69. Bottini, N., Musumeci, L., Alonso, A., et al. (2004) A functional variant of lymphoid tyrosine phosphatase is associated with type 1 diabetes. *Nat. Genet.* **36**, 337–338.
70. Smyth, D., Cooper, J. D., Collins, J. E., et al. (2004) Replication of an association between the lymphoid tyrosine phosphatase locus (LYP/*PTPN22*) with type 1 diabetes, and evidence for its role as a general autoimmunity locus. *Diabetes* **53**, 3020–3023.
71. Ladner, M. B., Bottini, N., Valdes, A. M., and Noble, J. A. (2005) Association of the single-nucleotide polymorphism C1858T of the *PTPN22* gene with type 1 diabetes. *Hum. Immunol.* **66**, 60–64.

72. Kawasaki, E., Hutton, J. C., and Eisenbarth, G. S (1996) Molecular cloning and characterization of the human transmembrane PTP homologue, phogrin, an autoantigen of type I diabetes. *Biochem. Biophys. Res. Commun.* **227**, 440–447.
73. Begovich, A. B., Carlton, V. E., Honigberg, L. A., et al. (2004) A missense single-nucleotide polymorphism in a gene encoding a PTP (*PTPN22*) is associated with rheumatoid arthritis. *Am. J. Hum. Genet.* **75**, 330–337.
74. Kyogoku, C., Langefeld, C. D., Ortmann, W. A., et al. (2004) Genetic association of the R620W polymorphism of PTP *PTPN22* with human SLE. *Am. J. Hum. Genet.* **75**, 504–507.
75. Velaga, M. R., Wilson, V., Jennings, C. E., et al. (2004) The codon 620 tryptophan allele of the lymphoid tyrosine phosphatase (LYP) gene is a major determinant of Graves' disease. *J. Clin. Endocrinol. Metab.* **89**, 5862–5865.
76. Mena-Duran, A. V., Togo, S. H., Bazhenova, L., et al. (2005) SHP1 expression in bone marrow biopsies of myelodysplastic syndrome patients: a new prognostic marker. *Br. J. Haematol.*, **129**, 791–794.
77. Fonatsch, C., Haase, D., Freund, M., Bartels, H., and Tesch, H. (1991) Partial trisomy 1q. A nonrandom primary chromosomal abnormality in myelodysplastic syndromes? *Cancer Genet. Cytogenet.* **56**, 243–253.
78. Zanke, B., Squire, J. Griesser, H. et al. (1994) A hematopoietic PTP (HePTP) gene that is amplified and overexpressed in myeloid malignancies maps to chromosome 1q32.1. *Leukemia* **8**, 236–244.
79. Schweiger, S. and Schneider, R. (2003) The MID1/PP2A complex: a key to the pathogenesis of Opitz BBB/G syndrome. *Bioessays* **25**, 356–366.
80. Ito, A., Koma, Y.-I., and Watabe, K. (2003) A mutation in protein phosphatase type 2A as a cause of melanoma progression. *Histol. Histopathol.* **18**, 1313–1319.
81. Chen, W., Possemato, R., Campbell, K. T., Plattner, C. A., Pallas, D. C., and Hahn, W. C. (2004) Identification of specific PP2A complexes involved in human cell transformation. *Cancer Cell* **5**, 127–136.
82. Tautz, L., Bruckner, S., Sareth, S., et al. (2005) Inhibition of *Yersinia* tyrosine phosphatase by furanyl salicylate compounds. *J. Biol. Chem.* **280**, 9400–9408.
83. Liang, F., Huang, Z., Lee, S.-Y., et al. (2003) Aurintricarboxylic acid blocks both *in vitro* and *in vivo* activity of YopH, an essential virulent factor from *Yersinia* that cause the plague. *J. Biol. Chem.* **278**, 41,734–41,741.
84. Lazo, J. S. and Wipf, P. (2003) Small molecule regulation of phosphatase-dependent cell signaling pathways. *Oncol. Res.* **13**, 347–352.
85. Ducruet, A. P., Vogt, A., Wipf, P., and Lazo, J. (2005) Dual specificity protein phosphatases: therapeutic targets for cancer and Alzheimer's disease. *Annu. Rev. Pharmacol. Toxicol.* **45**, 725–750.
86. Pellecchia, M., Becattini, B., Crowell, K. J., Fattorusso, R., Forino, M., Fragai, M., Jung, D., Mustelin, T., and Tautz, L. (2004) NMR-based techniques in the hit identification and optimization process. *Expert Opin. Ther. Targets* **8**, 597–611.
87. Umezawa, K., Kawakami, M., and Watanabe, T. (2003) Molecular design and biological activities of protein-tyrosine phosphatase inhibitors. *Pharmacol. Ther.* **99**, 15–24.

88. Li, X., Bhandari, A., Holmes, C. P., and Szardenings, A. K. (2004) Alpha,alpha-difluoro-beta-ketophosphonates as potent inhibitors of protein tyrosine phosphatase 1B. *Bioorg. Med. Chem. Lett.* **14**, 4301–4306.
89. Bialy, L. and Waldmann, H. (2005) Inhibitors of protein tyrosine phosphatases: next-generation drugs? *Angew. Chem. Int. Ed. Engl.*, **44**, 3814–3839.
90. Pei, Z., Liu, G., Lubben, T. H., and Szczepankiewicz, B. G. (2004) Inhibition of protein tyrosine phosphatase 1B as a potential treatment of diabetes and obesity. *Curr. Pharm. Des.* **10**, 3481–3504.
91. Black, E., Breed, J., Breeze, A.L., et al. (2005) Structure-based design of protein tyrosine phosphatase-1B inhibitors. *Bioorg. Med. Chem. Lett.* **15**, 2503–2507.
92. Kumar, S., Zhou, B., Liang, F., Wang, W. Q., Huang, Z., and Zhang, Z.-Y. (2004) Activity-based probes for protein tyrosine phosphatases. *Proc. Natl. Acad. Sci. USA* **101**, 7943–7948.



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