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PROTEIN TYROSINE PHOSPHATASES

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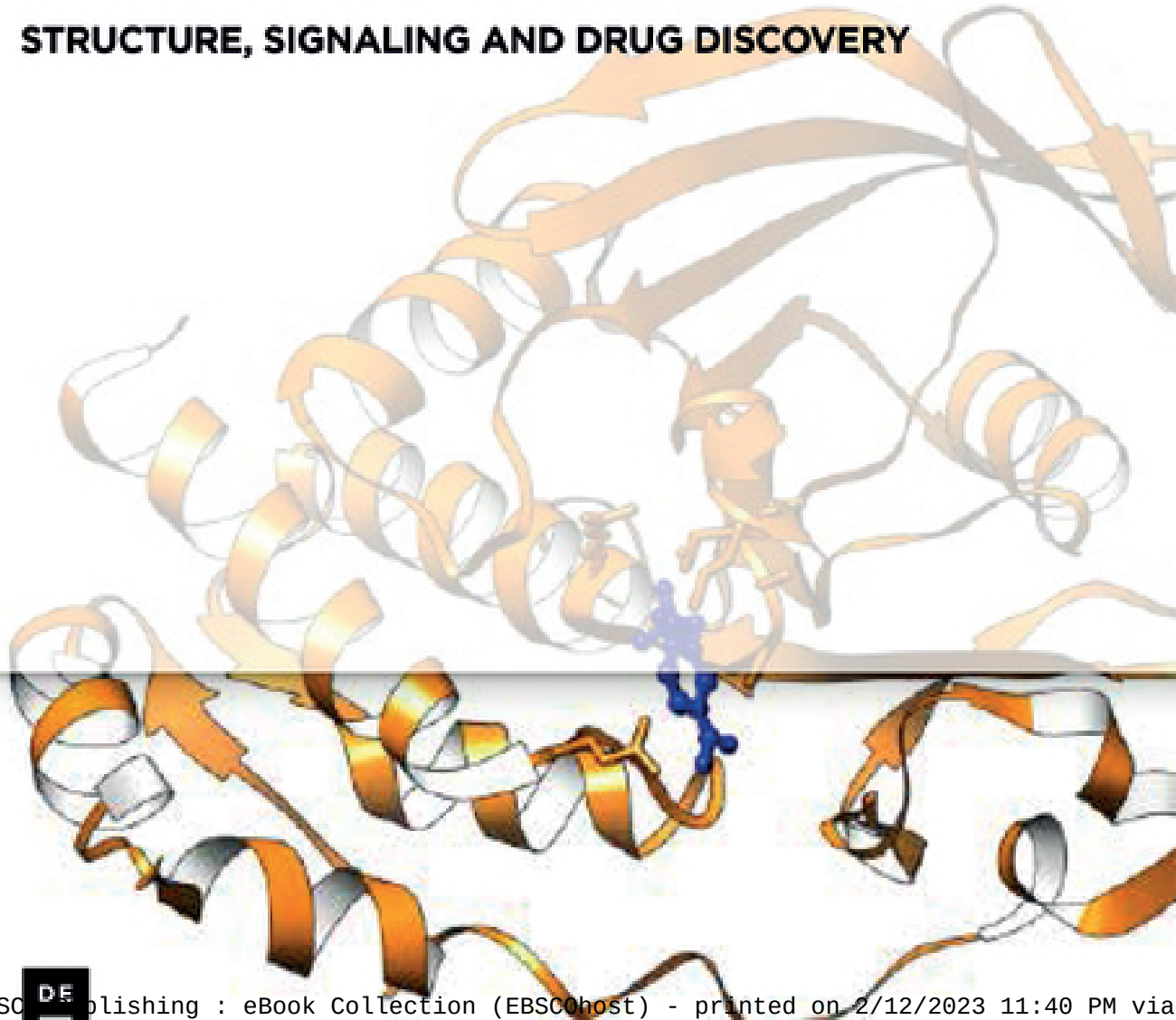

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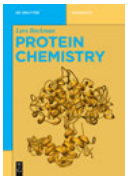

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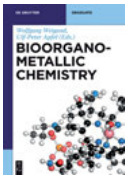

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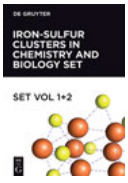

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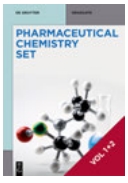

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Protein Tyrosine Phosphatases

Structure, Signaling and Drug Discovery

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Preface

Reversible protein phosphorylation-based signaling forms the basis of various physiological functions. Reversibility of phosphorylation is dependent on the opposing yet balancing actions of protein kinases (that phosphorylate proteins) and protein phosphatases (that dephosphorylate these target proteins). While the protein kinases are reported to be derivatives of a common ancestor, the protein phosphatases have evolved from distinct mechanistic ancestors that were also structurally discrete. This book describes protein phosphatases in cellular signaling, and the role of their structural underpinnings in their catalytic cycle. The book has been divided into eight chapters that allow the reader to systematically plough through the structural and mechanistic basis of protein phosphatase function. Special emphasis has been given to the largest protein phosphatase gene family: the protein tyrosine phosphatases. This gene family has evolved from a common ancestor and shares a conserved catalytic domain and mechanism. Ten conserved motifs in the protein structure allow for defining the molecular details of its domain's biochemical function. Understanding of their molecular regulation is key to dissecting their role in various pathophysiologies.

The first chapter of this book focuses on the discovery of tyrosine phosphorylation and its immense impact on the understanding of cellular signaling. Noteworthy are the earliest experiments that un-covered protein phosphorylation, but failed to make a mark in understanding human disease. The road to exploration of protein phosphorylation-based signaling saw both planned and some serendipitous discoveries that have allowed researchers to study cell signaling in its present form today. This chapter also describes the structure and mechanistic role of protein tyrosine kinases using Src as the model system. The crucial role of phosphotyrosine-recognition domains including SH2, PTB and atypical phosphotyrosine-recognition domains has been described with regard to their structure.

The second chapter of this book illustrates the diversity of the protein phosphatases. Their structural distinctness allows for their categorization into seven groups. The protein phosphoserine/phosphothreonine phosphatases make three groups, namely the phosphoprotein phosphatases, the metal-dependent protein phosphatases and the aspartate-based phosphatases. The other four groups are the evolutionary distinct classes of protein tyrosine phosphatases. These include the cysteine-based class I, II and III protein tyrosine phosphatases and the aspartate containing, haloacid dehalogenase related, class IV protein tyrosine phosphatases. This chapter describes the structural and mechanistic features of each group of protein phosphatases (except class I protein tyrosine phosphatases that are covered in the following chapters). Conserved structural features, biochemical mechanism and biological function of each group and its example member has been illustrated.

The third chapter focuses on the molecular details of the conserved class I protein tyrosine phosphatase catalytic domain. The catalytic domain is an enzymatic machine

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of about 280 amino acids that has a central β-sheet that accommodates the catalytic cysteine. Using a two-step mechanism requiring the concerted action of a conserved acid/base aspartate with the nucleophilic cysteine, the protein tyrosine phosphatase catalytic domain transfers the phosphatase of a phosphotyrosine residue onto a water molecule. Conserved glutamines are required to activate these water molecules and a conserved aromatic amino acid defines the access of the phosphotyrosine substrate to this molecular machinery. This chapter details the key structural and mechanistic elements of the catalytic domain, and how they participate in the culmination of its catalytic cycle. Emphasis has been given to the structural details of biochemical evidences of the catalytic mechanism. The later part of the chapter also describes the differences in the catalytic mechanism of the cysteine-based class II and class III protein tyrosine phosphatases. The unique class IV aspartate-based protein tyrosine phosphatase catalytic domain and its catalytic mechanism is also explained.

The fourth chapter of this book is dedicated to the receptor protein tyrosine phosphatases. Eight types of receptor forms of protein tyrosine phosphatases have been described for their biological role and distinct features that allow for their classification. The extracellular domains of these proteins allow for their interaction with varied extracellular matrix components and modulate their role in cellular signaling. Each type of the receptor protein tyrosine phosphatase subgroup has been described and their most prominent members have been highlighted. The chapter provides a comprehensive insight into the varied roles of related receptor protein tyrosine phosphatases and also illustrates the role of mutations and polymorphisms in these proteins in human disease.

The fifth chapter of this book is about a special subset of receptor protein tyrosine phosphatases that harbor two catalytic domains in their cytosolic portions. Interestingly, phosphotyrosine phosphatase activity is almost always limited to the membrane proximal domains, whereas the membrane distal domains are inactive and serve as pseudophosphatases. This chapter explains the unique molecular evolution and role of these silent pseudophosphatase domains in regulating the role of their active, cognate catalytic domains in the bidomain receptor protein tyrosine phosphatases.

The sixth chapter focuses on PTP1B and TCPTP; members of the NT1 subclass of non-receptor protein tyrosine phosphatases. The nine subtypes of non-receptor protein tyrosine phosphatases are the cytosolic counterparts to the receptor forms of these enzymes that operate at the membrane. PTP1B is perhaps the most exhaustively studied protein tyrosine phosphatase and was also the first phosphotyrosine phosphatase to be discovered. The chapter focusses on the role of PTP1B in cancer and its overreaching impact as a therapeutic target. Molecular mechanism of substrate identification, role of a second aryl-binding pocket and sensitivity to oxidation of the active site of PTP1B have also been explained.

The seventh chapter is a continuation on detailing the non-receptor protein tyrosine phosphatases and explains the biological roles and molecular modes of the

remaining subtypes. Each subtype has been explained for its reported mechanism, structure and biological function. Noteworthy is the understanding of specific features in the distinct catalytic domains of these subtypes that allow for their discretion. Emphasis has been laid on explaining the biological roles of these subtypes and how their sequence variation contributes to their molecular function.

The last chapter of this book is about various strategies of drug development that target the protein tyrosine phosphatases. The chapter documents the various natural and synthetic compounds that serve as effective inhibitors of these proteins. Binding modes and structural esthetics of reversible, irreversible, bidentate and allosteric inhibitors have been explained. The chapter ends with explaining biologics-based methods for therapeutic targeting of protein tyrosine phosphatases.

At present, this book explains the molecular details and structural elements that are key to the biochemical and biological function of protein tyrosine phosphatases. Emphasis has been given to providing a graphical illustration of these modes using the most relevant and recent protein structures from the protein data bank. In the present form of the book, research on protein dynamics and dynamics-based allostery of protein tyrosine phosphatases has not been covered. These concepts would provide the next level of understanding of these proteins and shall be included in future versions of this book.

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June, 2018
San Diego, United States

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Dr. Lalima G. Ahuja (formerly Lalima L. Madan) is a Post-doctoral scientific research fellow at the University of California San Diego, School of Medicine in San Diego, California, USA. Her doctoral work included the extensive biophysical, biochemical and structural characterization of the catalytic domains of Protein Tyrosine Phosphatases. She has obtained her doctoral degree in molecular biophysics from the Indian Institute of Science, Bangalore, India under the joint M.S.-Ph.D. program in Biological Sciences. Her undergraduate degree is in Biochemistry from the University of Delhi, New Delhi, India. She is the author of numerous research publications in the fields of protein chemistry, structural biology and enzymology. She has presented her work at various international platforms including the Salk Institute of Biological Sciences, Gordon Research Conferences – USA, Biophysical Society meetings, American Society of Chemistry meetings, American Society for Biochemistry and Molecular Biology meetings and meetings of the Protein Society.

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Contents

1 Tyrosine phosphorylation in cell signaling: Discovery and beyond

1.1 Protein phosphorylation

Protein phosphorylation serves as the currency of cellular signaling and allows for protein functional regulation and spatial control. It is shown to be a key biological process in both prokaryotes and eukaryotes [1–4], and forms the basis of signaling pathways as we understand today. Protein phosphorylation is a covalent modification wherein a phosphate (PO_4^{3-}) group is chemically attached to certain residues in target proteins. The negative charge of the phosphate allows for an alteration in the conformation of the said protein, thus allowing for a modulation of its function. The protein's conformation change depends on its structural context and directly affects its activation/in-activation, protein–protein interaction with other cognate partners and also its own recycling in the cell. Many cellular receptors, adaptors, enzymes, transcription factors, DNA-binding modules and also cytoskeletal proteins are regulated for their spatiotemporal responses using a simple phosphorylation–dephosphorylation switch. As much 30% of all proteins in cells are speculated to be phosphorylated at any given time [5], and alterations in these phosphorylation states of these proteins are being increasingly linked to diseases and pathophysiology [6].

Protein phosphorylation predominantly occurs on the serine (Ser), threonine (Thr) and tyrosine (Tyr) amino acid side chains of proteins that form acid-stable phosphomonoesters using their hydroxyl side chains (Figure 1.1). Histidine (His), arginine (Arg) and lysine (Lys) residues use their basic side chains to form acid-labile phosphoramidates. Acidic residues, namely, aspartate (Asp) and glutamate (Glu) make acyl-phosphates. Cysteine (Cys) residues are phosphorylated on their sulfhydryl side chains to form thiophosphates. Multisite protein phosphorylation on serine/threonine and tyrosine residues is a key feature of many eukaryotic signaling processes [7]. Histidine and aspartate phosphorylations are a hallmark of the two-component system and multicomponent signaling systems that connect extracellular stimuli of osmolarity and nutrients to gene regulation in both bacteria and plants [8, 9]. Histidine phosphorylation is known to be crucial in mammalian gene regulation with specific relevance in cardiovascular physiology and heart diseases [10, 11]. Phosphoproteome analysis of the mammalian heart mitochondria has identified histidine and cysteine phosphorylations on pyruvate dehydrogenase and sarcomeric mitochondrial creatine kinase [12]. Histidine and cysteine phosphorylation are also shown to be important for the regulation of phosphoenolpyruvate-dependent carbohydrate transport system in prokaryotes [13]. As the phosphoramidates of arginine and lysine are difficult to detect, their significance is presently understudied. These nitrogen phosphorylations have been detected in histone proteins [14], but their biological role is still uncertain.

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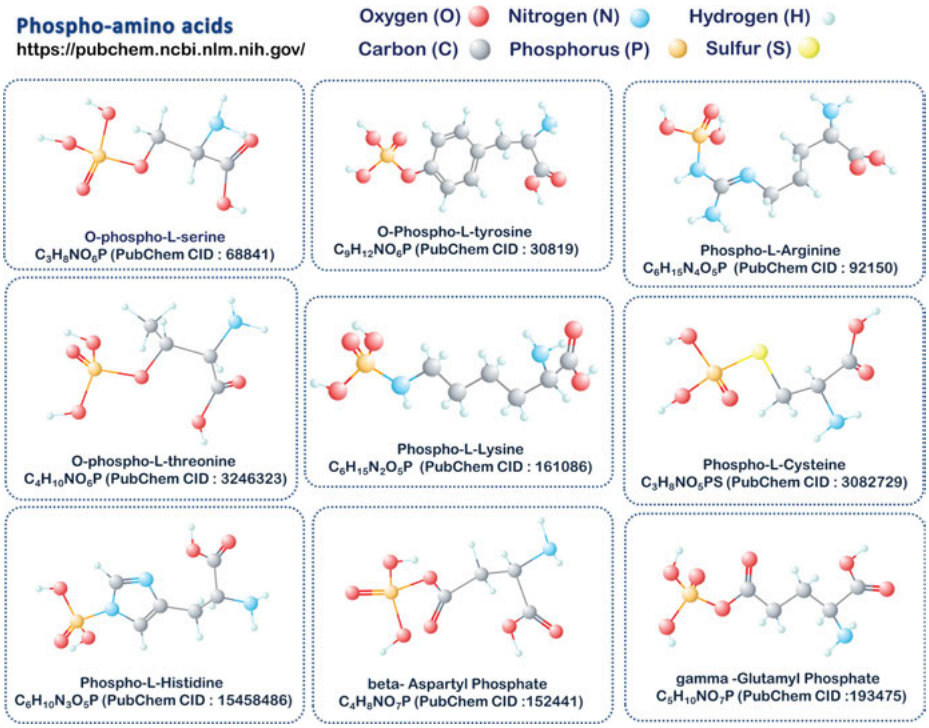

Figure 1.1: Molecular structure of phosphoamino acids.

The protein phosphorylation realm is composed of four components (Figure 1.2). The first component comprises of protein kinases (also called the eukaryotic protein kinases [EPKs]) that transfer the terminal γ-PO_4^- from the high-energy molecule adenosine 5′ tri-phosphate (ATP) onto the target proteins [15]. This superfamily of enzymes constitutes only ~2% of the human genome, but controls all of protein phosphorylation-based signal transduction. On the basis of their biochemical features, the EPKs are classified into seven groups. These are the serine/threonine-specific AGC kinases; the calcium (Ca^{2+})/calmodulin-dependent CaM kinases; CDK, MAPK, GSK3 and CLK-containing CMGC kinases; tyrosine kinases (TKs); tyrosine kinase-like kinases (TLK); STE kinases that are homologues of yeast sterile kinases and casein kinases. All EPKs have a conserved kinetic core that is mechanically equipped to carry out the phosphotransfer process on their target proteins. These target proteins, also called as the "substrate pool" or simply substrate proteins, form the second component of phosphorylation-based signaling realm.

These substrate proteins include, but are not limited to, cytoplasmic receptor tails, transcription factors, transcriptional regulators, ion channels, adaptor proteins,

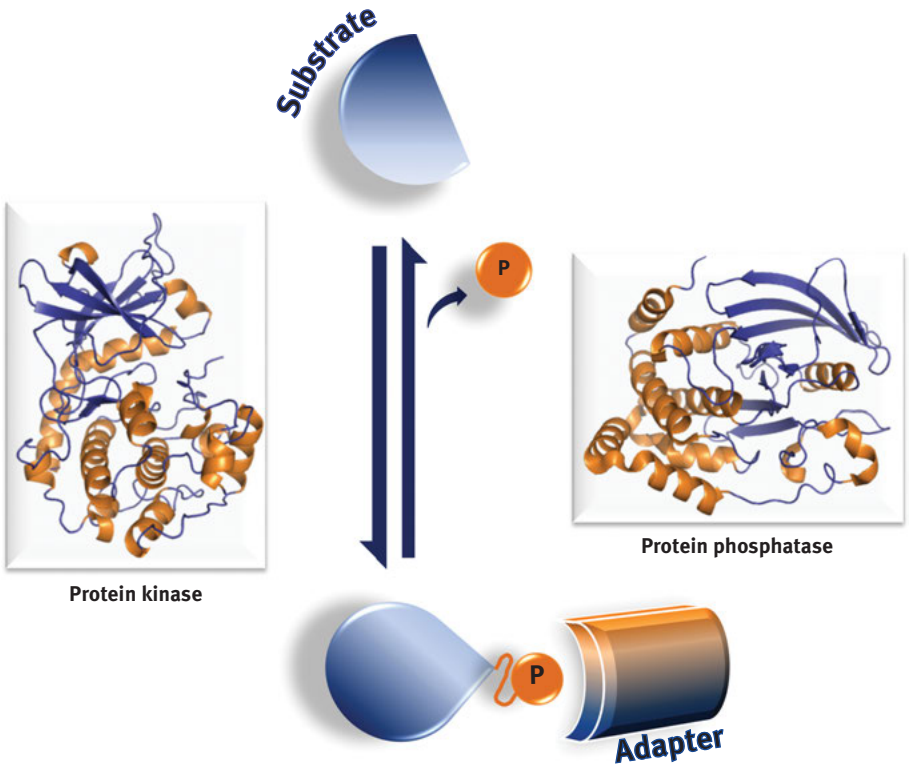

Figure 1.2: The realm of reversible protein phosphorylation.

cytoskeletal proteins and also protein kinases themselves [6]. Autophosphorylation of protein kinases forms an exquisite mode of self-regulation, wherein phosphorylation of cognate protein kinases gives rise to signaling cascades that regulate multiple cellular processes (Figure 1.3) [16, 17]. The third competent of the protein phosphorylation realm is composed of adaptor proteins that contain domains that recognize and bind to phosphorylated substrate proteins, and form the links between various cellular processes. These specialized domains recognize only specific phosphorylated sequences on proteins to elicit an exact response. Phosphoserine, phosphothreonine and phosphotyrosine-specific binding domains are found in various subcellular proteins and have been keenly studied [18]. The fourth component of the phosphorylation-based signaling is the enzymes that reverse protein phosphorylation and hence shut down signaling processes. These enzymes are called protein phosphatases, and depending on the residues that they hydrolyze, these are classified into phosphoserine/phosphothreonine phosphatases, dual-specificity phosphatases, protein tyrosine phosphatases and protein histidine phosphatases [19–22].

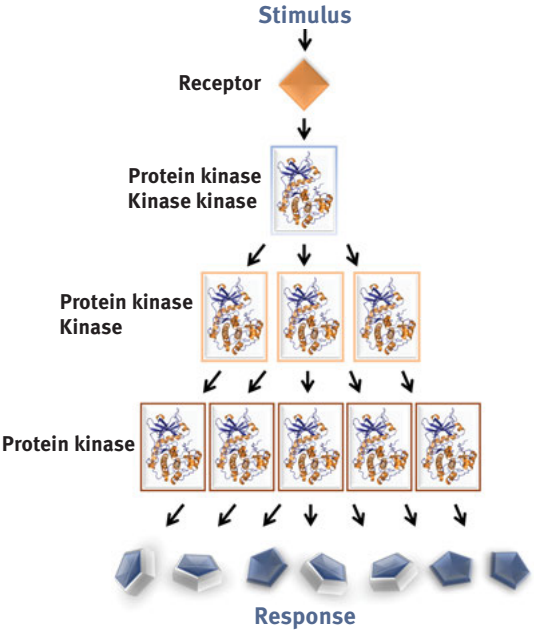

Figure 1.3: Signaling cascades as regulated by protein kinases.

1.2 Origins and early work

The presence of protein phosphorylation was first detected in the egg yolk protein, vitellin, by P.A. Levene and C.L. Alsberg from Rockefeller Institute of Medical Research at New York, United States, as early as in 1906 [23]. It took another 26 years to elucidate that this phosphorylation of vitellin was a phosphoserine [24]. Protein phosphorylation was mainly associated with silent functions, wherein the negative charge provided by the phosphate group was thought to keep proteins like vitellin and casein (milk proteins) evenly dispersed in work media. In 1930, the proteolytic enzyme pepsin was reported to contain stoichiometric amounts of phosphate per gram of protein [25]. At this time, protein phosphorylation was thought as a metabolic reaction, and it was not until 1954 that it was established to be a reversible enzymatic process [26]. In 1940s, Carl and Gerti Cori, while working on glycogen metabolism, showed that the enzyme glycogen phosphorylase could exist in phosphorylated and unphophorylated forms [27]. They also reported an *in vitro* interconversion between these two forms, which they called *phosphorylase a* and *phosphorylase b* [28]. As no *in vivo* evidence or significance of this reaction was elucidated by them, this first interconversion reversible reaction went unappreciated. In the 1950s, Earl Sutherland and Thomas Rall, at Case Western Reserve University, were working on glycogen phosphorylase in the liver. They found that both epinephrine and insulin elicited a common response by increasing the amounts of 3′,5′-cyclic-adenosine monophosphate (cAMP) in liver cells. This indicated that cAMP was a "second messenger," a mediator of cellular response

that relayed the action of external hormones into the insides of the cell [29]. At the same time, Edwin Krebs and Edmond Fischer focused on the interconversion of *phosphorylase a* and *phosphorylase b* in muscle extracts. They found that the energy molecule ATP was required for the conversion of inactive *phosphorylase b* into the active form *phosphorylase a* [30]. In addition, phosphorylation of p*hosphorylase b* occurred on a specific serine residue, and required a divalent metal cofactor for the process. The enzyme that catalyzed the conversion of *phosphorylase b* into *phosphorylase a* was called phosphorylase kinase, whereas the enzyme catalyzing the reverse reaction was named phosphorylase phosphatase. Consequently, the work of Krebs and Sutherland converged into the identification of phosphorylase kinase as a substrate for a cAMP-dependent protein kinase A (named as PKA). In 1968, D. Walsh purified PKA from rabbit skeletal muscle [31], and in the next few years it was established that PKA was the same as the glycogen synthase kinase discovered in 1963 by Joseph Larner and Daniel Friedman [32]. The implications of these studies were multifold. These studies elucidated a novel system of cellular communication that linked hormone action to protein phosphorylation. The widespread abundance of PKA in mammalian tissues prompted J.F. Kuo and Paul Greengard to postulate the unifying theory of cAMP-mediated cell signaling by protein kinases [33]. This forms the basis of cellular signaling as we understand today. The realization of multiple substrates for PKA hinted toward the idea of protein kinase cascades that regulated protein phosphorylation. Multisite protein phosphorylation as suggested by Philip Cohen [34] was later known to be the norm rather than the exception. Finally, combined with the discovery and studies on G-protein heterotrimeric receptors by Alfred Gilman, Martin Rodbell, and Robert Lefkowitz [35], these studies formed the outlines of canonical signal transduction pathways that use protein phosphorylation as a core post-translational modification mechanism for cellular control.

1.3 Tyrosine phosphorylation: Discovery and beginnings

While the role of serine and threonine phosphorylation was well established in 1950s and 1960s, tyrosine phosphorylation remained undiscovered till late 1970s. Phosphotyrosine was rather serendipitously discovered in 1979 via the study on tumor viruses, namely, polyomavirus T antigen, the Rous sarcoma Src protein, and the Abelson leukemia virus Abl protein [36–38]. In a detailed commentary in the journal *Cell* [39], Tony Hunter and Walter Eckhart explain the role of aged running buffer for cellulose thin layer chromatography as being critical in the rather sudden discovery of phosphotyrosine from the polyomavirus middle T antigen. A subtle drop in the pH of the buffer from 1.9 to 1.7 units allowed for a differential migration of the phosphotyrosine in between phosphoserine and phosphothreonine markers (Figure 1.4). As phosphotyrosine was unreported at that time, Tony Hunter devised a two-dimensional separation system for the phosphoamino acids, even synthesizing

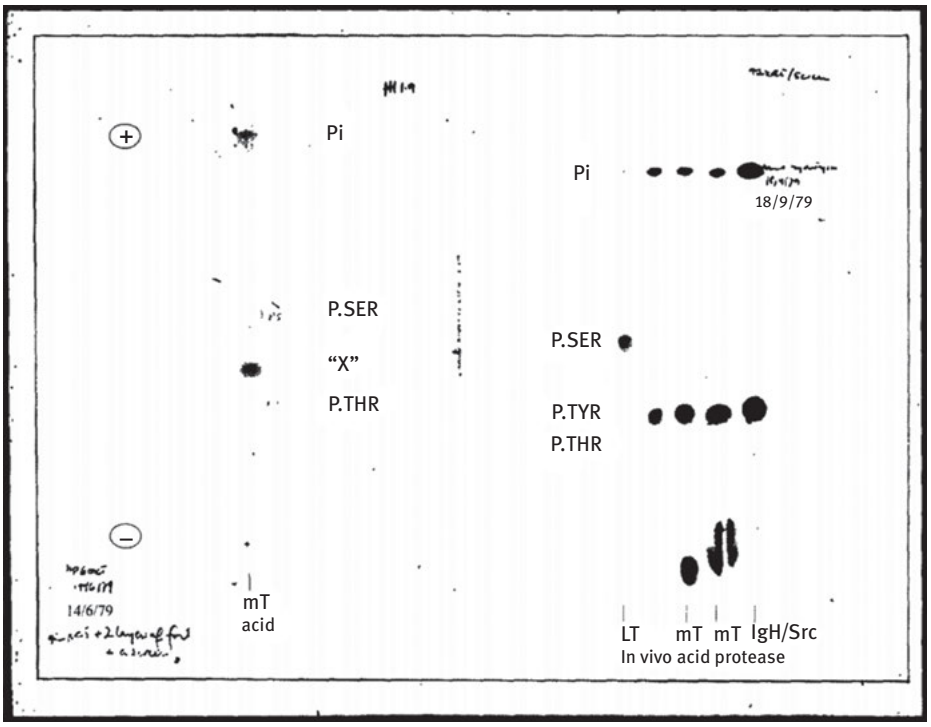

Figure 1.4: Historic moment leading to the discovery of phosphotyrosine. Adapted from Ref. [39].

his own phosphotyrosine marker by mixing tyrosine and $POCl_3$. Subsequently, Tony Hunter and Bart Sefton reported their findings on v-Src and c-Src tyrosine phosphorylation both *in vitro* and *in vivo* [37]. In 1980, Marc Collett, Tony Purchio and Ray Erikson purified v-Src and demonstrated its ability as a protein tyrosine kinase (PTK) for a diverse variety of substrate proteins [38, 40]. However, specialized techniques were now used to distinguish phosphotyrosine from phosphothreonine that migrated closely on cellulose chromatograms. This prompted many researchers to revisit the previously reported phosphothreonine kinases. One such protein was the epidermal growth factor receptor (EGFR) that was reported to be protein threonine kinase in 1978 by Stanley Cohen and coworkers [41]. The authors went back on the assays and indeed discovered EGFR to be phosphorylated at a tyrosine residue. This study [42], while accepting the scientific process of revalidation, also provided a vital link between growth factor signaling and retroviral oncoprotein-based signaling. The string of events that followed these studies laid the steps for the discovery of the first protein tyrosine phosphatase.

As the phosphotyrosine abundance in cells is much less than phosphoserine and phosphothreonine (~0.05% for phosphotyrosine compared to 90% phosphoserine and 10% phosphothreonine), phosphotyrosine remained undetected from whole

cells extracts for a long time. In 1980, temperature-sensitive mutants of Src were studied for the analysis of phosphotyrosine-based signaling in transformed cells. Levels of phosphotyrosine were seen to increase at permissive temperatures, but rapidly decreased to basal state with a shift to nonpermissive temperatures [43]. This hinted toward the presence of protein tyrosine phosphatases (PTPs) that must operate alongside the PTKs to maintain basal phosphotyrosine in the homeostatic cell. Initial efforts to identify PTPs focused on studying the then-known phosphoserine and phosphothreonine phosphatases for their ability to hydrolyze a phosphotyrosine. Protein phosphatase PP1 was initially speculated to be the first PTP, but was eventually shown to be devoid of any phosphotyrosine hydrolase activity [44]. However, PP2A and PP2C were shown to harbor PTP activity under nonphysiological Mg^{2+} levels and high pH [45]. PP2B/calcineurin was shown to hydrolyze the phosphotyrosine of the EGF receptor at similar kinetics for phosphoserine/phosphothreonine hydrolysis [46]. Multiple cell and tissue fractions were purified by diverse groups to have varying levels of PTP activity [47–50]. Interestingly, PTP biochemistry including its inhibition by Vanadate [51] and Zn^{2+} [52] were discovered much before the first PTP itself. PTP dependence on reducing agents for proper catalysis was also realized through these preliminary studies [53, 54].

The discovery of the first PTP was laden with various problems, mostly owing to the lack of tissue homogenates for their proper identification [55]. While enriched tissue fractions were available for analysis, PTP activity was mostly only monitored by the cleavage of the small molecule *para*-Nitrophenyl Phosphate (*p*NPP). As recombinant DNA technology was not available at that time, it was difficult to produce the known substrates from hormone receptors to identify PTPs with certainty. Researchers tried to use tyrosine-phosphorylated samples of Bovine Serum Albumin (BSA) and phosphorylase as more generous substrates [48, 52]. However, the poor solubility of highly charged proteins, nonspecific phosphorylations, multiple tyrosine phosphorylations and nonstoichiometric labeling were major challenges for assay conditions. In 1986, Ronald Kohanski and Daniel Lane used chemically modified lysozyme as a potent substrate of the insulin receptor kinase. They then used these tyrosine-phosphorylated chemically modified lysozyme fractions as a direct substrate to identify PTPs [56]. This indirect, but methodical, approach cleared a vital bottle-neck in the identification of cellular PTPs. In the late 1980s, Nicholas Tonks and coworkers started to focus on the human placenta a source for PTPs based on literature reports highlighting high PTK activity in them [57]. Indeed the first PTP was purified from the human placenta in 1988 [58]. The anion-exchange method used for the purification yielded two major peaks in the salt gradient. The first fraction at a low salt of 50–70mM NaCl was named PTP1A and the second peak eluting a higher salt of 100 mM NaCl was named PTP1B. Thio-tyrosine-phosphorylated EGF and insulin receptor (IR) fragments were used to immobilize and enrich the DEAE fractions eluted with high salt to obtain monomeric homogenous and 20,000 times purer fractions of PTP1A and PTP1B [58]. Both PTP1B and PTP1A were biochemically

distinguished from phosphoserine/phosphothreonine phosphatases by chemical assays and peptide mapping. Unlike phosphoserine/phosphothreonine phosphatases, PTP1A and PTP1B were inhibited by vanadate, molybdate and also Zn^{2+}. These were unaffected by NaF and thermostable inhibitors of protein phosphatase1 (PP1). In the subsequent year, PTP1B was sequenced by Edman degradation and a new superfamily of enzymes was detailed for the research community [59]. This book focuses specially on the structure, function, regulation and biological implications of this PTP superfamily of proteins.

1.4 Tyrosine phosphorylation: Current significance

An enormous body of work is now available for understanding the role of tyrosine phosphorylation in cellular signaling. The major milestones in this work are tabulated in Table 1.1. The emergence of the tyrosine phosphorylation is associated with cellular communication that evolved in metazoans. The simplest PTK genes have been identified for sponges [60], coelenterates [61] and also *Dictyostelium* [62]. The evolutionary

Table 1.1: Major milestones in phosphotyrosine-based signaling research.

Timeline	
1979	– Discovery of “phosphotyrosine” on polyoma virus middle T-antigen
1980	– v-Src and c-Src are PTKs
	– v-Abl is a PTK
	– EGFR is a protein tyrosine kinase
1981	– IR is a PTK
1982	– v-Src signaling is related to PKA signaling
	– Platelet-derived growth factor receptor (PDGFR) is a PTK
1983	– Polyoma virus middle T activates Src
1985	– Bcr-Abl fusion detected in chronic myelogenous leukemia
	– Src is regulated by tyrosine phosphorylation
1986	– Src is phosphorylated at Tyr527 (segment deleted in v-Src)
	– Trk human tumor oncogene encodes a PTK
1988	– PTP1B is purified and sequenced
	– CD45 lymphocyte receptor is related to PTP1B
1989	– Cdc2 is regulated by tyrosine phsophorylation
1990	– Yersinia plasmid encodes a PTP
	– SH2 domains bind phopshotyrosine
1991	– c-Src knockout causes osteoporosis
1992	– Structure of SH2 bound to phosphotyrosine containing peptide
1994	– Structure of PTP1B
1995	– Structure of IR PTK domain
1997	– Structure of c-Src in the inactive state
	– Structure of PTB domain

significance of the use of phosphotyrosine for signaling over phosphoserine/phosphothreonine is not immediately obvious. For a protein pool to be regulated by phosphorylation, there must be an optimal balance between the action of kinases and phosphatases to allow for distinct basal and excited states [63]. The intrinsic kinetic activity of phosphoserine/phosphothreonine kinases and phosphatases is inherently similar. Also the levels of phosphoserine/phosphothreonine kinases and phosphatases in cells are fairly same. This indicates that differential substrate affinities of kinases and phosphatases are being utilized for maintaining a balance in the basal phosphoserine/phosphothreonine levels in the cell. In contrast, the PTPs are over three order of magnitude faster than PTKs [64]. This explains the low levels of phosphotyrosine (~0.05% of the total phosphorylations) found in cellular extracts [65]. Phosphotyrosine signaling is hence both transient and specific and low basal levels of phosphotyrosine substrates are maintained in the signaling pool. This allows the system to be accurately inducible with maximal sensitivity and desired amplitude. Another aspect of phosphotyrosine signaling is the evolutionary emergence of phosphotyrosine recognition domains like SH2 and PTB domains that allow for signaling relays for efficient signal transfer. The higher binding energy for a bulkier phosphotyrosine (when compared to a phosphoserine/phosphothreonine) may have allowed for the creation of more complex networks of protein–protein interaction and cellular response.

1.5 The protein tyrosine kinases

The EPK family consists of ~518 genes [15], of which PTKs form 18% (~95 genes) and protein TLKs constitute 8% (~45 genes) of the total complement (Figure 1.5). PTKs can further be categorized into membrane-bound forms (receptor tyrosine kinases [RTKs]) and the cytoplasmic proteins TKs (non-receptor tyrosine kinases [NRTKs]). The human genome contains 58 RTKs that can be further divided into various subfamilies [66] (Figure 1.6). All of the RTKs share a general molecular architecture. This is made of three regions: extracellular domains that look to the outside of the cells for ligand binding, transmembrane regions that span the cell membrane and intracellular regions containing the catalytic kinase domain. Many of the hormone receptors like the IR, EGFR, fibroblast growth factor receptor, PDGFR, nerve growth factor receptor (NGF) and also the vascular endothelial growth factor receptor (VEGF) are all RTKs. The diverse array of their extracellular regions is composed of various globular domains that allow for ligand binding and also receptor dimerization. These domains include the immunoglobulin (Ig-like) domains, fibronectin type III-like domains, cysteine-rich domains and also cadherin and discoidin. The intracellular portion consists of a juxta-membrane region, the kinase catalytic domain and a C-terminal region. Substantial sequence diversity is seen in the juxta-membrane and the C-terminal regions. The PDGF family of RTKs has a ~100 residue insertion in-between its juxta-membrane region and the kinase catalytic

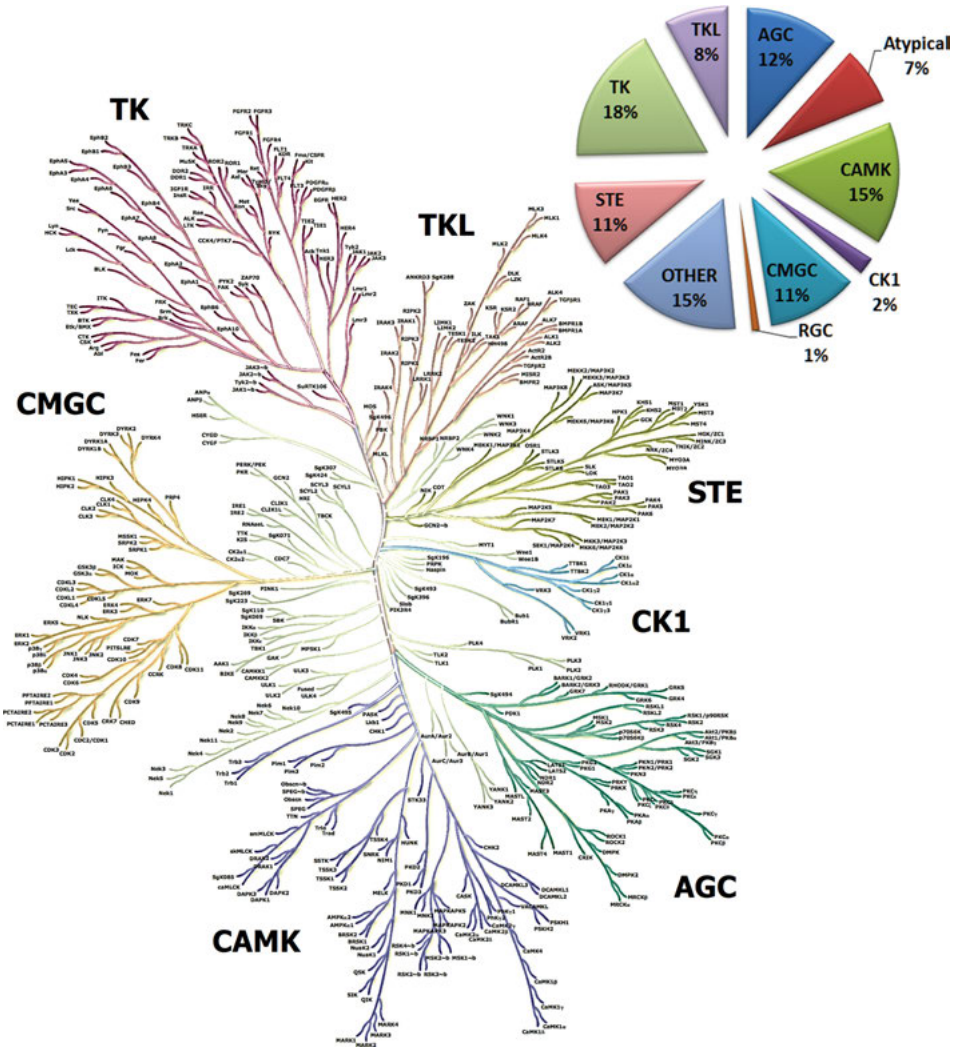

Figure 1.5: Protein kinase superfamily of proteins and their evolutionary relationships with each other. Kinase tree adapted from http://kinase.com/human/kinome/.

domain. The cytosolic NRTKs subfamily of PTKs consists of various oncogenes including Src kinase, Abl kinase and also the Janus kinases (Jaks). Many of the NRTKs contain accessory domains other than their kinase domains to assist in signal amplification or regulation by protein–protein, protein–lipid and also protein–DNA interactions (Figure 1.7). These domains include the Src homology domain (SH2) that itself recognizes and binds to a phosphotyrosine. The Abl kinase contains a nuclear localization signal that allows for its presence in both the cytosol and the nucleus. Abl kinase also contains F actin-binding and DNA-binding

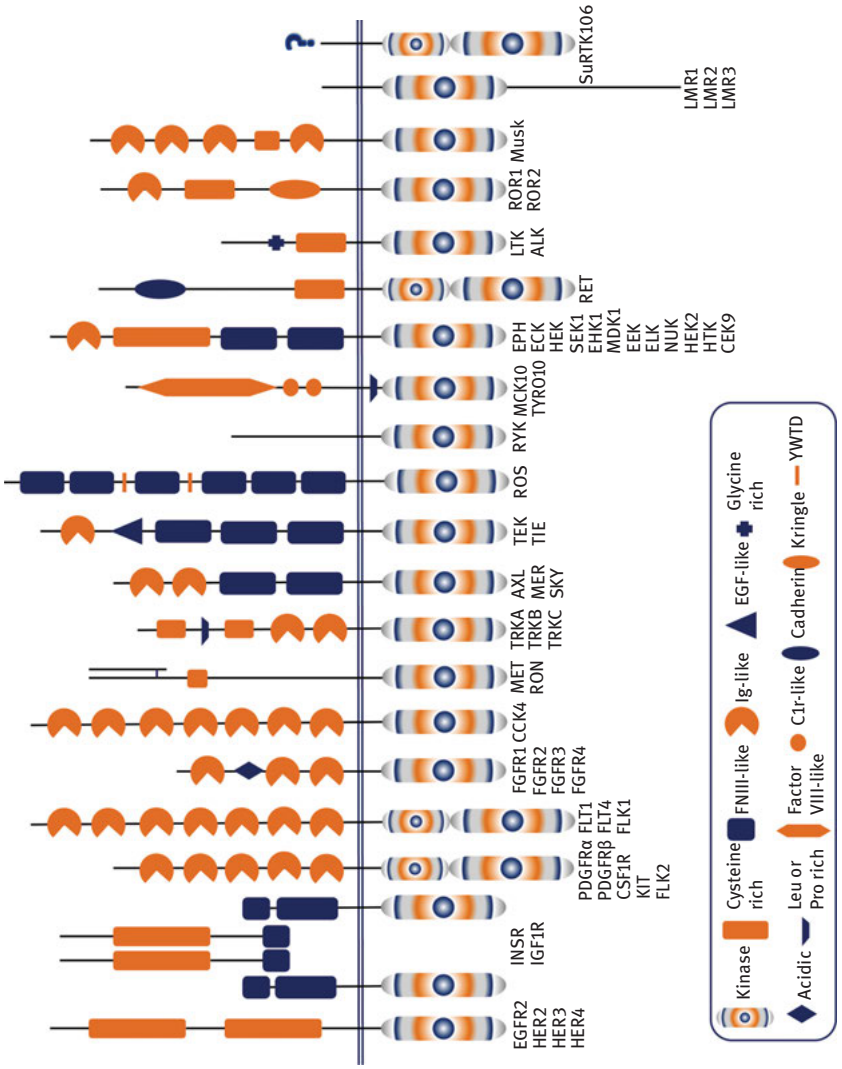

Figure 1.6: Classification of RTKs.

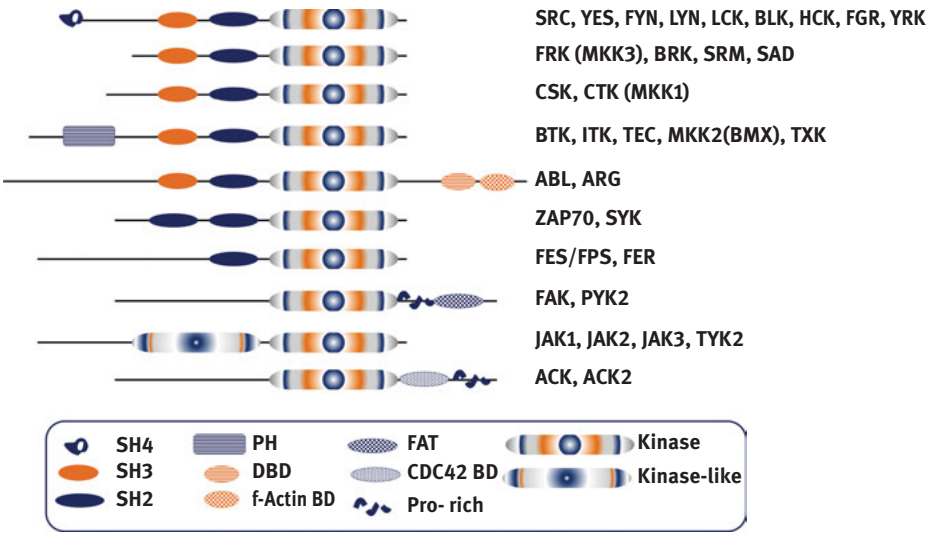

Figure 1.7: Classification of NRTKs.

domains. The JAKs have an N-terminal kinase-like domain that lacks any phosphotransfer activity. This domain occurs in tandem with a fully functional protein kinase domain.

1.5.1 The protein tyrosine kinase catalytic domain

Crystal structures of the catalytic domain of various RTKs and NRTKs are available in the Protein Data Bank (PDB). The overall structure is highly conserved and is a signature of the EPKs superfamily in general [67]. These catalytic domains have a bilobal structure with a small N-lobe and a larger C-lobe (Figure 1.8). The C-lobe is made primarily of α-helices and the N-lobe is made of five stranded β-sheet and a conserved αC helix. The active site is formed between the two lobes that bind both the nucleotide (ATP) and peptide/protein substrate. An activation loop connects the two lobes. A conserved Asp–Phe–Gly motif (DFG motif) is situated at the base of this activation loop. A conserved salt bridge (Lys72–Glu91 in PKA; Lys298–Glu313 in Src) is a signature of the protein kinase catalytic domain. A glycine-rich loop lies between the β1–β2 that contains a conserved hydrophobic residue (Phe/Tyr). This loop folds over the nucleotide ATP to close the catalytic domain during catalysis. The peptide substrate binds in an extended conformation spanning the active site left and the C-lobe. The extended conformation allows for the positioning of the target tyrosine to face the γ-PO_4 of ATP during catalysis. Catalysis requires a concerted action by key residues from a conserved loop called the catalytic loop

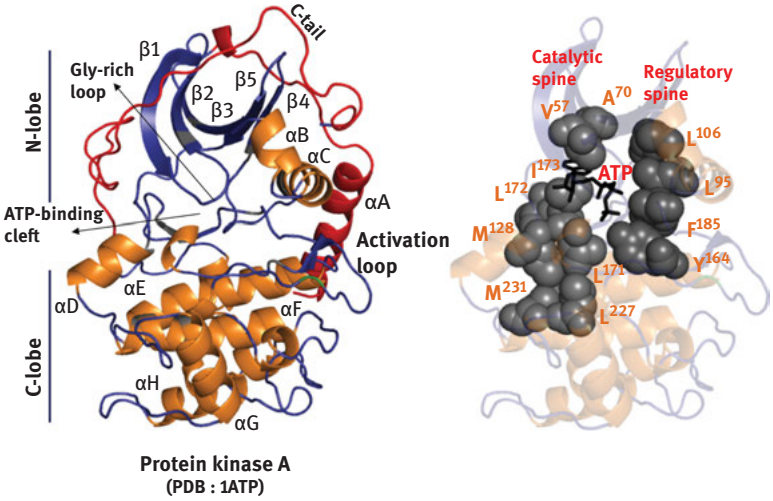

Figure 1.8: Structure of the conserved protein kinase domain. The hydrophobic core of the domain contains the regulatory and catalytic spines.

that forms the base of the active site. Conserved aspartate (Asp166 in PKA) engages with the hydroxyl group of the substrate peptide while a conserved asparagine (Asn171 in PKA) allows for its proper orientation. Another conserved aspartate (Asp184 in PKA) is responsible for binding the divalent metal ions. As a part of the conserved DFG motif, this asparagine's own optimal conformation is regulated by the conformation of the activation loop.

There are two important conformational transitions required for the kinase catalytic domain to function. The first transition requires the conversion of the inactive kinase domain to an active conformation that is catalytically competent. Activation of a kinase domain requires changes in the conserved αC helix, release of inhibitory domains and assembly of the inner hydrophobic skeletal network. Phosphorylation of the activation loop between the N- and C-lobes of the kinase domain forms an almost universal mechanism of PTK regulation [66]. For example, in the IR kinase domain, its activation loop must be phosphorylated at three tyrosines to be activated [68, 69]. In the inactive unphosphorylated state, the activation loop folds into the active site to occlude both nucleotide and peptide binding. Autophosphorylation of this activation loop allows for the opening of this active site and proper orientation of the DFG motif residues required for catalysis. Once the active conformation is achieved, the kinase is able to toggle between the "Open" and "Closed" states that are associated with the phosphotransfer process. The apo, activated enzyme is in the open conformation that binds the nucleotide-Mg^{2+} to form a catalytic intermediate. Binding of the peptide allows for the kinase to access the closed conformation that supports the transfer of the phosphate from the ATP to the tyrosine of the peptide. Once the products are formed,

the kinase releases the phosphorylated peptide/protein and the nucleotide ADP to cycle back to the open conformation.

The PDB contains various structures of protein kinases in their active and/or inactive forms. For example, Src has been crystalized and solved in both inactive (PDB ID: 2SRC) and active (PDB ID: 3DQW) conformations. Analysis of these various inactive versus active structures of protein kinases has allowed for the identification of a hydrophobic core skeleton in the kinase core by advanced computational methods [70]. Overall 18 amino acids at the core of the kinase domain form this hydrophobic skeleton (Figure 1.9). Kornev et al. used local spatial alignment tools to identify four nonconsecutive residues that aligned as a "spine" in only the active kinase structures. This spine was called the regulatory or the R-spine. The R-spine contains elements from the conserved αC helix and also the Phe from the conserved Asp–Phe–Gly motif. The R-spine interacts with an aspartate (Asp220 in PKA; Asp447 in Src) in the conserved αF helix at the kinase core. The R-spine contains two residues from the N-lobe (Leu106 in PKA; Leu325 in Src and Leu95 in PKA; Met314 in Src) and two residues form the C-lobe (Phe185 in PKA; Phe405 in Src and Tyr164 in PKA; His384 in Src). Another motif comprised of eight residues is called the catalytic or the C-spine. This has two residues from the N-lobe (Val281 and Ala293 in Src) and six residues from the C-lobe (Leu346, Ile392, Leu393, Val394, Leu451 and Leu455 in Src). The C-spine is completed by the binding of the nucleotide ATP in the active site cleft; hence, this spine is called the catalytic spine. The completion of the C-spine primes the kinase for catalysis. Three remaining residues of the hydrophobic skeleton are

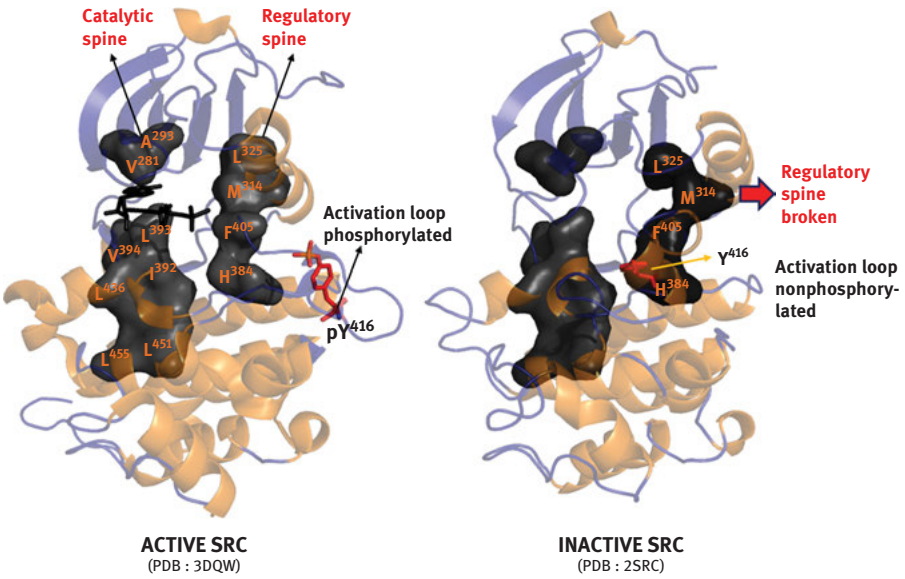

Figure 1.9: An assembled regulatory spine is a characteristic feature of an active kinase.

called the “shell” residues. Their importance in supporting the R-spine was established by site-directed mutagenesis and biochemical analysis [71]. One of these shell residues is also called the “gate keeper” (Met120 in PKA; Thr341 in Src) as it guards access to small inhibitors to a conserved pocket adjacent to the adenine binding pocket.

1.5.2 Protein tyrosine kinases: Src as a model

Protein kinase activity needs to be tightly regulated to prevent havoc in the cell by nonspecific or overphosphorylation of substrate proteins. While kinase-specific mechanisms exist for this regulation, related kinases are seen to have similar modes of modulation. For example, receptor dimerization is an important process for the RTKs including the IR kinase, PDGF receptor and also EGF receptors [69]. Dimerization of the extracellular domains and their association with ligands forms the requisite conformation for the autophosphorylation and activation of these RTKs. Sometimes, like in the case of Erb3 EGF receptor, higher order complexes may be required for downstream signaling [69]. PTPs not only counter the signal of these RTKs, but also help in inactivating these RTKs themselves by removing the phosphates from the tyrosine(s) of their activation loops [55]. Final downregulation of RTKs also occurs through various processes including endocytosis [72] and ubiquitination followed by proteolysis [73].

In NRTKs, various modulatory domains are found adjacent to their catalytic domains (Figure 1.7). Many of these domains not only function in downstream signaling, but also in the regulation of the NRTK activity itself. The domain organization and gene structure of Src family of kinases is shown in Figure 1.7. This family includes nine NTKs, including Src, Lck, Hck, Fyn, Blk, Lyn, Fgr, Yes and Yrk. All of these have a conserved domain organization that contains the kinase domain (SH1), SH2, SH3 and SH4 domains. The SH4 domains serve as a membrane targeting domain through its myristylation or palmitoylation [74, 75]. A unique sequence adjacent to the SH4 domain distinguishes the various family members. A unique feature of this family of kinases in the presence of a tyrosine phosphorylation site in their C-terminal that serves as an inhibitory site [76]. While activation of Src family of kinases requires the phosphorylation of their activation loop (Tyr 416 in Src), phosphorylation of the C-terminal tail tyrosine (Tyr 527) is a signature of the inactive state. In addition, the activation loop tyrosine is an autophosphorylation site, while the carboxyl-terminal tyrosine is a substrate site for another regulatory kinase. For Src this site is phosphorylated by Csk or its homologue Chk [77, 78]. Phosphorylation of this tyrosine causes the SH2 and SH3 domains of Src to fold over the kinase domain to lock it into an inactive conformation. In the resting state, Src is phosphorylated on its carboxyl-tail at Tyr 527. A deletion of this carboxyl-tail as seen in v-Src or its substitution to a phenylalanine causes it to be constitutively active [79, 80]. Both these mutant forms of Src are seen to be highly

phosphorylated on their activation loop Tyr 416, indicating a reciprocal relationship in their phosphorylation states.

1.5.3 Regulation of Src tyrosine kinase

Src activation is regulated by a conformational "latch, clamp and switch" model. In the inactive state, Src is phosphorylated at Tyr 527 such that its latches onto its own SH2 domain. In addition, the SH2-linker spans the SH3 domain as it folds over the kinase domain (Figure 1.10). Although the linker lacks the signature PxxP motif required for SH3 domain binding, the sequence adopts a left-handed polyproline type II helical conformation to fit into the SH3 domain. The SH2 and SH3 domains pack against in the kinase domain of Src at the opposite side of the catalytic cleft. The SH3 domains pack against the N-lobe, while the SH2 domain binds the C-lobe of the

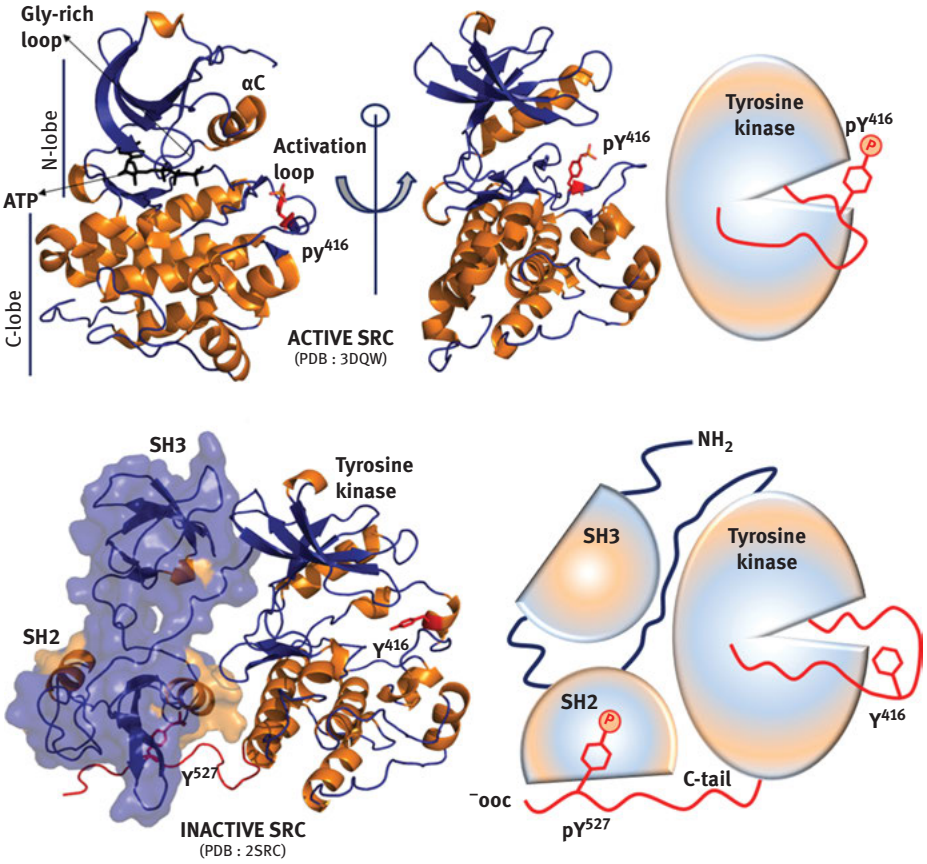

Figure 1.10: Activation and regulation of Src kinase.

kinase domain. This is the clamped inactive conformation. These interactions perturb the catalytic kinase domain so that the conserved lysine (Lys295) and the αC helix's glutamate (Glu310) are distorted into an inactive conformation. Also the activation loop adopts an α-helical conformation that occludes the autophosphorylation of Tyr 416 or the substrate protein.

Unlatching of the SH2 domain from pTyr527 forms the prerequisite of the activation process. This can happen through a random probability of equilibrium dissociation of the pTyr527 from SH2 domain; or if the SH2 domain is sequestered away by interacting proteins or substrates. In this unique way, the domain organization of Src family of kinases allows for an intrinsic coupling of its activation to signal targeting [81]. Release of Src from the inactive confirmation is aided by the sequestering of its SH2 or SH3 domain by cognate partners [80].

Dissociation of the SH2 domain from pTyr527 allows it to be accessible to various tyrosine phosphatases to prevent its reassembly into the clamped position. Candidate tyrosine phosphatases that act on pTyr527 include cytosolic PTP1B and SHP1 and also the receptor-bound tyrosine phosphatase domains of PTPα, PTPλ and CD45 [82]. Following the dephosphorylation of Tyr527, the clamped position is released, and the activation loop Tyr416 is autophosphorylated. This finally switches Src from an inactive to an active kinase state.

Src forms an interesting model system for the use of targeting domains for autoregulation. The SH2 and SH3 domains have moderate affinity for the Src sequences itself, but are sufficiently strong to bind Src in an inactivate conformation. However, both the SH2 and SH3 domains become activated by strong binding domains that exist in other proteins. These stronger binding partners readily out-compete the intramolecular interactions to make cognate complexes with Src. Simultaneously, the weak intramolecular interactions make sure that only strong binding partners are selected for activation and working of Src. Src uses its SH2 domain to bind to the phosphorylated forms of Cas, FAK, Paxillin and also growth factor receptors including EGFR and PDGF [83, 84]. SH3 domains of Src recognize and bind substrates like Shc, phosphatidylinositol 3-kinase (PI3K), Cas and Tks5 [85, 86]. In a cascading effect, activated Src can further phosphorylate these binding partners to create more binding sites for molecular adapters that can amplify the signal.

Deactivation of Src essentially requires the reversal of the "latch, clamp and switch" mechanism. Switching of Src to inactive state is dependent on the dephosphorylation of pTyr416. The established tyrosine phosphatase for this role is PTP-BAS that is a ubiquitously expressed cytosolic enzyme with a PTP domain, FERM domain and also five PDZ domains [82]. The reversal of the release of the clamp can, however, override the reversal of the switching mechanism. This means that even when Tyr416 is phosphorylated, phosphorylation of Tyr527 by Csk and Chk can push Src into a clamped position [87]. In this clamped position of the doubly phosphorylated Src, pTyr527 is masked by the SH2 domain. This makes pTyr416 selectively prone to dephosphorylation by phosphatases including PTP1B and PTPα to inactivate

Src [88]. In summary, four phosphorylated forms of Src exist: (a) unphophorylated Src, (b) pTyr527 Src, phosphorylated only at the C-terminal tail (this is the inactive latched clamped conformation); (c) pTyr416 Src, phosphorylated at the activation loop (this is the active, catalytically competent Src) and (d) pTyr527 and pTyr416 Src, phosphorylated at both the C-terminal tail and the activation loop. Phosphorylation at the C-terminal Tyr527 excludes phosphorylation at Tyr416 due to folding of the Src domains on itself in an inactive clamped conformation. Phosphorylation at the activation loop Tyr416 is indifferent to phosphorylation of Tyr527, which continues to be a substrate site for Csk and Chk kinases. The formation of the doubly phosphorylated Src attempts to fold the molecule into the clamped inactive conformation that facilitates the selective dephosphorylation of pTyr416 .

1.6 Phosphotyrosine recognition domains

The phosphotyrosine recognition domains provide for a reversible spatiotemporal regulation of protein–protein interaction in signal transduction. These domains bind to interacting proteins as decided by the affinity of their binding sequences [89]. These domains help to tie-in the signaling web of phosphotyrosine-based signal transduction. The domains are found in a variety of proteins with diverse catalytic and non-catalytic functions. Their recruitment to phosphotyrosine sites allows for the activation and functioning of their host proteins that mediate downstream signaling. At present, there are essentially two kinds of phosphotyrosine recognition domains known to the scientific community. These are the SH2 domains and the phosphotyrosine interacting domain (PTB). The human genome contains about 121 member of the SH2 domain family [90] and about 60 proteins that contain phosphotyrosine recognition domains [91] (Figure 1.11). A number of these domains are associated with hypothetically annotated proteins whose functions still need to be discovered. Nonetheless, the large numbers of these domains and diversity of protein kinases in the genome allow for a tight modulation of signaling, preventing it from misfiring. It ensures that only a few necessary substrates are utilized in the need of the cell and that the signal is either amplified or nullified depending on the binding of the modular partner.

The phosphotyrosine recognition domains ensure a basal level of cell signaling and activation following a stimulus for protein kinases. Mutations in these domains or their binding sequences have hence been increasingly linked to various human diseases and pathophysiologies. Mutation in the SH2 domain of Bruton tyrosine kinase is linked to the atypical XChromosome-linked agammaglobulinemia [92]. XChromosome-linked lymphoproliferative syndrome is associated with a mutation in the SH2 domain of the SH2 domain containing protein 1A (SAP) [93]. Noonan syndrome that is characterized by congenital heart defects and reduced postnatal growth is linked to mutations found in the SH2 domain of the protein phosphatase SHP-2 [94]. While this set of mutations in SHP-2 are “gain of function” and activate the phosphatase, less

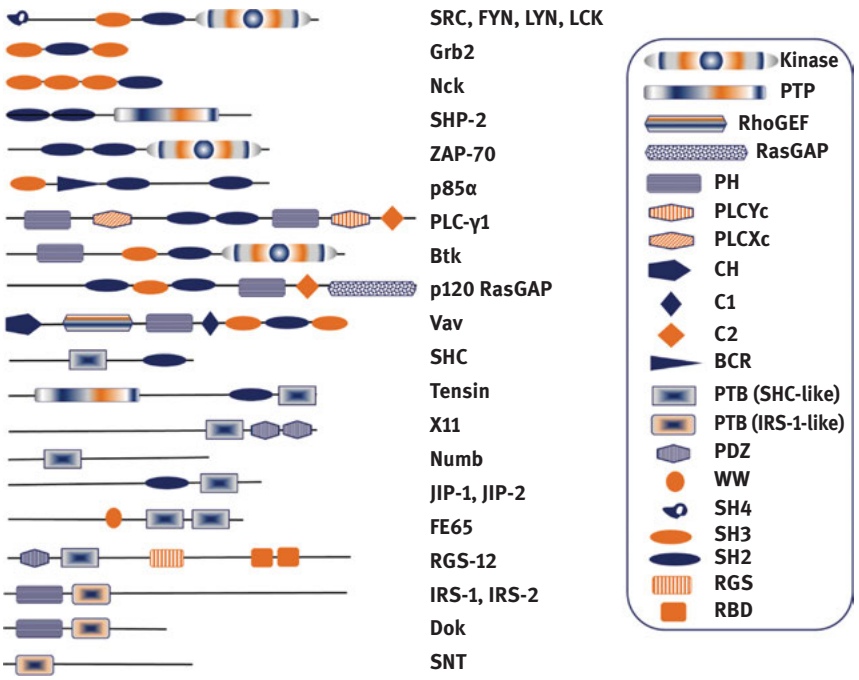

Figure 1.11: Domain organization of SH2 and PTB domain-containing proteins.

conservative mutations in the same regions of the SH2 domain of SHP2 are linked to juvenile myelomonocytic leukemia [95]. As mentioned previously, mutation in the C-terminal tail of Src kinases prevents its autoregulation mediated by the SH2 domain and is sufficient to cause malignancies [96]. Efforts are being made to pharmaceutically target the SH2 domains of Grb-2, Src, SHP-2, ZAP-70, Lck and also PI3K as potential treatments for cardiac disease, osteoporosis and cancer. In a rat model of parathyroid-hormone-induced bone resorption, inhibitors against the SH2 domain of Src have been shown to be effective against osteoclast activity [97].

1.6.1 SH2 domains: Discovery and selectivity

The discovery of the SH2 domain opened unprecedented opportunities for the appreciation of phosphotyrosine-mediated signaling and how it affects various aspects of human development and disease. The domain was serendipitously discovered in 1986 as a 100 amino acid insert required for the oncogenic activity of the *v-Fps/Fes* gene of Fujinami sarcoma virus [98]. Mutagenesis of this insert resulted in impaired cell transformation of Rat-2 cells with impaired protein kinase activity of the v-fps polypeptide *in vivo*. However, overexpression of the mutant insert containing gene in bacterial expression systems showed no effect on kinase activity of the gene *in vitro*.

This meant that the insert was regulating the kinase activity of the gene in the environs of the eukaryotic cell. Analysis of the insert sequence in related protein kinases allowed for its identification as a conserved sequence, N-terminal to the kinase domain of the Src family of tyrosine kinases. As the kinase domain was described as the SH1 domain, this insert was named as the Src homology 2 (SH2) domain [99].

In 1993, it was determined that SH2 domain binding to phosphotyrosine regions was decided by the sequence flanking the phosphotyrosine residue itself [89, 100]. Affinity of SH2 domain for its target sequence was measured to be between 0.2 to 5.0 μM. This is about a 100-fold better over a random sequence that binds with only ~25 μM affinity. Variations in the canonical binding mode of SH2 domains to their interacting sequence gives rise to diversity as well as selectivity [100, 101]. For example, the Src family of SH2 domains bind the pYEEI sequence, the Grb2 protein SH2 domains bind pYXNX sequence and SH2 domains from PI3K bind the pY φXφ (where φ is a hydrophobic residue) [89, 102]. Phosphotyrosine recognition alone accounts for the major energetic contribution for SH2 domain binding [103]. This allows for a strong discrimination between the phosphorylated and nonphosphorylated sequences. Residues at the C-terminus of the phosphotyrosine contribute additional weak interactions that generate cumulative binding free energy. The distributed nature of these binding interactions allows for quickly terminating the phosphotyrosine signal once the sequence dissociates from the SH2 domain and is promptly dephosphorylated by PTPs.

1.6.2 SH2 domains: Structure and function

Structural studies on the SH2 domain show a conserved fold consisting of a four or three stranded antiparallel β-sheet flanked by two α-helices [104] (Figure 1.12).

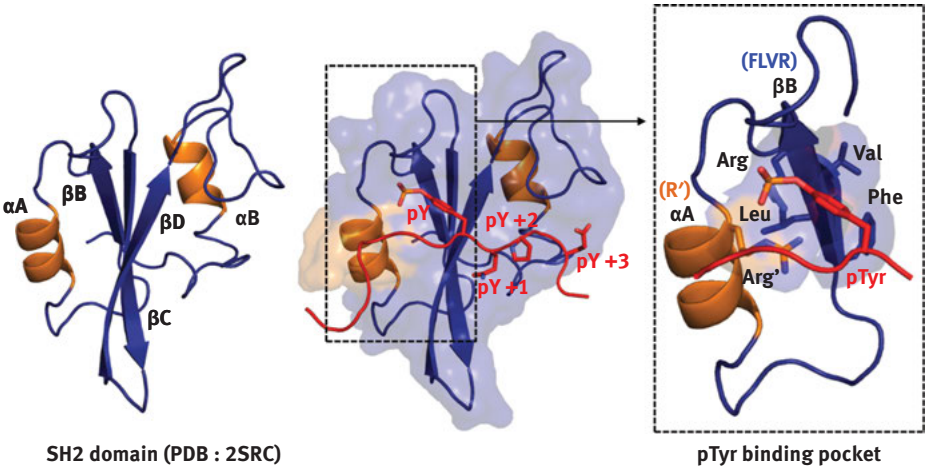

Figure 1.12: Structure and mode of binding of phosphotyrosine containing peptide to SH2 domains.

Key residues required for phosphotyrosine recognition are also conserved in a Phe-Leu-Val-Arg motif. The surface of the domain is highly positively charged so as to efficiently bind the negatively charged phosphotyrosine. The bound phosphopeptide spans this β-sheet in a perpendicular and extended conformation. The phosphotyrosine lies in a moderately deep binding cleft and is held in position by an elaborate network of hydrogen bonds and electrostatics [105]. Two arginine residues, one from the αA and the other from the βB strand, fold the phosphotyrosine in the binding pocket. Residues at the C-terminal of the phosphotyrosine are recognized by the BG and EF loops. On the basis of the structural features of these loops and the binding surface of the SH2 domain, three classes of SH2 domains have been proposed. The first is the phospholipase C-γ1 (PLC-γ1) class that uses a long hydrophobic cleft to select from aliphatic residues from pTyr+1 to pTyr+5 positions. The second class is of the Src family of kinases including the SH2 domains of Src, Fyn, Hck and Nck. These domains select for negatively charged residues at pTyr +1 and pTyr+2 positions. A hydrophobic pocket is used to select for an aliphatic residue at the pTyr+3 position. A single point mutation in the Src class of SH2 domains can switch them to the Grb-2 class of SH2 domains. This third class of SH2 domains has bulky tyrosine in the EF loop that blocks the pTyr+3 position, forcing the binding peptide to make a β-turn. SH2-domain–peptide binding is facilitated by an asparagine residue at the pTyr+2 position [104]. As an exception to this mode of peptide binding, the SH2 domain of Grb10 and SAP proteins is reported to use residues both that the N- and the C-terminal of the phosphotyrosine residue [106]. Their unusual pTyr-2 binding pocket allows for a three-pronged binding mode that allows even the nonphosphorylated peptides to bind the SH2 domain.

Tandem SH2 domains are also seen in proteins including Pt3K, Shp2 and Zap-70 [107]. These two tandem SH2 domains can bind two closely spaced phosphotyrosine residues as are reported to occur on the RTK tails. The affinity of the double-phosphorylated sequence is 20- to 50-fold greater to these SH2 domains as compared to the binding of a single phosphotyrosine to a single SH2 domain. The spacing between the two phosphotyrosines is seen to account for this enhanced affinity. In the case of Zap-70, the first phosphotyrosine motif binds to the SH2 domain in a canonical manner; however, the second phosphotyrosine motif binds the tandem SH2 domains such that it contacts both the SH2 domains in a non-conventional manner [108]. This is only possible if there is a specific spacing between the two phosphotyrosine motifs and a defined relative orientation of the SH2 domains. In the case of the Shp2 tandem domains, once the first phosphotyrosine motif binds, the orientation of the two domains is stabilized at the interface using disulfide bonds and this conformational rigidity accounts for the high affinity for target sequence binding [109]. The tandem SH2 domains of PI3K are themselves under phosphorylation-dependent regulation during signal transduction. Phorbol ester stimulation of cells allows for serine phosphorylation of PI3K SH2 domains, which makes them incapable of binding phosphotyrosine [110].

While the overall structure and sequence of the SH2 domains is highly conserved, a unique SH2 domain is found in the E3 ligase Cbl. Cbl functions in switching off PTKs and targets to ubiquitination-based proteasomal degradation [111]. This Cbl Sh2 domain is only 11% homologous in sequence to the conserved SH2 domain family and lacks the conventional BG loop and secondary β-sheet [112]. This SH2 domain contains an EF hand domain and a calcium-binding four helix bundle. The extended Cbl SH2 domain recognizes both the N-and C-terminal of the phosphotyrosine motif in an (N/D)XpY(S/T)XXP sequence [113]. This sequence is found in various Cbl SH2 domain-binding partners including VEGF receptor, Zap-70, Src, EGFR and Syk. For binding to protein families Met and Plexin, Cbl SH2 domain uses a shorter sequence DpYR. The short sequence allows the Cbl SH2 domain to bind in both canonical forward orientation and the unique reverse orientation [114].

1.6.3 PTB domains: Discovery and selectivity

The PTB domain was discovered in 1994 in the protein Shc, which also harbors a C-terminal SH2 domain discussed earlier [115]. The domain was identified as a region in the protein, distinct from the SH2 domain, that bound the phosphotyrosine of the EGFR. Since then, about 60 PTB domains are known in the human genome and mutations in many are associated with various diseases including diabetes, cardiac diseases and Alzheimer's [91]. It is now also known that PTB domains bind the negatively charged phospholipid head groups, which allow for their localization on the membrane. This allows the PTB domains to easily access the juxta-membrane regions of various RTKs and to mediate their downstream signaling. The phospholipid binding pocket is lined with highly basic residues and binding to the membrane is a distinct event from PTB.

PTB domains bind to a target NPXY motif in their substrate binding partners (Figure 1.13). On the basis of structural and sequence studies, PTB domains are classified into three groups: (a) the pTyr-dependent Shc-like, (b) pTyr-dependent IRS-like and (c) pTyr-independent Dab-like [91]. The pTyr-dependent Shc-like and pTyr-dependent IRS-like domains bind the phosphorylated NPQpY and NPApY sequences, respectively. The pTyr-independent Dab-like domain binds the unphophorylated NPTY sequence or the NPXF sequence, where tyrosine is replaced by a phenylalanine. Peptide-binding modes of the three classes of PTB domains are also distinct.

The pTyr-dependent Shc-like PTB domain's peptide binding pocket is positively charged and forms a network of hydrogen bonds with the phosphate of the phosphotyrosine. Two arginine residues (Arg67 and Arg175 in Shc) and a lysine residue (Lys169 in Shc) triangulate the phosphate group. The pTyr-dependent IRS-like PTB domains also have a similar positively charged pocket where two arginine residues (Arg212 and Arg227 in IRS-1) bind the phosphotyrosine. The peptide binding pocket of pTyr-independent Dab-like PTB domains is less basic and much shallow as compared

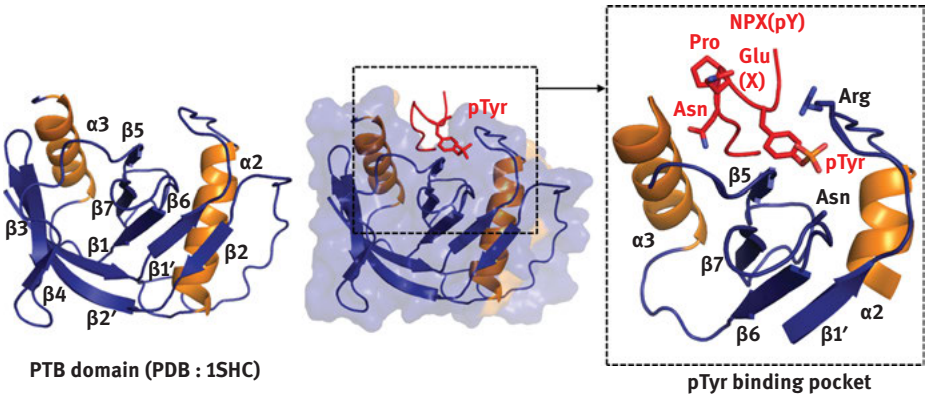

Figure 1.13: Structure and mode of binding of phosphotyrosine-containing peptide to PTB domains.

to the other two classes. Although phosphorylation of the tyrosine is not required for binding the peptide, the presence of an aromatic phenyl ring as a tyrosine or a phenylalanine is essential in the NPXY/F sequence in the binding peptide. A histidine (His136 in Dab1) makes *van der Wall* contacts with the phenyl ring of the tyrosine/phenylalanine and a glycine (Gly131 in Dab1) makes hydrogen bonds with the peptide. A number of hydrophobic contacts spread over the pTyr-independent Dab-like PTB domain maintain extensive binding interactions with the NPXY/F containing target peptides [91, 116].

1.6.4 PTB domains: Structure and function

The structure of the first PTB domain was solved in 1995, shortly after its identification in 1994 [117]. Since then a number of PTB domains have been solved, and despite their sequence variability, they all fold into the β-sandwich structure of the pleckstrin homology (PH) domain "superfold". In the case of PH domains, this β-sandwich binds to phosphoinositides and enabled/VASP homology domains [118]. The β-sandwich is made of one three-stranded and another four-stranded β-sheet placed orthogonally to each other at the core of the domain. The β-sandwich is capped by a conserved α-helix at the C-terminal. In the first two ligand-bound structures of the PTB domains, the phosphopeptide NPXpY was seen to adopt a type-I β-turn [117, 119]. The N-terminal region of the peptide near the conserved NPXpY sequence was seen in an extended conformation, making hydrogen bonding interactions with the β5 sheet (Figures 1.13 and 1.14). This mode of binding is called "antiparallel β-sheet augmentation" as the binding peptide augments β-sheet of the PTB domain [120]. The NPX sequence of the NPXpY motif functions to position the phosphotyrosine to interact with the basic residues on the surface of the PTB domain. These basic arginine residues occur in approximately similar places in the three-dimensional

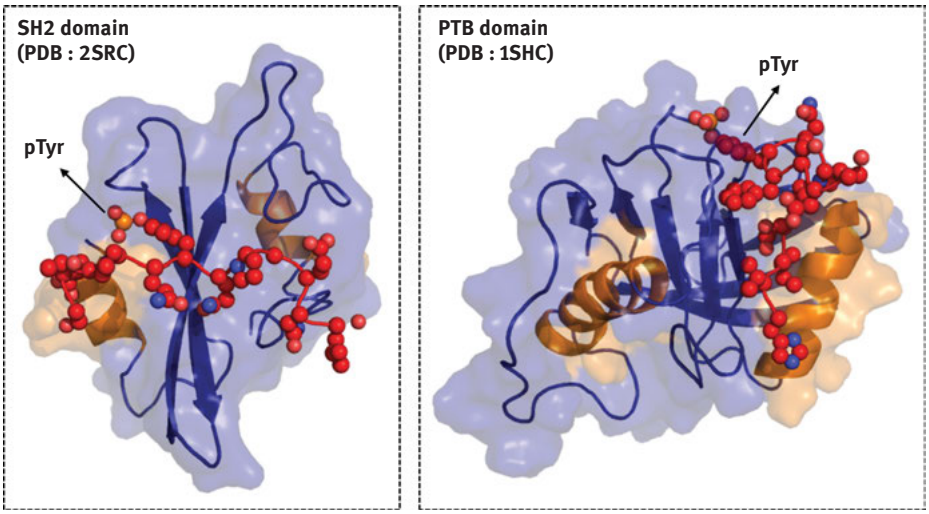

Figure 1.14: Comparison of mode of phosphotyrosine peptide binding by SH2 and PTB domains.

structure of Shc and IRS-1 PTB domains, although they are not comparable in sequence of the two domain types. Therefore, while the general mode of binding the peptide by β-sheet augmentation is the same in various PTB domains, the modes of engaging and binding the phosphotyrosine are variable.

Some studies suggest that phosphotyrosine recognition is a property of only a few PTB domains [121]. Biochemistry and structural biology of the PTB domains of X11, Numb, FRS2 and disabled-1 show their ability to bind nonphosphorylated peptides [122–125]. In each case, the peptide binds in between the β5 strand and the C-terminal helix, augmenting the β-sandwich of their PTB domains. In this way they show a peptide binding mode identical to the Shc and IRS-1 domains, irrespective of the phosphorylation states of the peptides. The X11 PTB domain binds to the myloid β-protein precursor protein (βAPP) at its cytoplasmic tail that contains the NPXY motif [122]. The structure of the bound peptide to the PTB domain shows the antiparallel β-sheet augmentation mode of binding. However also, the phosphorylation of the tyrosine in the NPXY motif as no effect on binding. The PTB domain of disabled (Dab)-1 protein binds the NPXY motif at the cytoplasmic tails of its target proteins, but its affinity is compromised if the tyrosine is phosphorylated [126]. The FRS2 PTB domains bind to distinct regions in the NGF receptor with appreciably high affinity. It binds to NPXpY site on the receptor that is phosphorylation dependent and also a site for Shc PTB domain interaction. However, FRS2 also binds a distinct site in the NGF receptor juxta-membrane region with the sequence AVHKLAKSIPLRRQVTVS that lacks tyrosine residues. Binding of FRS2 PTB to this site is phosphorylation independent [125]. NMR structure of the FRS2 PTB domain with the peptide of this sequence shows the canonical mode of binding of the peptide using β-sheet augmentation [127]. Peptide wrapping round the PTB domain and

additional interactions at the peptide–protein interface stabilize this complex. Similarly, the PTB domain of Numb binds strongly to the sequence GFSNMSFEDP as seen in the Numb-associated kinase (Nak) [123].

The PH domain fold of the PTB domains also allows some of these proteins to bind phosphoinositides. PTB domains of Shc and IRS-1 have both been reported to bind phosphoinositides, but with weak affinities [128, 129]. This suggests a role for their electrostatically polarized surfaces for their recruitment at cell membranes. The crystal structure of the Disabled (Dab)-1 PTB domain with the phosphoinositide headgroup phosphatidylinositol 4,5-bisphosphate (PIP2) shows phosphoinositide binding in the basic patch of the PTB domain [116, 126]. In this particular case, peptide and phosphoinositide binding are seen to be simultaneous and may even cooperate in locating the Disabled (Dab)-1 PTB domain at the membrane. In the reciprocal scenario, proteins containing PH domains have been shown to weakly bind various signaling peptides/proteins. The β-adrenergic receptor kinase PH domain is closest in its ability to function as a PTB domain [130]. The β-adrenergic receptor kinase PH domain binds to PIP2 and also the $G_{\beta\gamma}$ subunits of heterotrimeric G-proteins. These two interactions occur at distinct termini of the PH domains and both interactions are required for membrane association of the kinase. The kinase activity of the β-adrenergic receptor kinase remains unaltered in the presence of either PIP2 or the $G_{\beta\gamma}$ subunits [130]. Similarly, PH-like and PTB-like domains in various proteins have also been reported for their ability to bind protein targets. The FERM domain of Talin binds to β-integrin at the NPXY motifs in the cytoplasmic tail [131, 132]. Crystal structures of the FERM PH domains bound to the peptides of the cytoplasmic tails of β-integrin and ICAM-2 are similar to the X11 PTB domain in complex with its target peptide [133].

1.6.5 SH2 domains versus PTB domains

Structural studies are available on both the SH2 and PTB that reveal their molecular ways of binding to a phosphotyrosine-containing peptide. Although both domains consist of β-sheets that are surrounded by α-helices, their modes of phosphotyrosine recognition and binding are varied (Figure 1.14). Phosphopeptide recognition by the PTB domains is much more relaxed as compared to the SH2 domain, which primarily recognizes the phosphotyrosine. PTB domains appear to collaborate with peptide ligands that show a prominent propensity for β-sheet augmentation. If the peptide forms a β-strand antiparallel to the β5 strand of the PTB and joins the cleft between β5 strand and αC helix, phosphotyrosine recognition becomes almost unnecessary. In contrast, the presence of a phosphotyrosine is central to binding of nearly all SH2 domains. The exception to this rule is seen only in the case of the SAP protein that is altered in XChromosome-linked lymphoproliferative syndrome. The SAP SH2 domain is only known SH2 domain that is seen to bind tyrosine peptides independent of their phosphorylation state [134]. Molecular interactions between the SAP SH2 domain and

sequence of the peptide flanking both the N- and C-termini of the phosphotyrosine allow for efficient binding and recognition [134, 135].

1.6.6 Atypical phosphotyrosine recognition domains: HYB domain

The unique HYB domain has been identified in the Hakai protein that serves as an E3 ubiquitin ligase for E-cadherin in a tyrosine phosphorylation-dependent mechanism. Hakai protein resembles the c-Cbl E3 ligase in having a phosphotyrosine recognition region, a proline-rich region and also a RING finger motif [136] . The HYB domain is formed by the dimerization of two Hakai monomers mediated by Zn^{2+}-coordination. In this way, the HYB domain is distinct from the SH2 and PTB domains and is a feature of certain E3 ligases that are implicated in cell adhesion and cancer metastasis [137]. This includes the testis-specific ubiquitin ligase ZNF645 and the Numb-associated E3 ligase LNX. A 100-residue stretch including the RING finger motif of each Hakai monomer dimerizes to coordinate a total of six Zn^{2+} ions between their cysteine and histidine residues. A positively charged PTB pocket is created at the dimer interface. Phosphorylated E-cadherin binds the Hakai dimer using the sequence NVYpYY with a dissociation constant of ~7.2 μM [138].

1.6.7 Atypical phosphotyrosine recognition domains: PKCδ and PKCθ C2 domains

About 130 residue C2 domain is a β-sandwich of eight antiparallel strands. This domain family includes about 200 members in the human genome and primarily binds to phosphatidylserine in cell membranes using a calcium-dependent mechanism [139]. Calcium binding is mediated by interstrand loops of the C2 domain β-sandwich. Recent work on the C2 domain of Ser/Thr kinases PKCδ and PKCθ demonstrates their ability to bind to phosphotyrosine peptides derived from CUB domain-containing protein 1 (CDCP1) [140]. CDCP1 is a transmembrane protein substrate of Src family of protein kinases and is seen to be overexpressed in various cancers [141]. The phosphotyrosine peptide binds the C2 domain in an extended conformation across its two β-sheets. The phosphotyrosine residue is bound by positively charged arginine and lysine residues in a deep pocket and its phenyl ring is stabilized by ring-stacking interactions with a histidine. Peptide binding is sequence dependent with a dissociation constant of ~240 nM [140].

References

[1] Deutscher J, Saier Jr MH. Ser/Thr/Tyr protein phosphorylation in bacteria – for long time neglected, now well established. J Mol Microbiol Biotechnol 2005;9(3–4):125–31.
[2] Cozzone AJ. Protein phosphorylation in prokaryotes. Ann Rev Microbiol 1988;42:97–125.

[3] Chang C, Stewart RC. The two-component system. Regulation of diverse signaling pathways in prokaryotes and eukaryotes. Plant Physiol 1998;117(3):723–31.
[4] Day EK, Sosale NG, Lazzara MJ. Cell signaling regulation by protein phosphorylation: a multivariate, heterogeneous, and context-dependent process. Curr Opin Biotechnol 2016;40:185–92.
[5] Cohen P. The origins of protein phosphorylation. Nat Cell Biol. 2002;4(5):E127–E30.
[6] Johnson LN. The regulation of protein phosphorylation. Biochem Soc Trans 2009;37(Pt 4): 627–41.
[7] Cohen P. The regulation of protein function by multisite phosphorylation – a 25 year update. Trends Biochem Sci 2000;25(12):596–601.
[8] Stock AM, Robinson VL, Goudreau PN. Two-component signal transduction. Ann Rev Biochem 2000;69:183–215.
[9] Grefen C, Harter K. Plant two-component systems: principles, functions, complexity and cross talk. Planta. 2004;219(5):733–42.
[10] Klumpp S, Krieglstein J. Reversible phosphorylation of histidine residues in vertebrate proteins. Biochim Biophys Acta 2005;1754(1–2):291–5.
[11] Wieland T, Attwood PV. Alterations in reversible protein histidine phosphorylation as intracellular signals in cardiovascular disease. Front Pharmacol 2015;6:173.
[12] Feng J, Zhu M, Schaub MC, Gehrig P, Roschitzki B, Lucchinetti E, et al. Phosphoproteome analysis of isoflurane-protected heart mitochondria: phosphorylation of adenine nucleotide translocator-1 on Tyr194 regulates mitochondrial function. Cardiovasc Res 2008;80(1):20–29.
[13] Stadtman TC. Emerging awareness of the critical roles of S-phosphocysteine and selenophosphate in biological systems. Biofactors 1994;4(3–4):181–5.
[14] Matthews HR. Protein kinases and phosphatases that act on histidine, lysine, or arginine residues in eukaryotic proteins: a possible regulator of the mitogen-activated protein kinase cascade. Pharmacol Ther 1995;67(3):323–50.
[15] Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. The protein kinase complement of the human genome. Science 2002;298(5600):1912–34.
[16] Avruch J, Khokhlatchev A, Kyriakis JM, Luo Z, Tzivion G, Vavvas D, et al. Ras activation of the Raf kinase: tyrosine kinase recruitment of the MAP kinase cascade. Recent Prog Horm Res 2001;56:127–55.
[17] Kisseleva T, Bhattacharya S, Braunstein J, Schindler CW. Signaling through the JAK/STAT pathway, recent advances and future challenges. Gene 2002;285(1–2):1–24.
[18] Pawson T, Scott JD. Signaling through scaffold, anchoring, and adaptor proteins. Science 1997;278(5346):2075.
[19] Mumby MC, Walter G. Protein serine/threonine phosphatases: structure, regulation, and functions in cell growth. Physiol Rev 1993;73(4):673–99.
[20] Stoker AW. Protein tyrosine phosphatases and signalling. J Endocrinol 2005;185(1):19–33.
[21] Denu JM, Dixon JE. A catalytic mechanism for the dual-specific phosphatases. Proc Natl Acad Sci USA 1995;92(13):5910–4.
[22] Baumer N, Maurer A, Krieglstein J, Klumpp S. Expression of protein histidine phosphatase in Escherichia coli, purification, and determination of enzyme activity. Methods Mol Biol 2007;365:247–60.
[23] Levene PA, Alsberg CL. The cleavage products of vitellin. J Biol Chem 1906;2(1):127–33.
[24] Lipmann FA, Levene PA. Serinephosphoric acid obtained on hydrolysis of vitellinic acid. J Biol Chem 1932;98(1):109–14.
[25] Northrop JH. crystalline pepsin: I. Isolation and tests of purity. J Gen Physiol 1930;13(6):739–66.
[26] Burnett G, Kennedy EP. The enzymatic phosphorylation of proteins. J Biol Chem 1954;211 (2):969–80.

[27] Green AA, Cori GT, Oncley WanbJL. Crystalline muscle phosphorylase: I. Preparation, properties, and molecular weight. J Biol Chem 1943;151(1):21–9.
[28] Cori GT, Cori CF. the enzymatic conversion of phosphorylase a TO b. J Biol Chem 1945;158 (2):321–32.
[29] Rall TW, Sutherland EW. formation of a cyclic adenine ribonucleotide by tissue particles. J Biol Chem 1958;232(2):1065–76.
[30] Fischer EH, Krebs EG. Conversion of phosphorylase b to phosphorylase a in muscle extracts. J Biol Chem 1955;216(1):121–32.
[31] Walsh DA, Perkins JP, Krebs EG. An adenosine 3′,5′-monophosphate-dependant protein kinase from rabbit skeletal muscle. J Biol Chem 1968;243(13):3763–5.
[32] Friedman DL, Larner J. Studies on UDPG-α-Glucan Transglucosylase. III. Interconversion of Two Forms of Muscle UDPG-α-Glucan Transglucosylase by a Phosphorylation-Dephosphorylation Reaction Sequence*. Biochemistry 1963;2(4):669–75.
[33] Kuo JF, Greengard P. cyclic nucleotide-dependent protein kinases, IV. Widespread occurrence of adenosine 3′,5′-monophosphate-dependent protein kinase in various tissues and phyla of the animal kingdom. Proc Natl Acad Sci USA 1969;64(4):1349–55.
[34] Cohen P. The regulation of protein function by multisite phosphorylation – a 25 year update. Trends in Biochemical Sciences.25(12):596–601.
[35] Lefkowitz RJ. Historical review: a brief history and personal retrospective of seven-transmembrane receptors. Trends Pharmacol Sci 2004;25(8):413–22.
[36] Eckhart W, Hutchinson MA, Hunter T. An activity phosphorylating tyrosine in polyoma T antigen immunoprecipitates. Cell 1979;18(4):925–33.
[37] Hunter T, Sefton BM. Transforming gene product of Rous sarcoma virus phosphorylates tyrosine. Proc Natl Acad Sci USA 1980;77(3):1311–5.
[38] Witte ON, Dasgupta A, Baltimore D. Abelson murine leukaemia virus protein is phosphorylated in vitro to form phosphotyrosine. Nature 1980;283(5750):826–31.
[39] Hunter T, Eckhart W. The discovery of tyrosine phosphorylation: it's all in the buffer! Cell 2004;116(2Suppl): S35–9, 1 p following S48.
[40] Collett MS, Erikson RL. Protein kinase activity associated with the avian sarcoma virus src gene product. Proc Natl Acad Sci USA. 1978;75(4):2021–4.
[41] Carpenter G, King L, Jr., Cohen S. Epidermal growth factor stimulates phosphorylation in membrane preparations in vitro. Nature 1978;276(5686):409–10.
[42] Ushiro H, Cohen S. Identification of phosphotyrosine as a product of epidermal growth factor-activated protein kinase in A-431 cell membranes. J Biol Chem 1980;255(18): 8363–65.
[43] Sefton BM, Hunter T, Beemon K, Eckhart W. Evidence that the phosphorylation of tyrosine is essential for cellular transformation by Rous sarcoma virus. Cell 1980;20(3):807–16.
[44] MacKintosh C, Garton AJ, McDonnell A, Barford D, Cohen PTW, Tonks NK, et al. Further evidence that inhibitor-2 acts like a chaperone to fold PP1 into its native conformation. FEBS Lett 1996;397(2):235–38.
[45] Chernoff J, Li HC, Cheng YS, Chen LB. Characterization of a phosphotyrosyl protein phosphatase activity associated with a phosphoseryl protein phosphatase of Mr = 95,000 from bovine heart. J Biol Chem 1983;258(12):7852–7.
[46] Chernoff J, Sells MA, Li HC. Characterization of phosphotyrosyl-protein phosphatase activity associated with calcineurin. Biochem Biophys Res Commun 1984;121(1):141–8.
[47] Chernoff J, Li HC. Multiple forms of phosphotyrosyl- and phosphoseryl-protein phosphatase from cardiac muscle: partial purification and characterization of an EDTA-stimulated phosphotyrosyl-protein phosphatase. Arch Biochem Biophys 1983; 226(2):517–30.

[48] Horlein D, Gallis B, Brautigan DL, Bornstein P. Partial purification and characterization of phosphotyrosyl-protein phosphatase from Ehrlich ascites tumor cells. Biochemistry 1982; 21(22):5577–84.
[49] Foulkes JG, Erikson E, Erikson RL. Separation of multiple phosphotyrosyl-and phosphoseryl-protein phosphatases from chicken brain. J Biol Chem 1983;258(1):431–8.
[50] Brunati AM, Pinna LA. Isolation and partial characterization of distinct species of phosphotyrosyl protein phosphatases from rat spleen. Biochem Biophys Res Commun 1985; 133(3):929–36.
[51] Swarup G, Cohen S, Garbers DL. Inhibition of membrane phosphotyrosyl-protein phosphatase activity by vanadate. Biochem Biophys Res Commun 1982;107(3):1104–9.
[52] Brautigan DL, Bornstein P, Gallis B. Phosphotyrosyl-protein phosphatase. Specific inhibition by Zn. J Biol Chem 1981;256(13):6519–22.
[53] Rotenberg SA, Brautigan DL. Membrane protein phosphotyrosine phosphatase in rabbit kidney. Proteolysis activates the enzyme and generates soluble catalytic fragments. Biochem J 1987;243(3):747–54.
[54] Okada M, Owada K, Nakagawa H. [Phosphotyrosine]protein phosphatase in rat brain. A major [phosphotyrosine]protein phosphatase is a 23 kDa protein distinct from acid phosphatase. Biochem J 1986;239(1):155–62.
[55] Tonks NK. Protein tyrosine phosphatases – from housekeeping enzymes to master regulators of signal transduction. FEBS J 2013;280(2):346–78.
[56] Kohanski RA, Lane MD. Kinetic evidence for activating and non-activating components of autophosphorylation of the insulin receptor protein kinase. Biochem Biophys Res Commun 1986;134(3):1312–8.
[57] Pike LJ, Eakes AT, Krebs EG. Characterization of affinity-purified insulin receptor/kinase. Effects of dithiothreitol on receptor/kinase function. J Biol Chem 1986;261(8):3782–89.
[58] Tonks NK, Diltz CD, Fischer EH. Purification of the major protein-tyrosine-phosphatases of human placenta. J Biol Chem 1988;263(14):6722–30.
[59] Charbonneau H, Tonks NK, Kumar S, Diltz CD, Harrylock M, Cool DE, et al. Human placenta protein-tyrosine-phosphatase: amino acid sequence and relationship to a family of receptor-like proteins. Proc Natl Acad Sci USA 1989;86(14):5252–6.
[60] Ottilie S, Raulf F, Barnekow A, Hannig G, Schartl M. Multiple src-related kinase genes, srk1-4, in the fresh water sponge Spongilla lacustris. Oncogene 1992;7(8):1625–30.
[61] Bosch TC, Unger TF, Fisher DA, Steele RE. Structure and expression of STK, a src-related gene in the simple metazoan Hydra attenuata. Mol Cell Biol 1989;9(10):4141–51.
[62] Tan JL, Spudich JA. Developmentally regulated protein-tyrosine kinase genes in Dictyostelium discoideum. Mol Cell Biol 1990;10(7):3578–83.
[63] Cohen P. Signal integration at the level of protein kinases, protein phosphatases and their substrates. Trends Biochem Sci 1992;17(10):408–13.
[64] Sun H, Tonks NK. The coordinated action of protein tyrosine phosphatases and kinases in cell signaling. Trends Biochem Sci 1994;19(11):480–85.
[65] Hunter T. Protein kinases and phosphatases: the Yin and Yang of protein phosphorylation and signaling. Cell 1995:80(2):225–36.
[66] Lemmon MA, Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell 2010;141(7): 1117–34.
[67] Huse M, Kuriyan J. The conformational plasticity of protein kinases. Cell 2002;109(3):275–82.
[68] Hubbard SR, Wei L, Ellis L, Hendrickson WA. Crystal structure of the tyrosine kinase domain of the human insulin receptor. Nature 1994;372(6508):746–54.
[69] Hubbard SR, Mohammadi M, Schlessinger J. Autoregulatory mechanisms in protein-tyrosine kinases. J Biol Chem 1998;273(20):11987–90.

[70] Taylor SS, Kornev AP. Protein kinases: evolution of dynamic regulatory proteins. Trends Biochem Sci 2011;36(2):65–77.
[71] Meharena HS, Chang P, Keshwani MM, Oruganty K, Nene AK, Kannan N, et al. Deciphering the structural basis of eukaryotic protein kinase regulation. PLoS Biol 2013;11(10):e1001680.
[72] Sorkin A, Waters CM. Endocytosis of growth factor receptors. Bioessays 1993;15(6):375–82.
[73] Mohapatra B, Ahmad G, Nadeau S, Zutshi N, An W, Scheffe S, et al. Protein tyrosine kinase regulation by ubiquitination: critical roles of Cbl-family ubiquitin ligases. Biochim Biophys Acta 2013;1833(1):122–39.
[74] Resh MD. Fatty acylation of proteins: new insights into membrane targeting of myristoylated and palmitoylated proteins. Biochim Biophys Acta 1999;1451(1):1–16.
[75] Oneyama C, Iino T, Saito K, Suzuki K, Ogawa A, Okada M. Transforming potential of Src family kinases is limited by the cholesterol-enriched membrane microdomain. Mol Cell Biol 2009; 29(24):6462–72.
[76] Cooper JA, Gould KL, Cartwright CA, Hunter T. Tyr527 is phosphorylated in pp60c-src: implications for regulation. Science 1986;231(4744):1431–4.
[77] Davidson D, Chow LM, Veillette A. Chk, a Csk family tyrosine protein kinase, exhibits Csk-like activity in fibroblasts, but not in an antigen-specific T-cell line. J Biol Chem 1997;272(2): 1355–62.
[78] Okada M. Regulation of the SRC family kinases by Csk. Int J Biol Sci 2012;8(10):1385–97.
[79] Jove R, Hanafusa H. Cell transformation by the viral src oncogene. Ann Rev Cell Biol 1987;3: 31–56.
[80] Cooper JA, Howell B. The when and how of Src regulation. Cell 1993;73(6):1051–4.
[81] Young MA, Gonfloni S, Superti-Furga G, Roux B, Kuriyan J. Dynamic coupling between the SH2 and SH3 domains of c-Src and Hck underlies their inactivation by C-terminal tyrosine phosphorylation. Cell 2001;105(1):115–26.
[82] Roskoski R, Jr. Src kinase regulation by phosphorylation and dephosphorylation. Biochem Biophys Res Commun 2005;331(1):1–14.
[83] Kypta RM, Goldberg Y, Ulug ET, Courtneidge SA. Association between the PDGF receptor and members of the src family of tyrosine kinases. Cell 1990;62(3):481–92.
[84] Sieg DJ, Hauck CR, Schlaepfer DD. Required role of focal adhesion kinase (FAK) for integrin-stimulated cell migration. J Cell Sci 1999;112(Pt 16):2677–91.
[85] Liu X, Marengere LE, Koch CA, Pawson T. The v-Src SH3 domain binds phosphatidylinositol 3′-kinase. Mol Cell Biol 1993;13(9):5225–32.
[86] Seals DF, Azucena EF, Jr., Pass I, Tesfay L, Gordon R, Woodrow M, et al. The adaptor protein Tks5/Fish is required for podosome formation and function, and for the protease-driven invasion of cancer cells. Cancer Cell 2005;7(2):155–65.
[87] Chong YP, Mulhern TD, Zhu HJ, Fujita DJ, Bjorge JD, Tantiongco JP, et al. A novel non-catalytic mechanism employed by the C-terminal Src-homologous kinase to inhibit Src-family kinase activity. J Biol Chem 2004;279(20):20752–66.
[88] Sun G, Sharma AK, Budde RJ. Autophosphorylation of Src and Yes blocks their inactivation by Csk phosphorylation. Oncogene 1998;17(12):1587–95.
[89] Songyang Z, Shoelson SE, Chaudhuri M, Gish G, Pawson T, Haser WG, et al. SH2 domains recognize specific phosphopeptide sequences. Cell 1993;72(5):767–78.
[90] Liu BA, Jablonowski K, Raina M, Arce M, Pawson T, Nash PD. The human and mouse complement of SH2 domain proteins-establishing the boundaries of phosphotyrosine signaling. Mol Cell 2006;22(6):851–68.
[91] Uhlik MT, Temple B, Bencharit S, Kimple AJ, Siderovski DP, Johnson GL. Structural and evolutionary division of phosphotyrosine binding (PTB) domains. J Mol Biol 2005;345(1): 1–20.

[92] Saffran DC, Parolini O, Fitch-Hilgenberg ME, Rawlings DJ, Afar DE, Witte ON, et al. Brief report: a point mutation in the SH2 domain of Bruton's tyrosine kinase in atypical X-linked agammaglobulinemia. N Engl J Med 1994;330(21):1488–91.
[93] Nichols KE, Harkin DP, Levitz S, Krainer M, Kolquist KA, Genovese C, et al. Inactivating mutations in an SH2 domain-encoding gene in X-linked lymphoproliferative syndrome. Proc Natl Acad Sci USA 1998;95(23):13765–70.
[94] Tartaglia M, Mehler EL, Goldberg R, Zampino G, Brunner HG, Kremer H, et al. Mutations in PTPN11, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. Nat Genet 2001;29(4):465–8.
[95] Bentires-Alj M, Paez JG, David FS, Keilhack H, Halmos B, Naoki K, et al. Activating mutations of the noonan syndrome-associated SHP2/PTPN11 gene in human solid tumors and adult acute myelogenous leukemia. Cancer Res 2004;64(24):8816–20.
[96] Martin GS. The hunting of the Src. Nat Rev Mol Cell Biol. 2001;2(6):467–75.
[97] Shakespeare W, Yang M, Bohacek R, Cerasoli F, Stebbins K, Sundaramoorthi R, et al. Structure-based design of an osteoclast-selective, nonpeptide src homology 2 inhibitor with in vivo antiresorptive activity. Proc Natl Acad Sci USA 2000;97(17):9373–8.
[98] Sadowski I, Stone JC, Pawson T. A noncatalytic domain conserved among cytoplasmic protein-tyrosine kinases modifies the kinase function and transforming activity of Fujinami sarcoma virus P130gag-fps. Mol Cell Biol 1986;6(12):4396–408.
[99] Jin J, Xie X, Chen C, Park JG, Stark C, James DA, et al. Eukaryotic protein domains as functional units of cellular evolution. Sci Signal 2009;2(98):ra76.
[100] Bradshaw JM, Waksman G. Molecular recognition by SH2 domains. Adv Protein Chem 2002;61:161–210.
[101] Wavreille AS, Garaud M, Zhang Y, Pei D. Defining SH2 domain and PTP specificity by screening combinatorial peptide libraries. Methods 2007;42(3):207–19.
[102] Rozakis-Adcock M, Fernley R, Wade J, Pawson T, Bowtell D. The SH2 and SH3 domains of mammalian Grb2 couple the EGF receptor to the Ras activator mSos1. Nature 1993;363 (6424):83–5.
[103] Moran MF, Koch CA, Anderson D, Ellis C, England L, Martin GS, et al. Src homology region 2 domains direct protein-protein interactions in signal transduction. Proc Natl Acad Sci USA 1990;87(21):8622–6.
[104] Waksman G, Kuriyan J. Structure and specificity of the SH2 domain. Cell 2004;116(2 Suppl): S45–48, 3 p following S8.
[105] Muller AJ, Pendergast AM, Havlik MH, Puil L, Pawson T, Witte ON. A limited set of SH2 domains binds BCR through a high-affinity phosphotyrosine-independent interaction. Mol Cell Biol 1992;12(11):5087–93.
[106] Kaneko T, Huang H, Zhao B, Li L, Liu H, Voss CK, et al. Loops govern SH2 domain specificity by controlling access to binding pockets. Sci Signal 2010;3(120):ra34.
[107] Ottinger EA, Botfield MC, Shoelson SE. Tandem SH2 domains confer high specificity in tyrosine kinase signaling. J Biol Chem 1998;273(2):729–35.
[108] Hatada MH, Lu X, Laird ER, Green J, Morgenstern JP, Lou M, et al. Molecular basis for interaction of the protein tyrosine kinase ZAP-70 with the T-cell receptor. Nature 1995;377 (6544):32–8.
[109] Eck MJ, Pluskey S, Trub T, Harrison SC, Shoelson SE. Spatial constraints on the recognition of phosphoproteins by the tandem SH2 domains of the phosphatase SH-PTP2. Nature. 1996;379 (6562):277–80.
[110] Lee JY, Chiu YH, Asara J, Cantley LC. Inhibition of PI3K binding to activators by serine phosphorylation of PI3K regulatory subunit p85alpha Src homology-2 domains. Proc Natl Acad Sci USA 2011;108(34):14157–62.

[111] Sanjay A, Horne WC, Baron R. The Cbl family: ubiquitin ligases regulating signaling by tyrosine kinases. Sci STKE 2001;2001(110):pe40.
[112] Meng W, Sawasdikosol S, Burakoff SJ, Eck MJ. Structure of the amino-terminal domain of Cbl complexed to its binding site on ZAP-70 kinase. Nature 1999;398(6722):84–90.
[113] Hu J, Hubbard SR. Structural basis for phosphotyrosine recognition by the Src homology-2 domains of the adapter proteins SH2-B and APS. J Mol Biol 2006;361(1):69–79.
[114] Ng C, Jackson RA, Buschdorf JP, Sun Q, Guy GR, Sivaraman J. Structural basis for a novel intrapeptidyl H-bond and reverse binding of c-Cbl-TKB domain substrates. EMBO J 2008; 27(5):804–16.
[115] Blaikie P, Immanuel D, Wu J, Li N, Yajnik V, Margolis B. A region in Shc distinct from the SH2 domain can bind tyrosine-phosphorylated growth factor receptors. J Biol Chem 1994;269 (51):32031–4.
[116] Stolt PC, Jeon H, Song HK, Herz J, Eck MJ, Blacklow SC. Origins of peptide selectivity and phosphoinositide binding revealed by structures of disabled-1 PTB domain complexes. Structure 2003;11(5):569–79.
[117] Zhou MM, Ravichandran KS, Olejniczak EF, Petros AM, Meadows RP, Sattler M, et al. Structure and ligand recognition of the phosphotyrosine binding domain of Shc. Nature 1995;378 (6557):584–92.
[118] Lemmon MA, Ferguson KM. Signal-dependent membrane targeting by pleckstrin homology (PH) domains. Biochem J 2000;350 Pt 1:1–18.
[119] Eck MJ, Dhe-Paganon S, Trüb T, Nolte RT, Shoelson SE. Structure of the IRS-1 PTB domain bound to the juxtamembrane region of the insulin receptor. Cell 1996:85(5):695–705.
[120] Harrison SC. Peptide-surface association: the case of PDZ and PTB domains. Cell 1996;86 (3):341–3.
[121] Margolis B. The PTB domain: the name doesn't say it all. Trends Endocrinol Metab 1999;10 (7):262–7.
[122] Zhang Z, Lee CH, Mandiyan V, Borg JP, Margolis B, Schlessinger J, et al. Sequence-specific recognition of the internalization motif of the Alzheimer's amyloid precursor protein by the X11 PTB domain. EMBO J 1997;16(20):6141–50.
[123] Zwahlen C, Li SC, Kay LE, Pawson T, Forman-Kay JD. Multiple modes of peptide recognition by the PTB domain of the cell fate determinant Numb. EMBO J 2000;19(7):1505–15.
[124] Stolt PC, Jeon H, Song HK, Herz J, Eck MJ, Blacklow SC. Origins of peptide selectivity and phosphoinositide binding revealed by structures of disabled-1 PTB domain complexes. Structure 2003;11(5):569–79.
[125] Ong SH, Guy GR, Hadari YR, Laks S, Gotoh N, Schlessinger J, et al. FRS2 proteins recruit intracellular signaling pathways by binding to diverse targets on fibroblast growth factor and nerve growth factor receptors. Mol Cell Biol 2000;20(3):979–89.
[126] Howell BW, Lanier LM, Frank R, Gertler FB, Cooper JA. The disabled 1 phosphotyrosine-binding domain binds to the internalization signals of transmembrane glycoproteins and to phospholipids. Mol Cell Biol 1999;19(7):5179–88.
[127] Dhalluin C, Yan KS, Plotnikova O, Lee KW, Zeng L, Kuti M, et al. Structural basis of SNT PTB domain interactions with distinct neurotrophic receptors. Mol Cell 2000;6(4): 921–9.
[128] Ravichandran KS, Zhou MM, Pratt JC, Harlan JE, Walk SF, Fesik SW, et al. Evidence for a requirement for both phospholipid and phosphotyrosine binding via the Shc phosphotyrosine-binding domain in vivo. Mol Cell Biol 1997;17(9):5540–9.
[129] Takeuchi H, Matsuda M, Yamamoto T, Kanematsu T, Kikkawa U, Yagisawa H, et al. PTB domain of insulin receptor substrate-1 binds inositol compounds. Biochem J 1998;334 (Pt 1):211–8.

[130] Pitcher JA, Touhara K, Payne ES, Lefkowitz RJ. Pleckstrin homology domain-mediated membrane association and activation of the beta-adrenergic receptor kinase requires coordinate interaction with G beta gamma subunits and lipid. J Biol Chem 1995;270(20):11707–10.
[131] Calderwood DA, Yan B, de Pereda JM, Alvarez BG, Fujioka Y, Liddington RC, et al. The phosphotyrosine binding-like domain of talin activates integrins. J Biol Chem 2002; 277(24):21749–58.
[132] Garcia-Alvarez B, de Pereda JM, Calderwood DA, Ulmer TS, Critchley D, Campbell ID, et al. Structural determinants of integrin recognition by talin. Mol Cell. 2003;11(1):49–58.
[133] Hamada K, Shimizu T, Yonemura S, Tsukita S, Tsukita S, Hakoshima T. Structural basis of adhesion-molecule recognition by ERM proteins revealed by the crystal structure of the radixin-ICAM-2 complex. EMBO J 2003;22(3):502–14.
[134] Li S-C, Gish G, Yang D, Coffey AJ, Forman-Kay JD, Ernberg I, et al. Novel mode of ligand binding by the SH2 domain of the human XLP disease gene product SAP/SH2D1A. Curr Biol 1999;9 (23):1355–62.
[135] Liu BA, Jablonowski K, Shah EE, Engelmann BW, Jones RB, Nash PD. SH2 domains recognize contextual peptide sequence information to determine selectivity. Mol Cell Proteomics 2010;9 (11):2391–404.
[136] Fujita Y, Krause G, Scheffner M, Zechner D, Leddy HE, Behrens J, et al. Hakai, a c-Cbl-like protein, ubiquitinates and induces endocytosis of the E-cadherin complex. Nat Cell Biol 2002; 4(3):222–31.
[137] Rodriguez-Rigueiro T, Valladares-Ayerbes M, Haz-Conde M, Aparicio LA, Figueroa A. Hakai reduces cell-substratum adhesion and increases epithelial cell invasion. BMC Cancer 2011;11:474.
[138] Mukherjee M, Chow SY, Yusoff P, Seetharaman J, Ng C, Sinniah S, et al. Structure of a novel phosphotyrosine-binding domain in Hakai that targets E-cadherin. EMBO J 2012;31(5): 1308–19.
[139] Zhang D, Aravind L. Identification of novel families and classification of the C2 domain superfamily elucidate the origin and evolution of membrane targeting activities in eukaryotes. Gene 2010;469(1–2):18–30.
[140] Benes CH, Wu N, Elia AE, Dharia T, Cantley LC, Soltoff SP. The C2 domain of PKCdelta is a phosphotyrosine binding domain. Cell 2005;121(2):271–80.
[141] Wortmann A, He Y, Deryugina EI, Quigley JP, Hooper JD. The cell surface glycoprotein CDCP1 in cancer – insights, opportunities, and challenges. IUBMB Life 2009;61(7):723–30.

2 Protein phosphatases: Classification and domain architecture

2.1 The protein phosphatases

Regulation of signaling response in the interior environment of the cell requires the concerted action of protein kinases and protein phosphatases. As outlined in the first chapter, protein kinases are a superfamily of enzymes where all the members are extensively related to each other via their conserved hydrophobic core and the "spines" [1, 2]. Protein tyrosine kinases (PTKs), protein serine/threonine kinases (AGC kinases) and protein tyrosine-like kinases form three distinct branches of the same evolutionary tree (Figure 1.5). While human genomes encode about ~518 protein kinases, the number of protein phosphatases is far less (~150 genes) [3]. Also, unlike the protein kinases, protein phosphatases have evolved from structurally and mechanistically distinct ancestors.

Protein phosphatases can be structurally (not functionally) classified into seven classes (Table 2.1), which have evolved independently from different ancestral folds [4]. Bioinformatics studies indicate that these folds may have evolved from each other, where the sequence conservation or active site motifs have been conserved in evolutionary timescale. An older classification of protein phosphatases relied on their substrate specificity and classified them into pSer/pThr-specific, pTyr-specific or dual-specific phosphatases. This simplistic classification has since been revisited as subtle changes in structure influence substrate specificity and also similar substrate specificity of these enzymes could be achieved by subtle alterations in the structure [5, 6]. Also, the dual-specificity phosphatases (DUSP) included highly specific enzymes that used pTyr, pSer, phosphoinositides or mRNA as substrate. Nonetheless, this structural classification overlaps with a broad classification of these enzymes into two main classes: protein tyrosine phosphatases (PTPs) and the protein serine/threonine phosphatases (PSPs). There exist four distinct evolutionary families of PTPs: class I, II and III cysteine-based PTPs, and a distinct aspartate-based family of haloacid dehalogenase (HAD) phosphatases. The number of PTPs encoded in the human genome roughly matches the PTKs (~107 PTPs for ~95 PTKs). The number of PSPs is however much less (only ~30 genes). These PSPs exist as holoenzyme complexes comprising distinct catalytic (PSPs) and regulatory domains. While the number of catalytic subunit domains is quite less, extensive functional diversity is achieved by these phosphatases via combinatorial formation of holoenzymes with varied regulatory domains.

PSPs comprise three families: the phosphoprotein phosphatases (PPPs), the metal-dependent protein phosphatases (PPMs) and the aspartate-based phosphatases. Domain organization and representatives of each family are shown in Figure 2.1. Both PPPs and PPMs require a mechanistic role of metal ions at their active site. Metal ions

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Table 2.1: Classification of protein phosphatases.

	Phosphatase family	Examples
PSPs	1. PPP	PP1, PP2A, calcineurin, PP5
	2. PPM	PP2C
	3. FCP	FCP
PTPs	4. Class I Cys-based PTP	
	4.1 Classical PTPs	
	– Receptor PTPs	PTPα, CD45, CD148, GLEPP1
	– Non-receptor PTPs	PTP1B, TCPTP, SHP1, MEG2
	4.2 DSP or VH1-like PTPs	
	– MKPs	MKP1, MKP7, PAC1
	– Atypical DSPs	VHR, PIR, Laforin, VHZS
	– Slingshots	SH1, SSH2, SSH3
	– PRLs	PRL-1, PRL-2, PRL-3
	– CDC14s	CDC14A, KAP, PTP9Q22
	– PTENs	PTEN, TPIP, tensin, C1ten
	– Myotubularins	MTM1, MTMR1, MTMR3
	5. Class II Cys-based PTPs	CDC25A, CDC25B, CDC25C
	6. Class III Cys-based PTPs	LMPTP
	7. HAD family (Asp based)	Eya, CTD, cronophin

PTPs, protein tyrosine phosphatases; PSPs, protein Ser/Thr phosphatases; PPP, phosphoprotein phosphatase; FCP, aspartate-dependent phosphatase; PPM, metal-dependent phosphatase; LMPTP, low-molecular-mass protein tyrosine phosphatase; EyA, Eyes Absent; PRLs, phosphatases of the regenerating liver; PTEN, phosphatase and tensin homologue; VHZ, VH1-related phosphatase Z.

are used for activation of water that dephosphorylates the substrate phosphoserine/phosphothreonine. The aspartate-based phosphatases use their active site aspartate for this mechanism (hence their name). FCP (TFIIF-associating component of RNA polymerase II C-terminal domain [CTD] phosphatase) and SCP (small CTD phosphatase) are both aspartate-based protein phosphatases. Both FCP and SCP share a common substrate, the C-terminal domain of RNA polymerase II [7].

2.2 The phosphoprotein phosphatases

Protein phosphatase 1 (PP1), PP2A, PP2B (also known as calcineurin), PP4, PP5, PP6 and PP7 are all PPPs. All these members share high sequence homology and catalytic mechanism [8]. PP2C and pyruvate dehydrogenase phosphatase are both PPMs. Notably, although no sequence homology exists between PPPs and PPMs, both families have coevolved to share a metal-dependent dephosphorylation mechanism

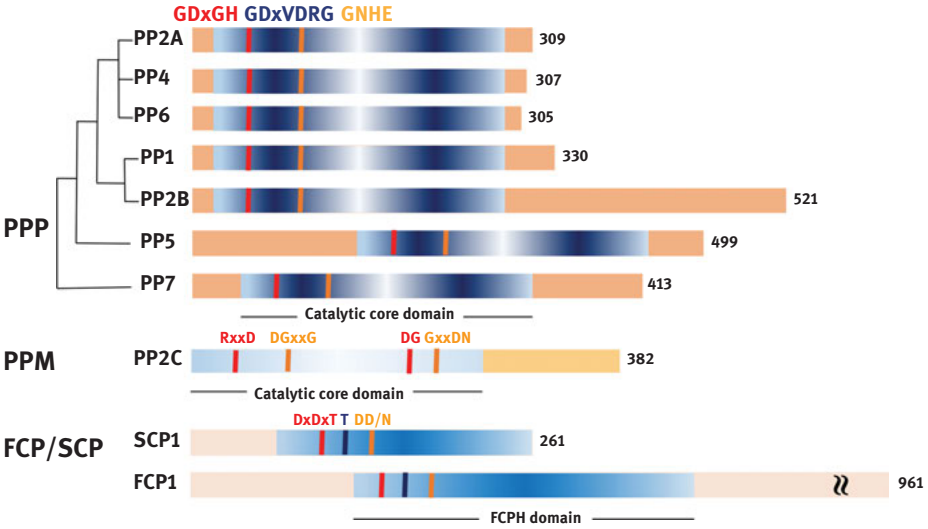


Figure 2.1: Domain organization of various protein Ser/Thr phosphatase gene families.

at their active sites. PPPs and PPMs also differ in domain organization, localization and cellular targeting. PPPs usually function as holoenzymes composed of a catalytic subunit and an associated regulatory subunit. Varied combinations of catalytic and regulatory subunits impart biological specificity to the holoenzyme. Differential posttransitional modifications of the catalytic and/or regulatory domains are used for enhanced regulation and specificity of these holoenzymes [9–11]. In contrast, the PPMs are generally reported to function as monomers [12]. Both PPPs and PPMs are implicated in various human diseases, including cardiovascular disorders, diabetes, Alzheimer's disease and also cancer [13–15]. This makes pharmacological targeting of these enzymes a lucrative strategy for dealing with these problems.

2.2.1 Protein phosphatase 1

PP1 is the major PSP present in all animal tissues. It functions in various cellular processes including cell division, cell death, protein synthesis, metabolism, regulation of membrane receptors and ion channels [16]. PP1 represents the historical phosphorylase phosphatase as first described by Gerty Cori in 1943 [17]. The three-dimensional structure of the first PP1 was almost simultaneously reported by two research groups in 1995 [18, 19]. The functional PP1 enzyme contains both a catalytic and a regulatory subunit. The sequence of the catalytic subunit shows a 70% conservation across family members with consistently placed active site residues and motifs. The backside of the catalytic subunit contains the residues required for regulatory subunit binding. About ~200 regulatory subunits have been predicted for the PP1 catalytic subunits based on

binding assays yielding proteins containing the common RVxF motif of the regulatory subunit [20]. Specific interactions between a defined catalytic and regulatory couple allow for compartmentalization of protein phosphatase activity to various cellular locations. These interactions also allow for modulation of the chemical kinetics of the catalytic subunit to accelerate specificity for certain substrates while simultaneously blocking access to others. Functionality of the PP1 enzyme complex hence depends on the specified catalytic and regulatory subunit pair.

The PP1 catalytic subunit is ~30 kDa protein that is conserved across all eukaryotes with a common reaction mechanism [18]. It adopts an α/β fold with β-sandwich structures surrounded by a large and a small α-helical domain (Figure 2.2). The joint of the β-sandwich and the two α-helical domains makes PP1 a Y-shaped groove that coordinates two metal ions required at the central active site. Mn^{2+} and Fe^{2+} are held together by three histidines, two aspartates and one asparagine residue. These residues form the conserved motifs G**D**xH**H**G, G**D**xVDRG and GN**H**E of the protein family that hold the metal ions required for efficient catalysis [18]. The metal ions bind and activate a water molecule that initiates a nucleophilic attack on the phosphate of the phosphoserine residue. Phosphatase activity of PP1 is inhibited by binding to inhibitory proteins expressed in the cells. These include the best studied DARPP32 [21,22] and inhibitor-1 and -2 proteins (also called I-1 and I-2) [23,24]. Inhibition of PP1 by these proteins requires their phosphorylation on a threonine residue that binds to lock the PP1 active site [24]. This provides an exquisite

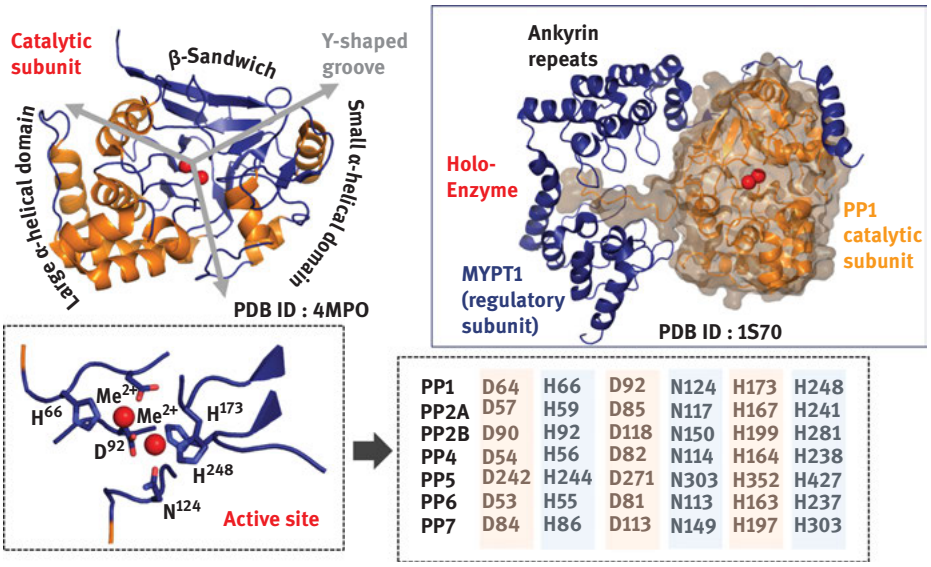


Figure 2.2: Molecular structure of PP1 phosphatase. The PPP family has a conserved active site with six amino acids that coordinate two metal ions. Each catalytic subunit associates with a regulatory subunit to form a holoenzyme.

mechanism by which protein kinases and protein phosphatases regulate each other's functional activities in the cell [25]. Inhibition of PP1 by inhibitor-1 and -2 proteins is specific and is the basis of classification of protein phosphatases into PP1 and PP2 subclasses. Both PP2A and PP2B remain uninhibited by inhibitor-1 and -2 proteins [26]. Separate endogenous inhibitor proteins exist for PP2A and PP2B. PP2A is inhibited by PHAP-1 (putative HLA class II-associated protein) [27]; while PP2B is inhibited by SET protein [28, 29]. PP1 and PP2 enzymes are also inhibited a variety of naturally occurring toxins such as okadaic acid that causes diarrhetic shellfish poisoning and microcystin, a live toxin produced by blue-green algae. The toxins bind in the catalytic channel of the Y-shaped PP1 and directly interrupt enzyme–substrate interactions at the phosphatase active site. Microcystin interacts with the two metal ions and okadaic acid binds the conserved arginine that coordinates the substrate phosphate [30].

Regulatory subunit binding to the catalytic subunit of PP1 occurs at the backside of the Y-shaped catalytic groove. Regulatory subunits contain a conserved RVxF/W sequence [31]. PP1-binding assays on proteins obtained from particulate fractions of cells for searching the interactome of PP1 identified about 78 novel proteins [20]. More sophisticated experiments have been conducted to identify a refined consensus sequence for regulatory subunit identification. A more robust sequence of [H/K/R][A/C/H/KM/N/Q/R/S/T/V][V][C/H/K/N/Q/R/S/T][F/W] has been proposed for the consensus RVxF/W sequence [32]. The highly conserved valine and phenylalanine/tryptophan residues are the anchors of the regulatory-catalytic subunit binding. Mutations in these residues to alanine directly abolish this specific interaction [31]. Variations in the other consensus sequence residues allow for identification and interaction between different catalytic and regulatory subunit pairs. Peptides corresponding to the RVxF/W are able to competitively disrupt catalytic and regulatory subunit pairs [31]. Residues of PP1 catalytic subunit interacting with the RVxF/W are conserved in all the PP1 isoforms but not in PP2 catalytic subunits. This provides regulatory subunit exclusivity to PP1 enzymes.

For over three decades, the main role of the regularity subunits is implicated in the cellular targeting of the catalytic subunits of PP1 [33]. Targeting enhances PP1 substrate specificity by making the phosphatase accessible to selected substrates in the vicinity of the targeting complex. However, in many cases, the PP1 holoenzyme formation can give rise to additional molecular surfaces that allow for altered and specific interactions of the substrate with the catalytic subunit. Structural studies of PP1 bound to the myosin phosphatase targeting subunit (MYPT1) shows how the binding of MYPT1 alters the Y-shaped surface groove of the active site of PP1. This also provides as example where regulatory subunits may use additional modes of binding to PP1 other than the conserved RVxF/W motif. MYPT1 binds PP1 in a tripartite mode using a TKVKF motif that interacts with a conserved hydrophobic patch on PP1, an ankyrin domain that caps the carboxyl terminus of PP1 and a hydrophobic helix at the MYPT1 N-terminal that docks into the PP1 surface [34]. Binding of MYPT1 to PP1 creates an additional

protein recognition surface for the incoming substrate. The ankyrin repeats of MYPT1 allow for enhanced substrate recognition by PP1 when present as an MYPT1-PP1 holoenzyme [35].

Protein phosphorylation is also used itself as a regulatory mechanism for PP1. Both catalytic and regulatory subunits of PP1 and also the inhibitor proteins are substrates for protein kinases including PKA, LMKTK2 and Nek2 [36, 37]. Phosphorylation of the catalytic subunit occurs at the P[V/I]TPP site at the C-terminal of PP1. Phosphorylation reduces the catalytic efficiency of the phosphatase, but serves as reversible switch that controls its action. Regulatory subunits including MYPT1 and GM are phosphorylated on multiple sites that directly alter substrate access or kinetics of the PP1 catalytic enzyme.

2.2.2 Protein phosphatase 2A

Protein phosphatases implicated in control of cellular metabolism have been classified into type I (PP1) and type II (PP2A, PP2B) [26]. PP2A is one of the most abundant cellular proteins and plays important roles in cellular signal transduction and control of cell cycle as a tumor suppressor [38]. PP2A was originally purified using histones and glycogen synthase as substrates and the first PP2A enzyme was studied in the recombinant form in late 1980s [39,40]. In cells, PP2A occurs in two forms – a heterodimeric form that functions as the core enzyme and a heterotrimeric form that functions as the holoenzyme [41]. The heterodimeric core enzyme is formed by a scaffolding subunit (A-subunit) and a catalytic subunit (C-subunit), both of which exist in two isoforms α and β. The α-isoform is more abundant than the β-isoform [42]. This heterodimeric core interacts with a variety of regulatory proteins (B-subunit) to form the heterotrimeric ABC holoenzyme. These regulatory PP2A B-subunits can be divided into four subfamilies: Family B (B55 or PR55), family B′ (B56 or PR61), family B″ (PR48/PR72/PR130) and family B‴ (PR93/PR110). Each mammalian B-subunit gene is encoded by various isoforms and also has splice variants. This provides a wide diversity to the regulatory subunits in the needs of the cell. Expression of required isoforms and splice variants of regulatory proteins provides cell and tissue-specific PP2A signaling. PP2A holoenzymes exhibit both spatially and temporarily regulated functions alongside an exquisite substrate specificity. Specificity is achieved by the various regulatory B-subunits. Holoenzymes comprising B family (B55 or PR55) of regulatory subunits but not B′(B56 or PR61) or family B″ (PR48/PR72/PR130) participate in dephosphorylation of the Tau proteins that bind microtubules in the cell [43–45]. Hyperphosphorylation of Tau proteins leads to the formation of neurofibrillary tangles in the brain, thus becoming one of the primary causes of Alzheimer's disease. Mutagenesis studies on the Tau protein has unveiled regions in its microtubule-binding repeats that

interact with the B-subunits of PP2A. Similarly, B′ (B56 or PR61) family of regulatory subunits is specific in binding to the conserved kinetochore protein shugoshin [46, 47].

The PP2A catalytic subunit (C-subunit) is analogous to the PP1 enzyme and has the same arrangement of a β-sandwich flanked by two α-helical domains (Figure 2.3). Two metal ions are coordinated at the center of Y-shaped groove at the surface of the protein that participates in activating a water molecule for the dephosphorylation reaction. The PP2A catalytic subunit also has a unique C-terminal tail (residues 294–309) that harbors a motif $TPDY_{307}FL_{309}$. This motif is also present in other PP2A-like phosphatases including PP4 and PP6. Methylation of the terminal Leu 309 allows for the recruitment of B-subunits to A- and C- subunit heterodimers [48, 49]. Deletion of the C-terminal tail abolishes this recruitment, compromises the function of PP2A and contributes to Alzheimer's disease pathogenesis [50, 51]. Methylation of Leu 309 is also crucial for the maintenance of G0/G1 and G1/S boundaries in cell cycle [52]. The C-subunit of PP2A is a potent target of tumor-inducing toxins including okadaic acid and microcystin. Both inhibitors interact with the same set of residues in the active site of the C-subunit [53]. Okadaic acid is about 100-fold potent in inhibiting PP2A than PP1 as the hydrophobic of the PP2A C-subunit is better defined as compared to the okadaic acid-binding site in PP1 [53]. PP2A catalytic subunit has been defined to be activated by various polycations including protamine, spermidine, spermine and Mg^{2+} ions [37]. The earlier "PCM" nomenclature of the PP2A enzymes indicated that they were polycation modulated.

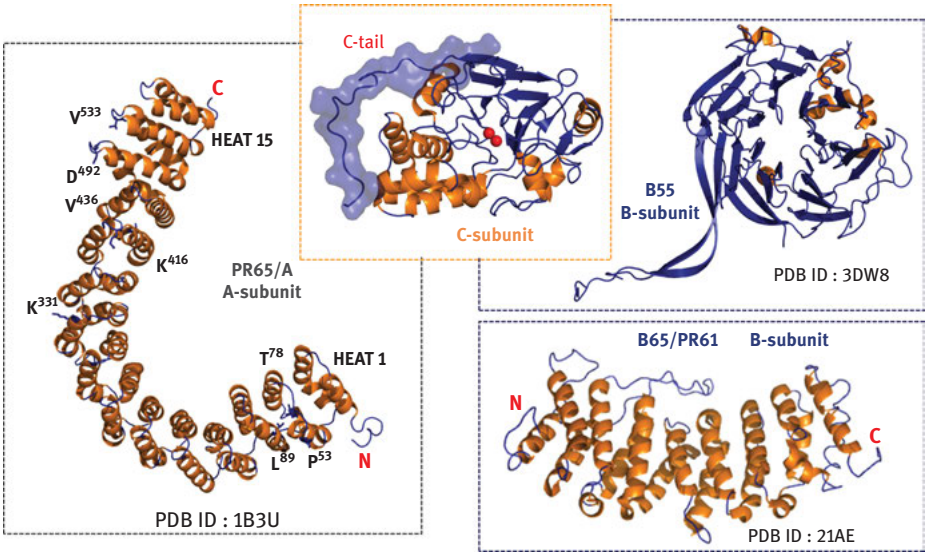
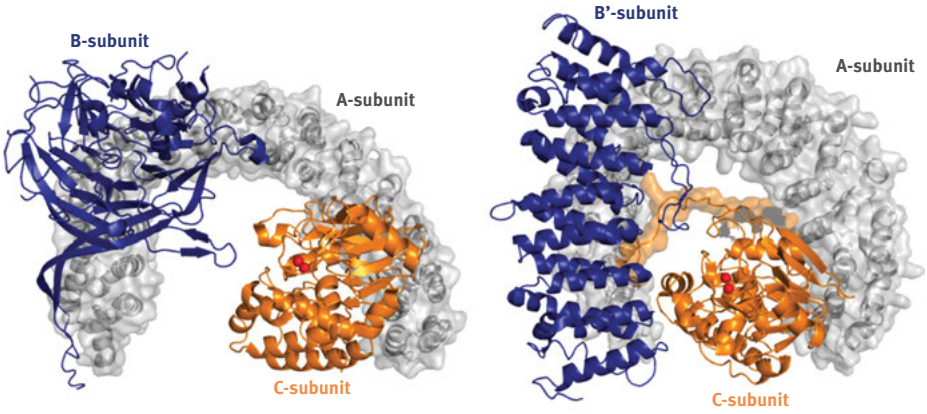


Figure 2.3: Three different constituents of PP2A holoenzyme. Shown are the molecular structures of the A-subunit, B-subunit, B'-subunit and C-subunit.

Although the PPP catalytic subunits share high sequence similarity in general, association of the scaffolding A-subunit is specific for the catalytic C-subunit of only PP2A-type enzymes. This protein–protein interaction surface is absent in the catalytic subunits of PP1, PP2B, PP5 and PP7 and is heavily modified in PP4 and PP6. Association of the A- and C-subunits has been crucial for the recruitment of the B-subunit to form the ABC heterotrimer. Human lung and colon cancer mutations have been mapped to the A-subunit of PP2A that prevents B-subunit binding to AC core enzyme [54]. Tumor-derived mutations R418W and V454A have also been mapped to the A-subunit and are speculated to dismember the A- and C-subunit core enzyme interface [55, 56]. The A-subunit of PP2A is ~65 kDa and consists of 15 tandem repeats of the HEAT (Huntington elongation A subunit-TOR) domain that come together to form a horseshoe structure [57] (Figure 2.3). The HEAT domain is a globular domain containing primarily α-helices. The interhelical loops of the HEAT domain are conserved, and they form a ridge in the horseshoe-shaped structure that is exclusively recognized by the catalytic subunit of PP2A [58]. Formation of the core AC heterodimer harnesses the conformational flexibility of the HEAT domain arrangement. HEAT repeats 13–15 are shifted as over 20–30 Å when associating the catalytic C-subunit of PP2A [53]. Formation of the ABC holoenzyme uses the N-terminal HEAT domains of the A-subunit that twist and move by 50–60 Å to accommodate the regulatory B-subunit [43]. This remarkable conformational flexibility of the scaffolding A-subunit also plays a role in substrate recruitment by the PP2A holoenzymes.

The PP2A holoenzyme is formed by stoichiometric complexation of A-, B- and C-subunits. Both the regulatory B-subunit and the catalytic C-subunit dock onto the apical side of the horseshoe structure of the A-subunit via interactions with the interhelical loops [59]. The B-subunit recognizes the amino terminal HEAT repeats of the A-subunit. B55 (B-family) subunit identifies the HEAT 1–7 repeats while the B65γ1 (B′-family) subunit identifies the HEAT 2–8 repeats. The opposite side of the A-subunit horseshoe structure is occupied by the catalytic subunit through interactions with the HEAT 11–15 repeats [43]. Binding of the B- and C-subunits on the A-subunit causes the HEAT repeats 10 and 11 to pivot as much as 35 Å such that the N- and C-termini of the A-subunit are now closer to each other in the heterotrimer as opposed to AC heterodimer [60]. Extensive interactions between the B65γ1 (B′-family) and the C-subunit ensure a tighter holoenzyme that is more compact and rigid as compared to the B55 (B-subunit) containing PP2A holoenzyme (Figure 2.4). The substrate-binding site in the ABC holoenzyme is formed at the top proximal face of the B-subunit that faces the catalytic subunit.

Just like their substrate specificity, the different B-family subunits are drastically different in their structures. The B55 (B-subunit) makes a seven-bladed β-propeller where each blade is made of four antiparallel β-strands. The four strands designated as A, B, C and D radiate from central torus-like structure of the WD40 domain [61]. In addition to this β-propeller structure, B55 also has two β-hairpins and two α-helices. These elements are located on the top face of the β-propeller that forms a highly acidic groove



PP2A holoenzyme with B55 subunit
PDB ID : 3DW8

PP2A holoenzyme with B65/PR61 subunit
PDB ID : 2IAE

Figure 2.4: Comparison of ABC and AB'C-type holoenzyme of PP2A.

for substrate recruitment. Beta-strands C and D of blade 2 of the β-propeller extend out of the propeller structure that makes a β-hairpin to contact the A-subunit at HEAT repeats 1 and 2. The bottom face of the β-propeller structure binds HEAT repeats 3–7 of the A-subunit. Interactions between the B55 and A-subunit are tighter as opposed to the interactions between B55 and the catalytic subunit. This indicates that the presence of the C-subunit may not be required for the formation of a complex between the A-subunit with the B55 protein [60]. Dephosphorylation assays have allowed for the identification of Tau-binding regions on the B55 protein in the holoenzyme [60]. Mutations in the central groove of the β-propeller B55 structure make the PP2A inefficient in dephosphorylating Tau. Biochemical analysis of overlapping Tau peptides indicates that Tau uses two segments: residues 197–259 and residues 265–328 to binding to the PP2A holoenzyme.

In contrast to the β-propeller structure of B55, B65γ1 takes a solenoid shape made of about eight α-helical bundles that closely resemble HEAT repeats despite no sequence similarity with them [62]. Long interhelical loop between repeats 1 and 2 allows for contacts between the B65γ1 and C-subunit. Binding of B65γ1 to the A-subunit is a loose packing that involves the binding of A-subunit HEAT repeats 2–8 to the convex side of the B65γ1 α-helical repeats. Relatively the interaction between the B65γ1 and the A-subunit is weak and forms a loose AB complex. The methylated C-tail of the C-subunit is crucial in binding together the B65γ1 containing heterotrimer. This C-tail of the C-subunit interacts with the pseudo-HEAT repeats 4–6 of the B65γ1 subunit. In the unmethylated form, the affinity between the AC core heterodimer and the B65γ1 is not enough to allow ABC complex formation. The carboxyl group of Leu309 in the C-subunit repels the negatively charged cluster on the A-subunit comprising Glu62, Asp63, Glu64 and Glu101. Methylation of Leu309 by phosphatase methyltransferases neutralizes this charge repulsion, allowing the C-tail to settle down on the A-subunit

and creating a docking site for the B65γ1 subunit (Figure 2.4). Phosphorylation of the C-subunit C-tail at Tyr307 by tyrosine kinases like Src and of B65γ1 by Erk kinase allows for regulation of PP2A holoenzyme formation and activity [63, 64]. The complex between B65γ1 containing PP2A holoenzyme has been characterized for its binding to the substrate shugoshin (Sgo 1) [65]. Utilizing the helical structure of the B65γ1, Sgo 1 binds the holoenzyme as a homodimer of coiled coils. The N- and C-terminals of the Sgo 1 coiled coil make discrete interactions with B65γ1 and the C-subunit. Each binding surface uses its own hydrophobics, hydrogen bonds and electrostatic interactions to make a tight holoenzyme–substrate complex.

2.2.3 Protein phosphatase 2B

Protein phosphatase 2B is also known as calcineurin that participates in various calcium-dependent cellular signaling. These include the biological processes of neurodevelopment, memory formation, immune response and cardiac health [66]. The PP2B enzyme is composed of a catalytic subunit called calcineurin A (CNA) and a regulatory subunit known as calcineurin B (CNB). The CNA catalytic subunit is structurally similar to the PP1 enzymes and uses similar metal coordination at the active site [67, 68]. These metal ions have been identified as Zn^{2+} and Fe^{3+} for CNA. Other than the phosphatase domain, CNA contains an autoinhibitory element, a Ca^{2+}-calmodulin-binding motif and CNB-binding helical domain (Figure 2.5). The

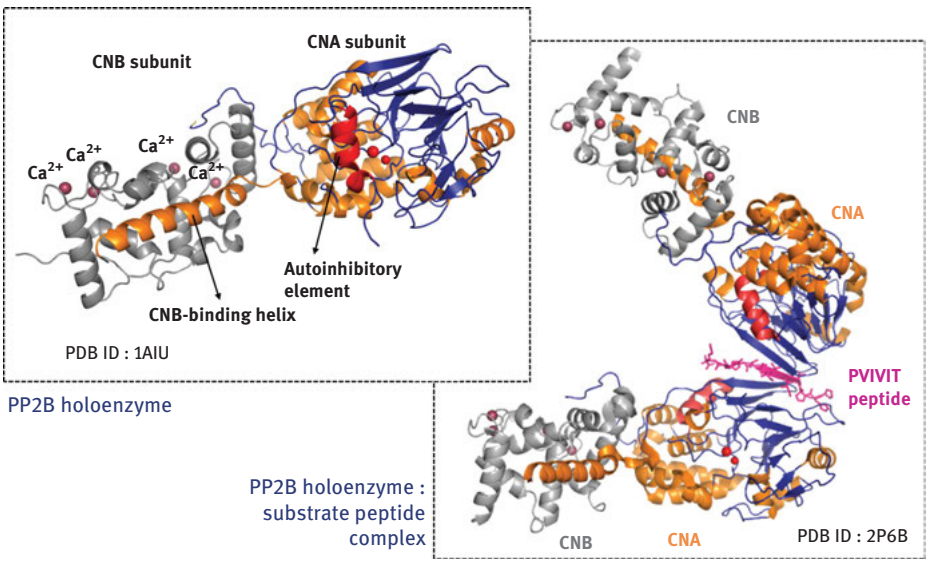


Figure 2.5: Structure of PP2B (calcineurin) showing the CNA and CNB subunits. The PP2B holoenzyme binds the substrate PVIVIT peptide by β-augmentation.

CNB domain contains two calcium-binding domains, each containing two helix-loop-helix EF-hand motifs that coordinate a total of four Ca^{2+} ions per CNB molecule. The CNB-binding helix of CNA subunit binds into the hydrophobic face of the CNB subunit in the middle of these two calcium-binding domains.

In the inactive state, the autoinhibitory element of CNA subunit blocks access to the active site of the catalytic subunit [68]. Docking of the autoinhibitory element uses a combination of hydrogen bonds and *van der Waal* contacts. Displacement of this element away from the active site of calcineurin is key to its activation but is not clearly understood. Also, activation of calcineurin by Ca^{2+}-calmodulin is a topic of intense scientific study, but a definitive answer is a long way ahead. Biochemical and structural studies have revealed some clues to this activation. While Ca^{2+}-calmodulin exists as a monomer, the Ca^{2+}-calmodulin-binding motif of calcineurin forms a continuous α-helix that can coordinate the complexation of Ca^{2+}-calmodulin in a head-to-tail dimer [69]. These studies indicate that the activated calcineurin probably functions as a dimer along with Ca^{2+}-calmodulin. Biochemical studies suggest that it is possible for the substrate peptide to bind calcineurin, Ca^{2+}-calmodulin in a 2:2:1 ratio. Calcineurin recognizes its substrate proteins using the consensus recognition sequence PxIxIT motif [70,71]. Two molecules of calcineurin can bind a single substrate peptide by β-segmentation allowing the peptide to bind the edge of their β-sheet as a β-strand [72]. Variations in the conserved PxIxIT motif allow for a wide range (0.5–250 μM) of binding affinities between calcineurin and its substrates.

2.2.4 Protein phosphatase 5

PP5 forms a unique system of PPP where the catalytic and regulatory domains are encoded in the same polypeptide chain. PP5 was identified late as compared to other members of the PPP family [73]. PP5 is ubiquitously expressed in all mammalian tissues. It plays key roles in processes of cell proliferation, cell differentiation, cell migration and cell death [74]. PP5 is most widely studied for its effect on glucocorticoid receptor (GR) signaling. PP5 controls GR signaling by directly interacting with the Hsp90-GR complex [75]. Two truncated versions of PP5 exist in the form of p56 and p50. The full-length PP5 protein is 58 kDa. The p56 isoform is generated by cleavage of the N-terminus, while the p50 isoform is generated by the proteolytic cleavage of a C-terminal region [76]. Formation of p50 removes the nuclear localization signal present at the C-terminus (residues 476–491) of PP5, thus limiting the presence of p50 isoform to the cytosol.

The phosphatase domain of PP5 lies in its C-terminal region and possesses all the key elements of the conserved PPP domain [77]. Residues Arg275, Asn303, His304 and Arg400 allow for direct binding of phosphoamino acids of the substrate proteins at the PP5 active site. However, sequence of the PP5 phosphatase domain is only about 40% similar to the phosphatase domains of PP1 or PP2A and PP2B. This low-sequence

homology between the phosphatase domains had prompted a separate classification of PP5 into a unique class in the 1990s [78]. PP5 also contains other structural elements unique to its sequence that include a peptidyl-prolyl *cis–trans* isomerase-like domain and three consecutive tetratricopeptide repeat (TPR) domains N-terminal to phosphatase domain. TPR domains are protein–protein interaction domains of about 34 amino acids that fold into a pair of antiparallel α-helices [79]. Adjacent TPR domains contact each other to pair these pairs of antiparallel α-helices generating a groove that serves as the interacting protein docking surface (Figure 2.6). The consecutive TPR domains of PP5 are known to bind the C-terminal sequence of Hsp90 at the acidic Glu-Glu-Val-Asp motif. Basic residues of PP5 namely Lys32, Arg74, Lys97 and Arg101 are crucial for this interaction. The second TPR domain of PP5 also contributes to its autoinhibition by interacting with its own C-terminal region (αJ, residues 490–499) [80]. The αJ segment binds in the surface groove of the TPR domains causing it to fold over the phosphatase domain and occlude substrate binding. Truncation of the TPR domains in the p56 isoform is speculated to allow for its constitutive activation, perhaps with altered substrate binding. Competition assays using a peptide of Hsp90 show that it binds the isolated TPR domains more strongly than the PP5 enzyme [81]. Structural studies on PP5 bound to Hsp90 peptide show an induced conformational shift in the α7 helix of its TPR domain that seems incompatible with intra-chain αJ binding [82].

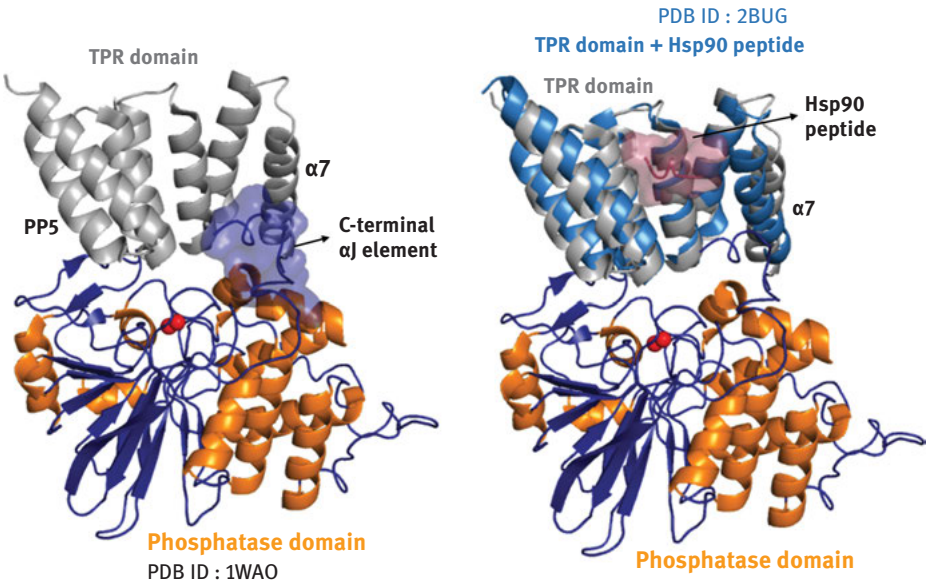


Figure 2.6: Molecular structure and working of PP5. Binding modes of αJ and HSP90 peptide are nonoverlapping and mutually exclusive.

The agility of PP5 TPR domains to accommodate ligand-induced conformational change is central to its function in the environment of cell. PP5 uses its TPR domain to interact with various G-proteins including $G_{\alpha 12}$, $G_{\alpha 13}$ and also the GTPase Rac [83, 84]. These interactions stimulate the phosphatase activity of PP5 by releasing the inhibitions that account for its low basal activity. Other than proteins, long-chain polyunsaturated fatty acids such as arachidonic acid can also bind the TPR domains of PP5 [85]. This indicates that PP5 function is also regulated by lipid second messengers such as arachidonyl-CoA, making PP5 a lipid-stimulated protein phosphatase.

2.2.5 Natural toxins as inhibitors of PPP catalytic subunits

Small-molecule modulators of PPPs have allowed for the elucidation of their complex signaling processes, also allowing for identification of their substrate preferences. More than a dozen natural toxins have studied as potent inhibitors of PPPs (Table 2.2). PP1 and PP2A proteins are seen to be most sensitive to these toxins, followed by PP4, PP5 and PP6. PP2B (calcineurin) and PP7 are seen to be less sensitive or responsive to these inhibitors such as okadaic acid, calyculin A, nodularin and tautomycin. These toxins target the highly conserved catalytic center of PPPs. This includes the six conserved amino acids and the two metal ions coordinated at the active site.

Table 2.2: Natural toxin inhibitors of PP1 and PP2A.

Phosphatase		Toxin	PDB ID
PP1	α-Isoform, catalytic subunit	Microcystin	**1FJM**
PP1	α-Isoform, catalytic subunit	Nodularin-R	**3E7A**
PP1	α-Isoform, catalytic subunit	Tautomycin	**3E7B**
PP1	γ-Isoform, catalytic subunit	Okadaic Acid	**1JK7**
PP1	γ-Isoform, catalytic subunit	Calyculin	**1IT6**
PP1	γ-Isoform, catalytic subunit	Motupurin	**2BCD**
PP1	γ-Isoform, catalytic subunit	Dihydromicrocystin-LA	**2BDX**
PP2A	AB′C holoenzyme with Shugoshin	Microcystin-LR	**3FGA**
PP2A	α-Isoform, A-subunit with α-isoform, C-subunit	Microcystin-LR	**2IE3**
PP2A	α-Isoform, A-subunit with α-isoform, C-subunit	Okadaic Acid	**2IE4**
PP2A	α-Isoform, A-subunit with α-isoform, C-subunit	Dinophysistoxin-1	**3K7V**
PP2A	α-Isoform, A-subunit with α-isoform, C-subunit	Dinophysistoxin-1	**3K7W**

One of the natural toxins frequently used to modulate PPPs is microcystin. Microcystin is a family cyclic hexapeptides generated by cyanobacteria [86]. The most well-characterized member of this hexapeptide is microcystin-LR, although about 80 known variants are found in nature. Microcystin inhibits the catalytic subunits of PP1 and PP2A with an equipotent inhibition constant of about 0.7 nm [87, 88]. Microcystin sits

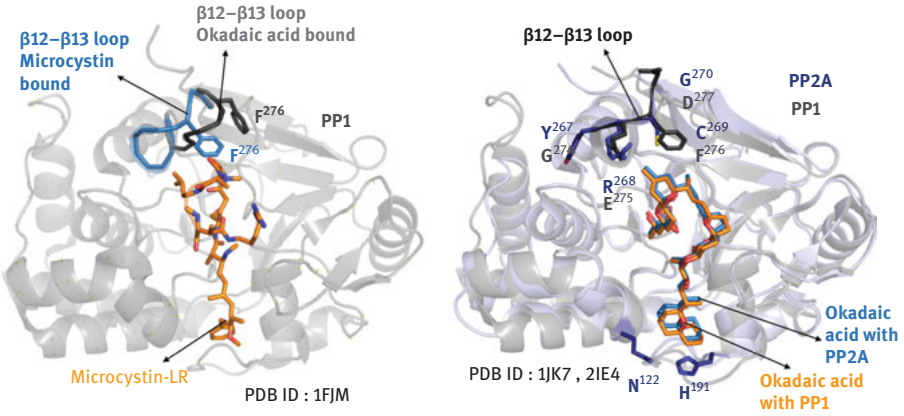


Figure 2.7: Mode of binding of natural toxins to structures of PP1 and PP2A. The β12–β13 loop decides the modes of binding of microcystin and okadaic acid to PP1. Binding of okadaic acid to PP2A is enhanced by interactions with N121 and H191 at the bottom of the binding cleft.

through the hydrophobic groove emanating from the PPP bimetal active site center and forms a covalent bond with Cys of the β12–β13 loop (Figure 2.7). However, mutagenesis of the Cys is shown to have little effect on microcystin binding and could be an after-reaction upon prolonged incubation with the inhibitor [89, 90].

The other common natural toxin of PPPs is okadaic acid that causes diarrhetic shellfish poisoning [91]. Okadaic acid is a monocarboxylic acid that inactivates the PPPs by binding to their bimetal active site. Okadaic acid forms a cyclic structure connecting its hydrophobic spiroketal moiety and acidic moiety by using an intramolecular hydrogen bond. The hydrophobic spiroketal moiety accounts for the high potency of okadaic acid. Okadaic acid is most potent against PP2A (IC_{50} = 0.1 nM) as opposed to PP1 (IC_{50} = 15.0 nM). It is a much poor inhibitor of PP2B calcineurin with IC_{50} of only 4.0 μM [92]. Mutagenesis and structural studies have shown that residues Arg96, Arg221, Tyr272 and Phe276 of PP1 are crucial for toxin binding (Figure 2.7) [93]. Difference in the potency of the toxin for various PPP is explained by the subtle differences in the active sites of PP1 and PP2A [53]. The difference lies in the β12–β13 loop, which also participates in binding to microcystin. Mutation of Phe276 of PP1 to Cys (as present in PP2A) enhanced the binding affinity for okadaic acid by about ~40-fold [94]. Also, residues Gln122 and His191 of PP2A contribute to binding of okadaic acid from the open end of the hydrophobic groove using *van der Waal* interactions.

2.3 The metal-dependent protein phosphatases

The founding member of this family of protein phosphatases was purified as the Mg^{2+}-dependent Ser/Thr phosphatase that activated liver glycogen synthetase

[95]. This enzyme was designated as PP2C, but now the family members are denoted as PPM1 followed by a letter (A, B, C, etc.). The human genome encodes 16 distinct PPM genes that give rise to at least 22 distinct PP2C isoforms [96]. Sequence analysis of PPM [97], its insusceptibility to natural toxins okadaic acid and microcystin and structural studies on the protein [98] confirm its distinct evolutionary lineage from the PPPs. Also, unlike the PPPs, PPMs are seen to function as monomers and no regulatory or inhibitory subunits are known. The primary role of PPMs is seen in association with stress-related protein kinases for the regulation of stress signaling, metabolism, apoptosis and cell survival [99]. It is noteworthy that the plant genome contains far more PPMs than the human genome (~80 genes in *Arabidopsis*) [100].

The catalytic core domain of PPMs contains a β-sandwich in the center with two flanking pairs of α-helices alongside the β-sheets (Figure 2.8) [98]. Two metal ions are located at the base of the cleft formed between the two β-sheets. Similar to the reaction mechanism of PPPs, PPMs function by the activation of a water molecule through the hexacoordinated active site metals using an S_N^2 mechanism. Three accessory α-helices present attached to the core domain may contribute to its regulation or participate in substrate selection. Compared to the human PPMs, bacterial PPMs use three metal ions at their active site. The structural core and the six active site residues of these bacterial homologues from *Mycobacterium*

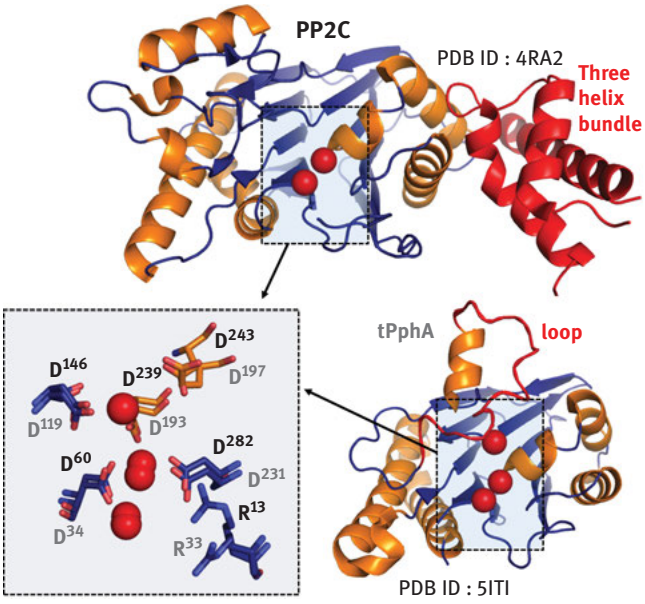


Figure 2.8: Structure of metal-dependent phosphatase (PPM), PP2C. The human structure has an accessory three helix bundle. The prokaryotic homologue, tPphA, has a conserved active site but binds three metal ions.

tuberculosis and *Streptococcus agalactiae* are highly conserved. These bacterial homologues also contain a loop that covers the active site and probably participates in allowing substrate access to the metal center [101, 102].

PPM isoforms exhibit distinct functions, cellular localizations and are highly varied in their domain organization and protein–protein interactions. PPM1A is activated by binding and allosteric modulation by a putative insulin second messenger INS2 [103]. PPM1 hence acts as the phosphatase that modulates glycogen synthetase of the liver and participates in insulin-regulated signaling. This indicates that the role of PPMs lies in hormone signaling in humans, much like in *Arabidopsis*. In plants, PPM1 heterodimer binds to the START family of receptors and increases their affinity for abscisic acid [104]. PPM1D phosphatase is also known as Wip1. Wip1 is an oncogene whose transcription is induced by the action of p53 in response to DNA damage [105]. In a feedback loop, Wip1 then serves to dephosphorylate p53. Reciprocally, overexpression of Wip1 is seen to increase the level of activated p53 protein in cells [106]. Wip1 also participates in the regulation of stress kinases including ATM, Chk2 and p38 mitogen-activated protein kinase (MAPK) [107]. PPM1G phosphatase is a part of the spliceosome complex and binds to YB-1 protein to modulate alternative splicing [108]. Phosphorylation of PPM1G enhances its binding to YB-1, hence providing an additional layer to its regulation. Some PPMs are called Pleckstrin homology (PH) domain leucine-rich repeat protein phosphatases (PHLPP) as they contain a PH domain for association with phosphoinositides. PHLPP1 dephosphorylates the kinase Akt at its Thr308 position and promotes the cells decision to apoptosis [109]. Other PHLPPs also promote apoptosis by dephosphorylating the kinase MST1 [110]. PHLPP hence serves as a tumor suppressor.

Inhibitors to PPM1A (PP2Cα) and PHLPP have been designed in attempts to study their downstream substrates and interacting proteins [111]. 1-Amino-9,10-dioxo-4-(3-sulfamoylanilino1) anthracene-2-sulfonic acid and (1,3-[[4-(2,4-diamino-5-methylphenyl) diazenylphenyl] hydrazinylidene]-6-oxocyclohexa-1,4-diene-1-carboxylic acid have been identified as PHLPP inhibitors with IC_{50} in the lower micro-molar range [112]. Nonphosphate-based inhibitors have been identified for PPM1A, but are weak in their binding affinities [113]. Phosphonothioic acid inhibits PPM1A with a K_i of 15.0 μM [114]. A natural plant alkaloid, sanguinarine, has been identified as a selective inhibitor of PPM1A. Sanguinarine serves to be specific for PPMs (IC_{50} = 2.5 μM) as compared to PP1 (IC_{50} = 42.5 μM), PP2A (IC_{50} > 100.0 μM) or PP2B (IC_{50} = 77.0 μM) [115].

Although a decent body of work is available for PPMs, more attention has been focused on their oncogenic or tumor suppressor functions. Wip1 (PPM1D) gene is known to be amplified in over 15% of breast cancer samples [116]. Overexpression of PPM1D is also seen in pancreatic adenocarcinomas, ovarian cancer and also gastric cancer [117]. Depletion of PPM1D in mouse models of breast cancer is seen to greatly increase their viability and PPM1D null mouse fibroblasts and reported to be resistant to transformation [118, 119]. Wip1 functions as a negative regulator of p53

and hence directly involves in the MAPK and p16 (Ink4-a)–p19(Arf) pathways [119]. Wip1 is a lucrative target for anticancer drug discovery initiatives. Phosphatase activity-based assays are being used to establish compounds that can be used as Wip1 inhibitors that do not cross-react with PP2A or PPM1A (PP2Cα) [120]. Substrate-inspired cyclic hexapeptides have also been designed as potential competitive inhibitors of Wip1 [121]. Contrary to PPM1D oncogene, the tumor suppressor PHLPP gene invigorates the discovery of small-molecule activators. This is a far more challenging research pursuit than the identification of inhibitors that would allow for trapping of the protein in inactive states. PPM1A gene encodes the PP2Cα phosphatase that also serves as a tumor suppressor. Rigorous studies have identified a small-molecule activator for PPM1A called NPLC0393 that has shown potential to prevent liver fibrosis by enhancing PPM1A activity. Activation of PPM1A negatively affects the transforming growth factor β pathway and rescues cells from fibrosis [122].

2.4 FCP/SCP phosphatases

The FCP/SCP family of protein phosphatases are a subset of the HAD superfamily of enzymes. These enzymes use the conserved aspartate in the sequence DxDxT/V to catalyze the transfer of a phosphate to a substrate protein using a single Mg^{2+} metal ion [123]. Another distinguishing feature of this family is their distinct reaction mechanism from the PPPs or PPMs. These phosphatases catalyze phosphotransfer by orchestrating a nucleophilic attack on the phosphate group by their active site aspartate leading to the formation of a phosphoaspartyl intermediate (Figure 2.9). This phosphoaspartyl intermediate of FCP/SCPs has been captured and confirmed by X-ray crystallography [124]. In the later step, a water molecule is used to break the phosphoaspartyl and release the free phosphate and regenerate the active site aspartate [7, 124]. This reaction mechanism resembles the mechanism of PTPs that use an active site cysteine to make a cysteinyl-phosphate intermediate. The metal ion Mg^{2+} neutralizes the negative charge on the phosphate and passively participates in catalysis; unlike in the PPPs and PPMs where the two metal ions are directly involved in the activation of water for the dephosphorylation reaction.

In a unique order of signaling, these FCP/SCP phosphatases have only one primary substrate. This substrate is the C-terminal domain (CTD) of RNA polymerase II that harbors Y**S**PTSP**S** repeat sequences. The number of these Y**S**PTSP**S** repeats varies from 26 repeats in yeast to 52 repeats as seen in the human RNA polymerase. [125]. Phosphorylation of the second and the fifth serine cycle during different rounds of transcription and their levels are kept under tight regulation. Phosphorylation of serine residue at the fifth position ($pSer_5$) is required in transcription initiation and early stages of transcription elongation. Phosphorylation of serine residue at the second position ($pSer_2$) is required in transcription elongation and transcription termination.

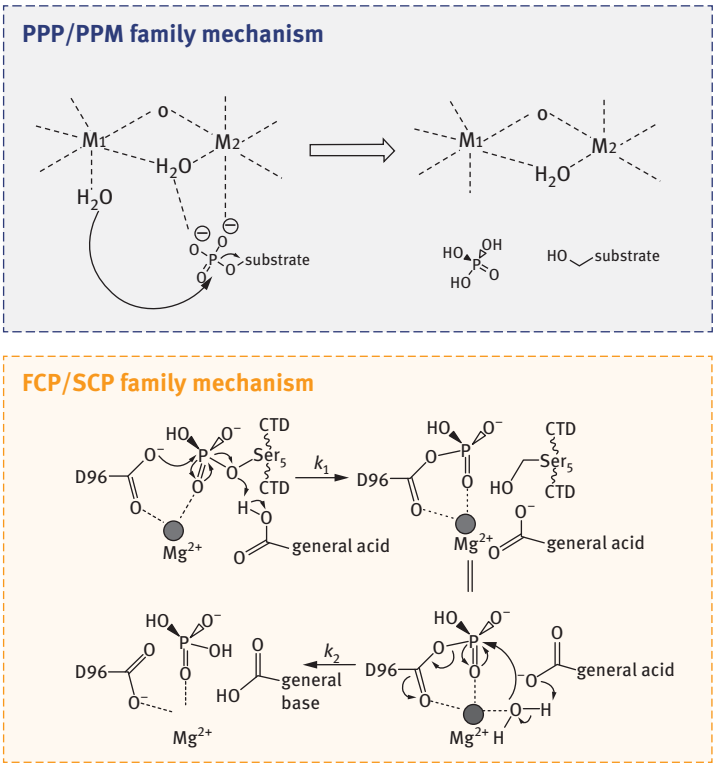


Figure 2.9: Catalytic mechanisms of PPP/PPM and FCP/SCP family of protein phosphatases. Figure adapted from https://commons.wikimedia.org/wiki/File:PP1_Mechanism_1.png and Ref [124].

Hence, a specific C-terminal code that uses various levels of phosphorylations on the second or the fifth serine constitutes the signal for effective transcription [126]. Enrichment of different phosphorylated states of CTD of RNA Polymerase II allows for its interaction with various regulatory proteins and transcriptional influencers [127, 128]. FCP/SCP hence regulate the cycling of RNA polymerase II and are essential for cell viability [129]. FCP can dephosphorylate both the phosphoserines on the CTD sequence, but show preference for the $pSer_2$ [129]. In *Saccharomyces*, this preference of FCP for $pSer_2$ over $pSer_5$ is about sixfold [130]. In contrast, SCP shows a 70-fold preference for $pSer_5$ over $pSer_2$, and is a CTD phosphatase found only in higher eukaryotes [131]. These unique substrate presences of SCP and FCP can allow for design of specific inhibitor for one that may not cross-react with the other [132].

Scp1 forms an α/β fold with a central β-sheet made of five β-strands (Figure 2.10) [7, 131]. The active site motif DxDxT is located C-terminal to the first β-strand in the core of the domain. An insertion domain made of three α-helices immediately follows the conserved motif. This domain of ~40 amino acids is the most divergent in SCP sequence and is reminiscent of WW domains that recognize prolines in interacting proteins.

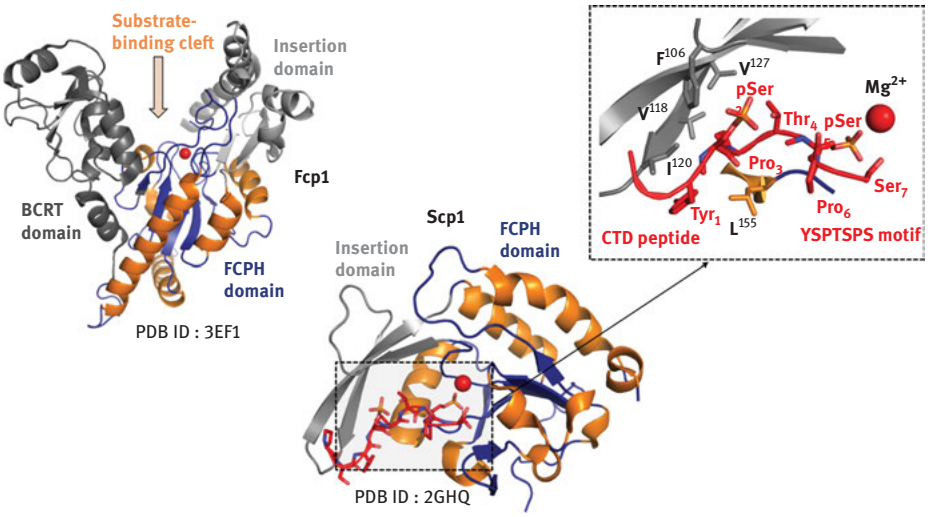


Figure 2.10: Structure and substrate-binding modes of Fcp1 and Scp1. The substrate contains tandem repeats of YSPTSPS motif.

Phosphoserine containing CTD peptides have been structurally characterized to bind between the active site cleft of the motif and the insertion domain. Substrate recognition is provided by the proline residue of the CTD sequence Y**S**PTSP**S** that interacts with the hydrophobic residues on the insertion domain. Mg^{2+} binds to the $pSer_5$ of the peptide and stabilizes the charges at the active site. Fcp1, on the other hand, forms a Y-shaped structure that has the FCP-Homology (FCPH) domain as its stem [133]. One arm of the Y-shaped molecule is formed by the BRCA1 C-terminal (BRCT) domain and the other arm is an insertion domain analogous to the insertion domain of Scp1. These two arms create a deep cleft in the Y-shaped molecule. Substrate peptide binds in the deep cleft to access the active site DxDxT motif at its base (Figure 2.10).

Recent work has suggested that Scp1 may use Smad protein as its substrate [134]. Another body of work has identified the role of Scp1 as a corepressor that associates with repressor element 1 silencing factor (REST) complex [135]. Both Scp1 and REST work toward silencing of specific genes in neuronal cells and their inhibition is a strategy for promotion of neuronal regeneration. It has been demonstrated that a dominant mutant of Scp1 (D96E or D98N) includes neuronal regeneration in mouse embryonic cells. Knockdown mutant of Scp1 directly promotes neural differentiation. Also, targeting of untranslated regions of Scp1 by micro RNA-123 can antagonize its antineural effect and promote neuronal differentiation [136, 137].

Emerging evidence has identified another member of HAD family of aspartate-based PSP that is distinct from FCP and SCP. Chronophin contains the conserved DxDxT active site motif and has a similar site to FCP/SCP. Chronophin removes phosphates from a protein cofillin which is a regulator of cytoskeletal dynamics in

cells [138]. While the structure of chronophin has been solved by x-ray crystallography, its mechanism of substrate identification, binding and processing remains to be elucidated.

2.5 Protein tyrosine phosphatases

The PTPs are a distinct superfamily that can be further divided into four separate subfamilies based on the amino acid composition of their catalytic domains (Figure 2.11) [3, 4]. Classes I–III include tyrosine phosphatases that use an active site cysteine that is placed in a conserved reaction center. The class I PTPs include the classical PTPs and DUSPs. The classical PTPs include both receptor and non-receptor tyrosine phosphatases. These proteins are explained in detail in the following sections of this chapter. The class II subfamily is represented by a sole member known as the low-molecular-mass protein tyrosine phosphatase (LMPTP). It is a cysteine-based phosphatase that is evolutionarily linked to the bacterial low-molecular-mass tyrosine phosphatase and uses phosphotyrosine as substrate [4]. The class III subtype consists of cysteine-based phosphatases that are related to rhodanese enzymes and use both phosphotyrosine and phosphothreonine as substrates. This subtype includes cell cycle regulating phosphatases that modulate the activity of cyclin-dependent protein kinases [139]. The class IV subtype includes the recently discovered aspartic acid-based tyrosine phosphatases that include the EyA (Eyes Absent) phosphatase of the HAD phosphatases. The HAD phosphatases use sugars, phospholipids, nucleotides and proteins as substrate and are very heterogeneous enzymes [140].

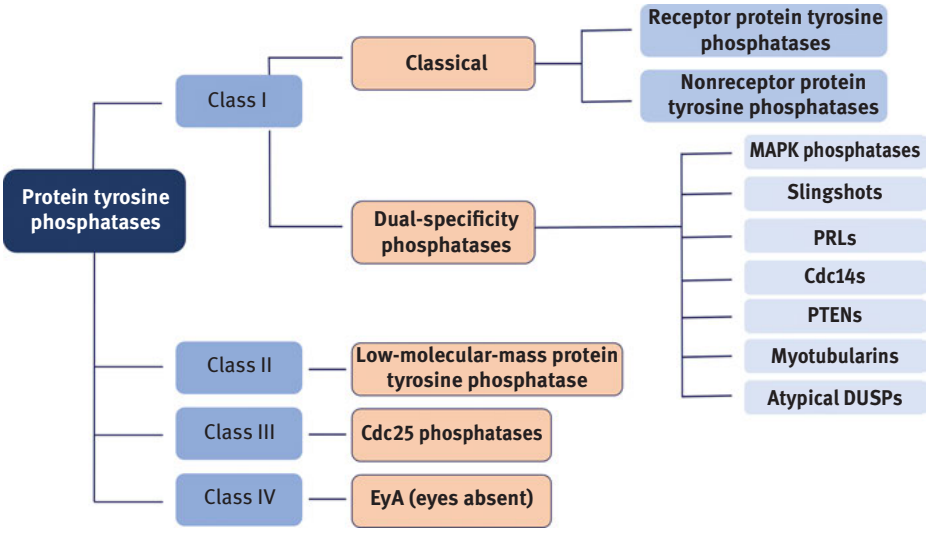


Figure 2.11: Classification of protein tyrosine phosphatase superfamily.

2.5.1 The dual-specificity phosphatases

DUSPs get their name from their unique ability to dephosphorylate the phosphotyrosine and phosphoserine/phosphothreonine residues within the same substrate. Their conserved active site contains the HCxxxxxR motif with a nucleophilic cysteine that allows the formation of a stable phosphoryl intermediate [141]. As in the case of classical PTPs, a conserved aspartic acid functions as general acid/base to stabilize the said phosphoryl intermediate (see Chapter 3). The shallow active site of DUSPs allows them to accommodate more than one type of phosphoamino acid residue than their active site [142]. The human genome has about 61 genes that encode for the various DUSPs (Table 2.3). Members of this subtype include the MAPK phosphatases, slingshots, phosphatases of the regenerating liver (PRLs), Cdc14s, phosphatase and tensin homologues (PTENs), myotubularins and the atypical DUSPs. DUSPs are the most diverse group of phosphatases in the superfamily and have varied substrate preferences (Figure 2.12). The MAPK phosphatases dephosphorylate phosphothreonine/phosphotyrosine residues. Slingshots dephosphorylate phosphoserines while Cdc14s dephosphorylate phosphothreonine/

Table 2.3: Various dual-specificity phosphatases.

Dual-specificity phosphatases						
MAPK phosphatases	**Slingshots**	**PRLs**	**Cdc14s**	**PTENs**	**Myotubularins**	**Atypical DUSPs**
DUSP1	SSH1L	PTP4A1	Cdc14A	PTEN	MTMR1	Laforin
DUSP2	SSH2L	PTP4A2	Cdc14B	TNS1	MTMR2	PTPMT1
DUSP4	SSH3L	PTP4A3	CDKN3	TNS2	MTMR3	STYX
DUSP5			PTPDC1	TPTE	MTMR4	DUSP3
DUSP6				TPTE2	MTMR5	DUSP11
DUSP7					MTMR6	DUSP12
DUSP8					MTMR7	DUSP13A
DUSP9					MTMR8	DUSP13B
DUSP10					MTMR9	DUSP14
DUSP16					MTMR10	DUSP15
MK-STYX					MTMR12	DUSP18
					MTMR14	DUSP19
					MTM1	DUSP21
					SBF1	DUSP22
					SBF2	DUSP23
						DUSP26
						DUSP27
						DUSP28
						DUPD1

MAPK, mitogen activated protein kinase

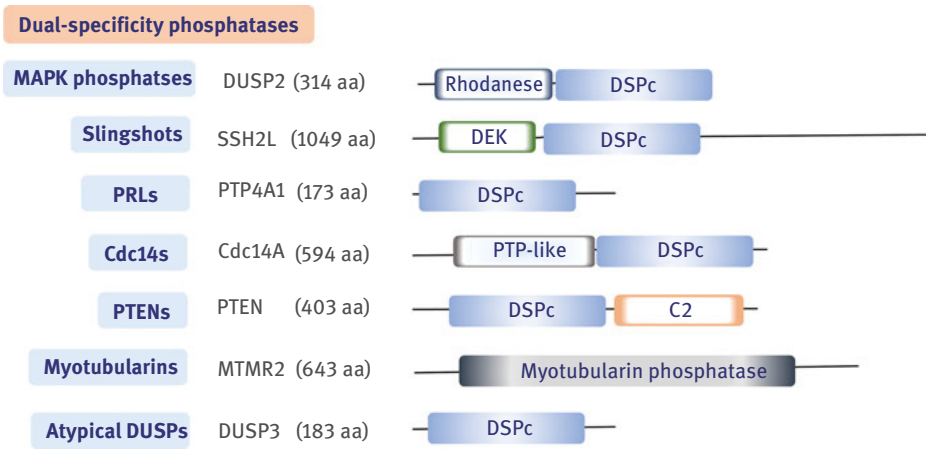


Figure 2.12: The dual-specificity phosphatases.

phosphoserine residues. Nonprotein substrate utilizing DUSPs include DUSP11 that dephosphorylates mRNA; PTENs that are specific for phosphoinositol; myotubularins that are specific for inositol-4-phosphatases; and laforin that uses carbohydrates as substrate.

The first discovered DUSP was VH1 from vaccinia virus [143]. The phosphatase was reported to be present in the encapsulated fluid of the virus to be released into the intracellular spaces of the host upon viral attack [144]. Inside the host cell VH1 dephosphorylates STAT1 and host cell proteins by targeting their phosphotyrosine, phosphoserine and phosphothreonine residues [145]. VH1 is reported to be critical for maintaining viability of the virus and also for dephosphorylating viral proteins that participate in viral morphogenesis [145, 146]. The human homologue of VH1 was discovered soon after and was named VHR [147]. VHR is reported to regulate signal regulating cell proliferation and differentiation. It dephosphorylates the phosphotyrosine and phosphothreonine residues of Erk and Jnk protein kinases and their signaling partners [148, 149].

2.5.1.1 MAPK phosphatases

The MAPK phosphatases are a well-characterized subgroup of DUSPs that simultaneously dephosphorylate the phosphothreonine and phosphotyrosine residues in a conserved TxY motif. These phosphatases target MAPK to antagonize their signaling cascades. These phosphatases have an N-terminal Cdc25-like (or Cdc25 homology 2)/Rhodanese domain that contains unique kinase interaction motifs. These motifs allow for specificity in the interaction between various MAPK and MAPK phosphatases (Table 2.4). The C-terminal region of these phosphatases has the conserved tyrosine phosphatase domain with the extended consensus sequence DX_{26}(V/L)X(V/I)HCXAG

Table 2.4: Interaction between various MAPK and MAPK phosphatases.

Phosphatase	Other names	MAP kinase association
DUSP1	MKP-1, CL100, hVH1,3CH134, PTPN10erp	p38 = JNK > ERK
DUSP2	PAC-1	ERK = p38 > JNK
DUSP4	MKP-2, hVHR-2, TYP1	ERK= JNK > p38
DUSP5	hVHR3, B23	ERK
DUSP6	MKP-3, Pyst1, rVH6	ERK > JNK = p38
DUSP7	MKP-X, Pyst2, B59	ERK > JNK = p38
DUSP8	hVH5, M3/6, HB5	JNK= p38 > ERK
DUSP9	MKP-4, Pyst3	ERK > p38 > JNK
DUSP10	MKP-5	p38 = JNK > ERK
DUSP16	MKP-7	JNK = p38 > ERK
MK-STYX	STYX-L1	———————

(I/V)SRSXT(IV)XXAY(L/I)M. The extended consensus sequence contains the nucleophilic cysteine for phosphoryl intermediate formation and also an upstream aspartate that allows for stabilizing the said intermediate [150, 151]. The structural folding of the active site region is highly conserved and mutation of the conserved cysteine residue abolishes all phosphatase activity (Figure 2.13) [141, 152, 153]. Production of reactive

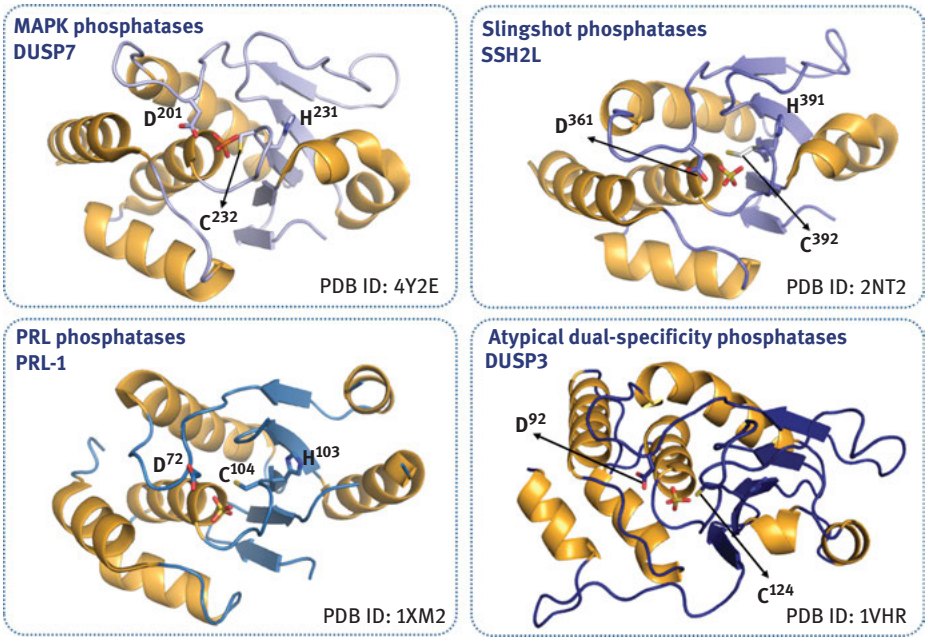


Figure 2.13: The dual-specificity phosphatase catalytic domain as seen in the various DUSP subtypes. Active site shows the nucleophilic cysteine and an inorganic phosphate.

oxygen species in the cells leads to oxidation-based inactivation of the nucleophilic cysteine that also leads to altered configuration of the MAPK phosphatase active site [154, 155]. One member of this subtype called MK-STYX (MAPK serine, threonine, tyrosine and tyrosine-specific phosphatase) has its nucleophilic cysteine substituted naturally for a serine residue. MK-STYX is an inactive phosphatase or a pseudophosphatase [156].

Expression and activity of MAPK phosphatases is critically regulated and is maneuvered in response to various stimuli in the cellular context [157–159]. Their expression is reported to be low in resting or unstressed cells and increases in response to stimulation by cytokines, growth factors or serum. Induction of their expression is also reported to be varied in various cell lines and tissues and is often correlated with MAPK activation in these cells. MAPK phosphatases hence work in a feedback mechanism with the MAPK to regulate mitotic signaling. The catalytic activity of some of these phosphatases is reported to be enhanced upon binding to their interacting MAPK [160]. This is suggested to occur via conformational changes in the active site, particularly around the general acid/base aspartate residue that stabilizes the phosphoryl intermediate [161]. MAPK phosphatases are also substrate of their target protein kinases and undergo phosphorylation. Erk is reported to phosphorylate DUSP1 at Ser359 and Ser364 [158]. Protein kinase p53 phosphorylates DUSP16 at Ser446 [162]. Phosphorylation of these MAPK phosphatases is reported to enhance their half-life in cells by sequestering them away from ubiquitin-mediated degradation. Expression of these phosphatases is also under epigenetic control by modifications of their promoter regions. Hyper-methylation of the DUSP1 promoter leading to its loss of expression has been reported in pancreatic cancer [163]. Acetylation of this promoter in response to Toll-like receptor stimulation serves to activate DUSP1 expression. DUSP1 then dephosphorylates p38 to decrease the innate immune response [164].

MAPK phosphatases are major regulators of some of the most critical biological processes. DUSP1, DUSP2 and DUSP10 are important regulators of immune signaling [165, 166]. DUSP1 inactivates p38 and Jnk, and plays critical roles in both adaptive and innate immune response [167–169]. DUSP2 is reported to regulate both p38 and Erk in in vitro experiments [170]. Knockout mouse models have provided a role for DUSP2 in cross-talk between the Erk and Jnk kinases [171]. DUSP10 functions to inactivate Jnk kinases and regulate inflammatory response [172]. Functional roles of DUSP4, DUSP6, DUSP7, DUSP10 and DUSP16 have been reported in various types of cancers [173–176]. DUSP6 is upregulated as a negative-feedback regulator or mitogenic signaling in cancers that have aberrant receptor tyrosine kinase and Ras/Raf signaling [177]. Expression profiles of these phosphatases are seen to be highly contest specific. For example, DUSP1 is reported to exhibit increased expression in glial, gastric and prostate cancer cell lines but decreased expression in ovarian and testicular cancer [178–182]. DUSP1 is reported to sensitize cancer cells to cisplatin-mediated apoptosis [183].

2.5.1.2 Slingshot phosphatases

Slingshot phosphatases were first discovered in the fruit fly *Drosophila melanogaster*, where their mutants caused disorganized epidermal cell morphogenesis [184]. The human slingshots include three members, namely SSH1L, SSH2L and SSH3L. All three slingshot phosphatases are widely expressed and are suggested to have a regulatory role in actin polymerization [185, 186]. These phosphatases suppress actin filament assembly by dephosphorylating phosphoactin depolymerization factor and phosphocofilin [187]. All the three slingshot phosphatases have a conserved catalytic domain that can use both phosphoserine and phosphothreonine residues as substrates (Figure 2.13). They also contain 14-3-3 binding motifs, and SH3 region and a C-terminal F-actin binding site [187]. These phosphatases negatively regulate actin polymerization induced by LIM domain kinase 1 (LIMK1) and the testis specific kinase (TESTK1). SSH1L is reported to directly dephosphorylate LIMK1 [187].

2.5.1.3 PRL phosphatases

The PRLs are a subgroup of DUSPs that include PRL-1, PRL-2 and PRL-3 proteins encoded by the *PTP4A1, PTP4A2* and *PTP4A3* genes [188, 189]. These are small ~20 kDa proteins that contain a PTP domain followed by a polybasic region. This polybasic region is suggested to facilitate the binding of these phosphatases to membrane lipid and also promote their localization in the nucleus [190]. A conserved CAAX sequence motif at the C-terminal end of these proteins allows for their farnesylation and consequent association with the plasma membrane and early endosomes [191]. Structural analysis of PRLs has been done using crystallographic and nuclear magnetic resistance-based methods [192–194] (Figure 2.13). PRLs are reported to regulate the activity of Src and Erk kinases [195, 196]. These phosphatases also regulate the Rho GTPases (RhoA and RhoC) and other DUSPs called the PTENs [197, 198]. PRL-3 is reported to dephosphorylate the protein Ezrin at pThr567 [199]. PRLs are reported to regulate cell proliferation, migration, transformation and invasion. All three PRLs are reported to be overexpressed in cancer cell lines [188]. PRL-1 and PRL-3 are reported to be associated with migration and invasion of epithelial cells [200, 201]. PRL-3 is reported to be upregulated in breast, gastric, liver and ovarian cancer and various other tumors [202–205].

2.5.1.4 Cdc14 phosphatases

The Cdc14 phosphatases are evolutionarily conserved from yeast to humans and function as key regulators of cell cycle progression. In yeast, Cdc14 dephosphorylates cyclin-dependent kinase substrates Cdh1 and Sic1 and allows cells to exit mitosis [206]. In humans, Cdc14 subgroup comprises four phosphatases: Cdc14A, Cdc14B, CDKN3 and PTP9Q22/PTPDC1 (annotated). These phosphatases are closely related to the class II Cdc25 phosphatases [3]. These Cdc14 phosphatases dephosphorylate the phosphoserine/phosphothreonine residues next to a proline residue (P + 1), as seen

in substrate sequences of proline-directed cyclin-dependent kinases and mitogen-activated kinases. The structure of their phosphatase region is unique in having tandem A and B DUSP domains that have the active site as a groove in between these two domains (Figure 2.14) [207]. The nucleophilic cysteine is contained in the CxxxxxR motif in the B-domain. The active site lies in the inter-domain region as a long groove. The acid–base aspartate containing loop and α5A/α6A loops of the A-domain line the groove on one end and the acid-base aspartate containing -loop of the B-domain lies at the opposite face.

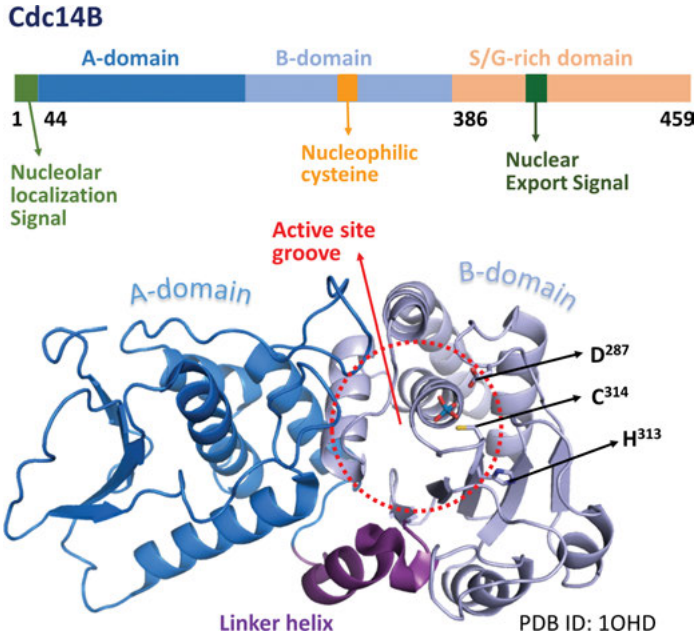


Figure 2.14: Domain organization in the Cdc14 DUSPs.

Three acidic residues (Glu206, Glu209 and Asp215 in Cdc14B) cluster to form an acidic patch at one end of the substrate groove. This acidic patch is suggested to engage the basic residues commonly seen at P+2 to P+4 positions in cyclin-dependent kinase substrates [208]. The Cdc14s are also suggested to work in concert with Pin1, the proline *cis–trans* isomerase, to allow for optimal binding of proline containing substrate at the active site [209].

Cdc14 phosphatases are reported to play crucial roles in spindle stabilization and centrosome maturation associated with cell cycle progression [210, 211]. Cdc14B is targeted to the nucleus during interphase and to the mitotic spindle during mitosis [212]. Cdc14A is localized at the centrosome during interphase and later at the spindle mid-zone. Overexpression of Cdc14A causes multipolar spindle formation because of

premature splitting of the centrosome, while downregulation of Cdc14A induced cell death [213]. Cdc14 phosphatases are reported to inhibit RNA polymerase and also to allow for chromosome disjunction during mitosis [214]. Substrates of Cdc14 phosphatases include Cdc25A and p53 protein kinases and the Rab5 activating protein RN-tre [215–217].

2.5.1.5 PTEN phosphatases

This subgroup of phosphatases gets their name from their member PTEN that dephosphorylates phosphoinositide substrates. The protein is coded by the *PTEN* gene located on chromosome 10 (band 10q23.31) [218]. PTENs contain a tensin-like domain (C2 domain) attached to their DUSP domain (Figure 2.15). The C2 domains have a CBR3 loop that allows PTEN to bind phosphatidylinositol-3-phosphate of membrane lipids [219]. The N-terminal region of PTEN contains a nuclear localization signal (residues 7–31) and also a cytoplasmic localization signal (residues 19–25) [220]. Residues 6–15 have a motif that binds phosphatidylinositol 4,5-bisphosphate (PIP_2) and is called the PIP_2 binding motif [221]. A naturally occurring variant of PTEN is formed by the alternate start site readout of the *PTEN* gene and makes PTEN long that has about 173 extra amino acids at the N-terminus [222]. PTEN also has a disordered region at its C-terminus (residues 353–403) that contains various phosphorylation sites and is responsible for modulation of its phosphatase activity [223].

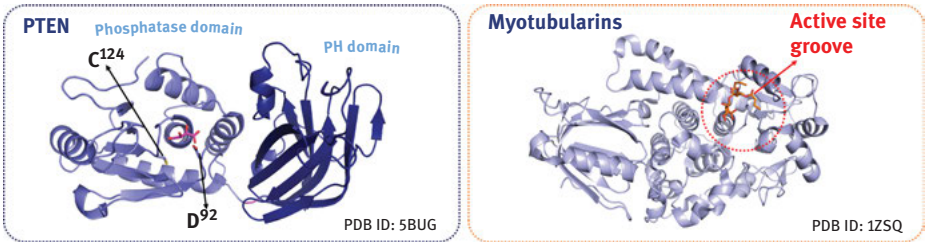


Figure 2.15: The distinct catalytic domains of PTEN and myotubularin DUSPs.

PTEN uses phosphoinositide substrates to control the levels and cellular signaling by the phosphatidylinositol 3,4,5-trisphosphate (PIP_3) [218]. PTEN removes the 3′ phosphate from PIP_3 to release PIP_2 and inhibit Akt signaling. PTEN has recently been shown to also use protein substrates including IRS1 and Dishevelled [224, 225]. The nucleophilic Cys214 is present in the phosphatase domain in a deep and wide pocket that accommodates the substrates of PTEN.

Mutations and deletions that lead to loss of function of PTEN are correlated with glioblastoma, and cancers of the liver, prostate, breast and endometrium. PTEN is reported to be the most common tumor suppressor gene lost in prostate and other cancers [226]. Deletions and mutations of the *PTEN* gene cause PTEN hamartoma

tumor syndrome that includes Cowden syndrome, Bannayan–Riley–Ruvalcaba syndrome and Proteus-like syndrome [227]. Deficient function of PTEN is reported in patients with learning disabilities and broad-spectrum autism [228].

2.5.1.6 Myotubularins

Myotubularins are also a subtype of DUSPs that, like the PTENs, dephosphorylate D3-phosphorylated inositol phospholipids [3, 229] (Figure 2.15). This subtype includes 15 gene products, about half of which are inactive phosphatases due to the lack of critical amino acid residues in their catalytic domains [230]. Missing residues include the active site nucleophilic cysteine, the conserved arginine or the acid/base aspartate. The role of inactive myotubularins is unclear, but they are suggested to work in concert with the active group members to regulate phosphoinositide-based signaling [231]. Myotubularins possess additional domains and motifs, other than their phosphatase domain, that allow for them to interact with other proteins and the plasma membrane. These include an N-terminal PH domain, a FYVE (Fab1p, YOTB, Vac1p, and EEA1) domain and a C-terminal coiled-coil domain [232]. These coiled coil regions allow the inactive myotubularins to make pairs with the active ones; like MTMR1 (active) with MTMR5 (inactive) and MTMR6 (active) with MTMR9 (inactive) pairs. Mutations in myotubularins leading to a loss of function are associated with neuromuscular disease [233].

2.5.1.7 Atypical DUSPs

The atypical DUSPs are a set of about 19 proteins that are phylogenetically distinct, but share characteristic features with MAPK phosphatases and the VH1 phosphatase originally discovered from vaccinia virus [145]. These proteins contain the DUSP domain but lack the Cdc25 Homology 2 (CH2) domain seen in the MAPK phosphatases [234]. The literature is crowded with conflicting reports regarding these phosphatases and these proteins have also been renamed and reclassified by various research groups (Table 2.5).

Table 2.5: Alternate names of atypical DUSPs in literature.

Atypical DUSP	Other names in literature
DUSP3	– VHR (VH1-related)
DUSP11	– PIR1 (phosphatase that interacts with RNA–ribonucleoprotein complex 1)
DUSP12	– GKAP (glucokinase-associated phosphatase)
DUSP13A	– TMDP (testis- and skeletal muscle-specific DUSP)
DUSP13B	– MDSP (muscle-restricted DUSP)
	– BEDP (branching-enzyme-interacting DUSP)
	– SKRP4 (stress-activated protein kinase pathway-regulating phosphatase-4)
DUSP14	– MKP-L (MKP-1-like protein tyrosine phosphatase)
	– MKP-6
DUSP15	– VHY (VH1-like member Y)

Table 2.5: (*continued*)

Atypical DUSP	Other names in literature
DUSP18	– DUSP20
	– LMWDSP20 (low-molecular weight DUSP20)
DUSP19	– DUSP17
	– SKRP1 (stress-activated protein kinase pathway-regulating phosphatase 1)
DUSP21	LMWDSP21 (low-molecular-mass DUSP21)
DUSP22	– VHX (VH1-related phosphatase-related X)
	– MKP-X
	– LMWDSP2 (low-molecular-mass DUSP2)
	– JKAP (JNK pathway-associated phosphatase)
	– JSP1 (JNK-stimulating phosphatase 1)
DUSP23	– VHZ (VH1-related phosphatase Z)
	– DUSP25
	– LDP-3 (low-molecular-mass DUSP3)
DUSP26	– DUSP24
	– MKP-8
	– LDP-4 (low-molecular-mass DUSP4)
	– SKRP3 (stress-activated protein kinase pathway-regulating phosphatase 3)
	– NEAP (neuroendocrine-associated phosphatase)

The first atypical DUSP to be discovered and structurally characterized is DUSP3 (or VHR as mentioned earlier) [235, 236] (Figure 2.13). DUSP3 is constitutively expressed and is localized to the nucleus. It plays an important role in regulation of cell cycle, MAP kinase activity and DNA damage and repair [237]. DUSP3 specifically dephosphorylates Erk 1/2 but not the p38 or JNK MAPKs [149]. It is also reported to dephosphorylate pTyr996 of the ErbB receptors [238]. DUSP3 shows dimerization-based regulation of activity and is also phosphorylated by ZAP-70 on Tyr138 [239, 240]. DUSP3 is reported to be overexpressed in cervix cancer cell lines, including SiHa, CaSki, C33 and HT3 [241]. It is also overexpressed in prostate cancers [242]. The DUSP23 phosphatase is similar to DUSP3 and is hence also known as VH1-related phosphatase Z. DUSP23 is the smallest DUSP with a single phosphatase domain [243]. It localizes to the centrosome and is crucial for regulation of the cell cycle. It is implicated in various cancers and is also required for placental development [244]. DUSP23 is reported to dephosphorylate the transcription factor glial cells missing homologue 1 [245]. In contrast to the MAPK phosphatases, DUSP23 is reported to enhance the activity of p38 and JNK proteins [246].

DUSP13 is expressed in the skeletal muscle, testes, heart and blood. Two alternate reading frames of the DUSP13 gene allow for generation of two isoforms: DUSP13A or TMDP (testes and skeletal-muscle-specific DUSP) and DUSP13B or muscle-restricted DUSP (also called branching enzyme interacting DUSP) [247]. DUSP13B is crucial for regulation of meiosis and spermatogenesis in the testes [248] and shows specific phosphatase activity for MAPKs, not shown by DUSP13A [249].

DUSP26 is expressed in many tissues including skeletal muscles, heart, ovaries and brain. It is reported to localize mainly to the nucleus and the Golgi complex [250]. It dephosphorylates and inactivates p38 MAPK and abrogates its apoptotic signal in Anaplastic thyroid cancer (ATC) cell lines [251]. DUSP26 is reported to associate with the KIF3 motor protein complex that regulates the transport of intracellular proteins. It is suggested to bind the kif3a subunit of the complex and dephosphorylate Kap3 [252]. DUSP26 is reported to dephosphorylate pSer20 and pSer37 of p53 kinase [253]. DUPD1 is another DUSP expressed in skeletal muscles, liver and adipose tissues [254]. The exact biological role of DUPD1 remains elusive, although its phosphatase domain has been characterized [255]. DUPD1 is referred to as DUSP27 in many places in literature; which is misleading as DUSP27 is a distinct gene product and another atypical DUSP. The actual DUSP27 is an evolutionarily conserved protein that is expressed in the heart and skeletal muscle. It is characterized by a long C-terminal region of around 700 residues and an inactive phosphatase domain where the nucleophilic cysteine is substituted to another residue (varies from fish to human).

Another inactive atypical DUSP is the serine/threonine/tyrosine interacting protein (STYX). The catalytic domain of STYX has the nucleophilic cysteine substituted by a glycine residue [256]. Reverse mutation of this Gly120 to a cysteine converts STYX into an active phosphatase. STYX binds to phosphotyrosine containing regions in proteins and competes with other phosphatases for their target substrates [257]. Important binding partners of STYX include Erk1/2 whom it assists in nuclear export and the testicular RNA-binding protein Crhsp-24 implicated in spermiogenesis [258, 259].

DUSP12 is characterized by the presence of a C-terminal Zinc-binding domain alongside its N-terminal functional DUSP domain [260]. This Zinc-binding domain is suggested to work as redox sensor that may protect the DUSP12 phosphatase domain from oxidative stress [261]. DUSP12 is suggested to be a putative oncogene that functions to regulate key cellular processes [262, 263]. Most notably, DUSP12 interacts with Hsp70 chaperone proteins to prevent heat shock-induced cell death [264]. Overexpression of DUSP12 is reported to block adipogenesis and its polymorphisms are associated with type II diabetes [265, 266]. The rat DUSP12 is reported to directly interact with glucokinase enzyme and enhance its rate of glucose phosphorylation [260]. DUSP12 is hence also known as glucokinase-associated phosphatase.

DUSP15 and DUSP22 are DUSPs that have an N-terminal myristoylation site (Gly2) that allows their targeting to the plasma membrane [267]. DUSP15 is transcriptionally regulated during oligodendrocyte differentiation where its role is to dephosphorylate Platelet-derived growth factor (PDGF)-receptor beta and SNX6 protein [268]. DUSP22 regulates cell migration and motility by dephosphorylating pTyr397, pTyr576 and pTyr577 of FAK protein kinase [269]. It also dephosphorylates pSer118 of estrogen receptor alpha [270]. It is also reported as a negative regulator of STAT3 signaling [271].

DUSP18 is a DUSP that is widely expressed and was first identified from the fetal brain [272]. It dephosphorylates JNK but not p38 or Erk MAPK [159]. DUSP19 interacts with MAPK MKK7 and inhibits JNK-mediated signaling [273]. It is also reported to interact with apoptosis signaling regulating kinase 1 or ASK1 [274]. DUSP14 is reported to negatively regulate tumor necrosis factor-induced activation of nuclear factor kappa B [275]. DUSP21 is a cancer testis antigen and is a therapeutic target for hepatocellular carcinoma [276].

DUSP11 was originally discovered as being associated with ribonuclear complexes and was called phosphatase interacting with RNA-ribonucleoprotein 1 or PIR1 [277]. DUSP11 is suggested to function in RNA splicing where it dephosphorylates the 5′ ends of RNA strands. Its catalytic domain is closely related to RNGTT protein which is an mRNA capping enzyme [278]. Another DUSP that uses nonprotein substrates is Laforin. Laforin or the Lafora disease phosphatase uses its N-terminal CBM20 carbohydrate-binding domain to target polyglycan bodies [279, 280]. It uses its C-terminal phosphatase domain to dephosphorylate complex carbohydrate glycans [281]. Epilepsy causing mutations have been reported in both domains of Laforin [281]. Laforin also serves as an adaptor protein and modulates PPPIR3D regulatory subunit of PP1 [282]. A study suggests that Laforin can dephosphorylate pSer9 of glycogen synthetase kinase 3 [283].

PTPMT1 is the first PTP reported to be localized to the mitochondrion where it dephosphorylates phosphatidylinositol-5-phosphate [284, 285]. It contains an N-terminal mitochondrial targeting sequence followed by a phosphatase domain that resembles PTEN. PTPMT1 is also reported to dephosphorylate phosphatidylglcerolphosphate to release phosphatidylglycerol in the cardiolipin biosynthesis pathway [286].

2.5.2 The class II protein tyrosine phosphatases

The class II PTPs are small-sized (~15–18 kDa) enzymes that are also called the low-molecular-weight protein tyrosine phosphatase (LMW-PTP). These are specific for using phosphotyrosine as substrate but not phosphoserine or phosphothreonine [287, 288]. These enzymes catalyze phosphotyrosine dephosphorylation under acidic conditions (pH ≤ 6.0) and are also classified as acid phosphatases (hence the name ACP) [289, 290]. These were first identified from red blood cells, but are now known to be ubiquitously expressed in various tissues and cell types [291–293]. Human express four LMW-PTPs that are actually isoforms created by alternate splicing of the transcript of the same *ACP1* gene [294]. Two of the four isoforms are reported to lack any catalytic activity. The active isoforms named IF1 (and HCPTPA) and IF2 (and HCPTPB) differ in the loop regions around their active site. These small differences provide them distinct substrate specificities and ligand-binding abilities [295, 296]. The inactive isoforms named SV3 and LMW-PTP-C are reported to regulate the activity of the active isoforms by binding and sequestering their substrate proteins [297, 298].

The catalytic domain of LMW-PTP has five α-helices flanking four β-sheets from either site (Figure 2.16) [299, 300]. The catalytic loop (or phosphate-binding loop, *P*-loop) containing the nucleophilic cysteine is located at the N-terminal region between the β_1-sheet and α_1-helix. This loop is surrounded by a V-loop (that varies in the isoforms) and another DPYY loop that contains the general acid/base aspartate for catalysis [301]. The V-loop and DPYY loop configure around the catalytic loop to create a compact active site that is similar but smaller than that of the classic class I PTPs. This is despite the catalytic domain of LMW-PTPs having no sequence similarity with the class I PTPs [300].

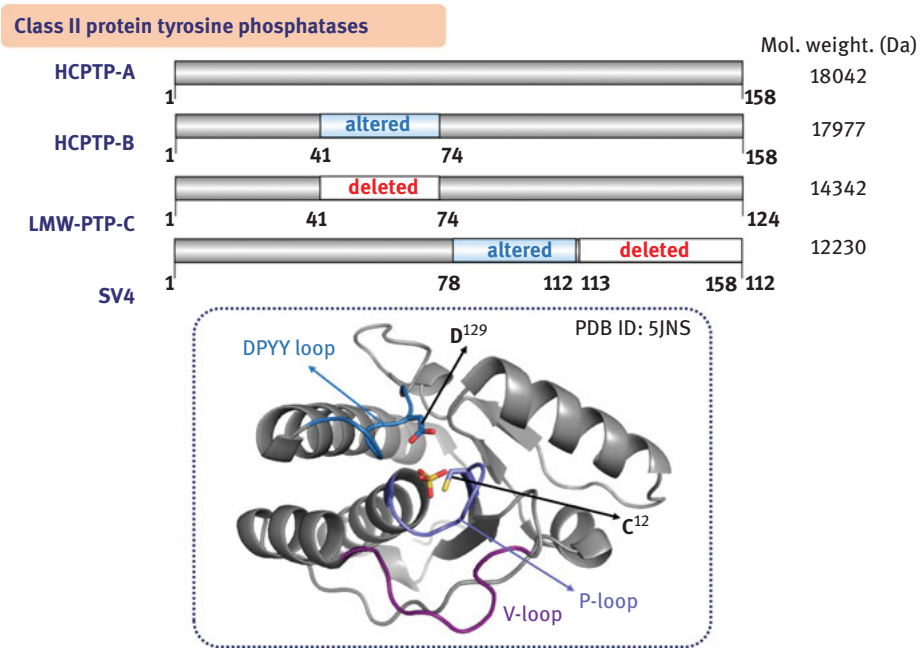


Figure 2.16: Domain architecture and active site of the class II protein tyrosine phosphatases.

LMW-PTPs regulate and inhibit signaling of various receptor PTKs including that of the insulin receptor, epidermal growth factor receptor, fibroblast growth factor receptor and the vascular growth factor receptor [287, 302–304]. Loss of function of LMW-PTP activity is implicated in various diseases including autoimmune disorders, allergy, asthma, Alzheimer's and also cancer [305–308]. Overexpression of active LMW-PTP in NIH3T3 cells is reported to cause larger tumors in nude mice. Accordingly, phosphatase-dead LMW-PTP is reported to inhibit tumor growth of NIH3T3 cells [309]. This transforming property of LMW-PTP is suggested to be due to the dephosphorylation of the EphA2 receptors [310]. The crucial role of LMW-PTPs in mitogenic signaling has made them a

target for cancer therapeutics [311–313]. Inhibitor development for LMW-PTPs has been especially challenging as inhibitory drugs have shown cross-reactivity with the classic PTP1B [314, 315].

2.5.3 The class III protein tyrosine phosphatases

The class III PTPs are controllers of cell division that function to activate cyclin-dependent protein kinases (Cdks) by removing their inhibitory phosphorylations [316]. Accordingly, these are also called as Cdc25 phosphatases and are reported to be expressed in all eukaryotes [316, 317]. Class III PTPs were first identified in fission yeast [139]. The human Cdc25 phosphatases are three proteins named as Cdc25A, Cdc25B and Cdc25C [318]. Cdc25 isoforms dephosphorylate the dually phosphorylated Thr-Tyr motifs in the N-terminal regions of cyclin-dependent protein kinases (Thr14–Tyr15 in Cdk1) [319, 320]. As these phosphatases use a phosphothreonine and a phosphotyrosine in a dual motif as substrates, they are sometimes referred to as DUSPs. However, structurally and evolutionarily, these are quite distinct from the class I DUSPs. It is noteworthy that while the Cdc25 phosphatases share the catalytic mechanism and active site structure with the class I PTPs, they are suggested to have evolved separately from independent ancestral genes [3]. Cdc25 proteins are related to the rhodanese-type sulfur transfer enzymes that may have evolved to become cysteine-based protein phosphatases [321, 322].

The size of Cdc25 phosphatases ranges from 300 to 600 residues and they have distinct N- and C-terminal regions. The N-terminal region is the most diverse in the various isoforms and contains phosphorylation, ubiquitination and protein–protein interaction sites [323–326]. The C-terminal regions contain the catalytic domain of about 200 residues with the CxxxxxR motif and the nucleophilic cysteine. The motif sits in a cradle-shaped conformation to allow optimal substrate binding at the active site (Figure 2.17) [327, 328]. This catalytic domain of the Cdc25 phosphatases contains less α-helices and β-sheets compared to the catalytic domains of class I and class II PTPs. Also, the loops surrounding the active site of class I and class II PTPs are absent from the structures of Cdc25 and indicate their distinct evolutionary lineages. Molecular dynamics simulations on the catalytic domain of Cdc25B has provided evidence for flipping of the phosphate-binding loop (*P*-loop) to accommodate an incoming ligand [329].

Class III PTPs are positive regulators of cell cycle progression and are hence implicated in various cancers as oncogenes [330]. At the same time, Cdc25 phosphatases are reported to be responsible for prevention of Cdk activation under conditions of DNA damage or incomplete replication in cells [331]. Cdc25 phosphatases are understandably targets of drug discovery efforts for anticancer therapeutics. Various Cdc25 phosphatase inhibitors have been reported in literature. These include natural products, electrophiles, phosphate mimetics and peptide-based inhibitor molecules [332].

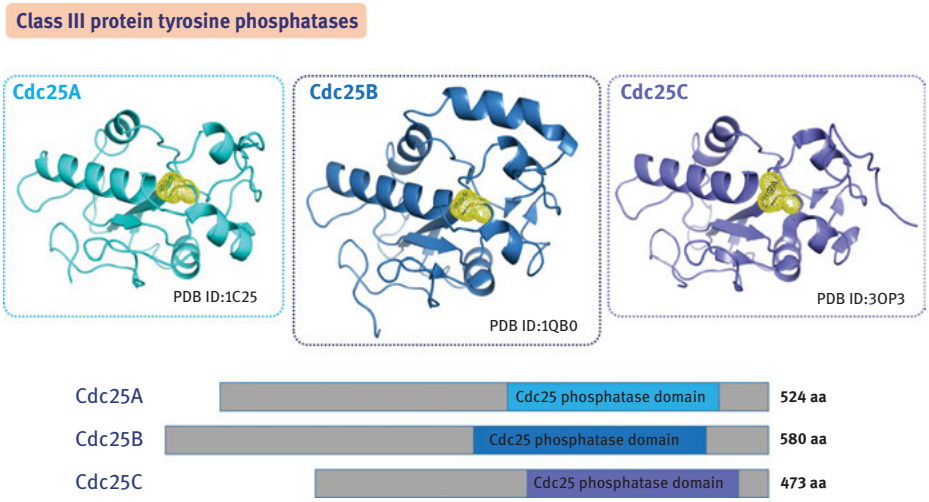


Figure 2.17: The catalytic domain of class III protein tyrosine phosphatases/Cdc25 phosphatases.

2.5.4 The class IV protein tyrosine phosphatases

The Eyes-Absent (Eya) proteins are a unique class of PTPs that use an aspartate-based catalytic mechanism [333]. Eya proteins are named after their functional role as a component of the SIX-EYA-DACH transcription factor network required for eye specification in *D. melanogaster* [334]. Eya proteins are critical for formation of various tissues and organogenesis and Eya mutations are linked to congenital disorders in humans [335]. These Eya proteins have provided the first biological example of a transcription factor having phosphatase activity [336, 337]. Humans have four paralogs of this family named as Eya1, Eya2, Eya3 and Eya4. The four proteins have a unique ~270 amino acid region that is specific to them and is known as the Eya domain (Figure 2.18). These domains allow Eya proteins to interact with transcription factors such as Dachshund homologue 1 (in mice) and the homeodomain proteins Sine oculis (in *Drosophila*) and Six (in vertebrates) (Figure 2.18). Increased levels of Eya and Six have been reported in breast and ovarian cancers and also in malignant peripheral nerve sheath tumors [338–340].

The catalytic domain of Eya phosphatases belongs to HAD superfamily of enzymes. These phosphatases dephosphorylate phosphotyrosine residues using an aspartyl-phosphate intermediate [341]. This is in contrast to the cysteinyl-phosphate intermediate-driven catalysis by the class I, II and III PTPs. The conserved catalytic core of the HAD phosphatase domains of Eya proteins is defined by four signature motifs and the presence of a bound Mg^{2+} ion [342]. Motif I contains the nucleophilic aspartate required for the aspartyl-phosphate intermediate formation. Another aspartate in this motif allows Eya to chelate an Mg^{2+} ion at the active site. The motif II has a

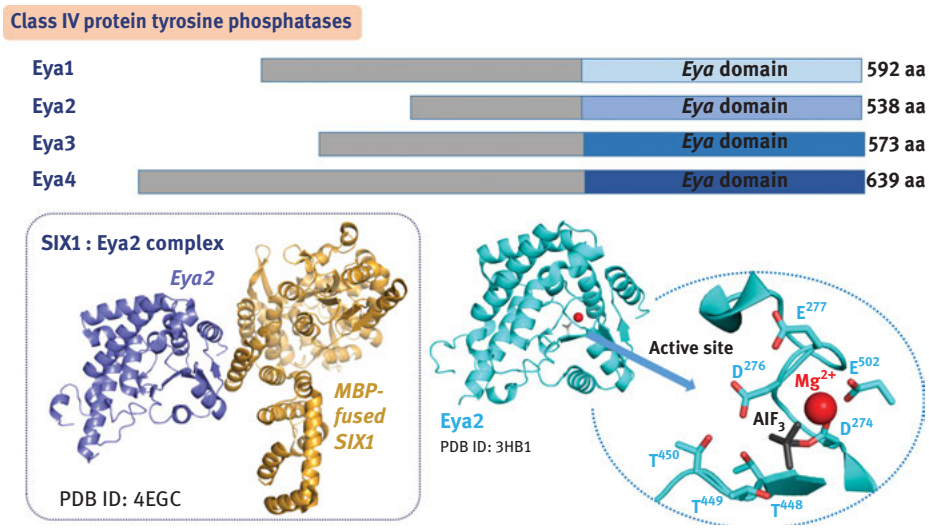


Figure 2.18: The aspartate-based class IV protein tyrosine phosphatase catalytic domain.

crucial serine/threonine residue that allows for proper orientation of the substrate at the active site. Motif III is essentially a critically placed lysine residue that stabilizes the aspartyl-phosphate intermediate. Motif IV works together with motif I in the active site to chelate the Mg^{2+} ion required for catalysis. The general acid/base that allows for hydrolysis of the aspartyl-phosphate intermediate and release of inorganic phosphate from the active site is harbored in Motif I [343].

Eya has been identified to dephosphorylate histone H2A.X and regulate the switch between DNA repair and apoptosis under conditions of DNA double-strand break and damage. Under normal conditions H2A.X is constitutively phosphorylated on its C-terminal Tyr142 (done by Williams Syndrome Transcription Factor (WSTF) tyrosine kinase) [344]. Under conditions of DNA damage, the Tyr142-phosphorylated H2A.X histone can recruit JNK1 and initiate the apoptotic signal. However, these conditions trigger ATM/ATR proteins to phosphorylate Eya3 on Ser219 and H2A.X on Ser139 (now called γH2A.X). This phosphorylation event allows the Eya1/Eya3 complex to dephosphorylate pTyr142 of H2A.X and also allow to recruit DNA repair enzymes. In this way a fine switch is created between DNA repair and apoptotic cell death in response to DNA damage [345, 346]. Dephosphorylation of γH2A.X is critical for resisting apoptotic cell death and can be crucial for mammalian organogenesis. Phosphothreonine dephosphorylation activity has been suggested for Eya4 in innate immune response to intracellular pathogens [347].

Mutations in Eya proteins are linked to various autosomal dominant human diseases. Mutations in Eya4 have been identified in deafness [348] and also dilated cardiomyopathy type I J [349]. Phosphatase activity of Eya proteins is reported to

be essential for promoting cell migration, invasion and transformation as seen in breast cancer cells [339]. Mutations in Eya that lead to its loss of interaction with the transcription factor SIX1 are associated with branchio-oto-renal syndrome [350].

References

[1] Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. The protein kinase complement of the human genome. Science 2002;298(5600):1912–34.
[2] Taylor SS, Kornev AP. Protein kinases: evolution of dynamic regulatory proteins. Trends Biochem Sci 2011;36(2):65–77.
[3] Alonso A, Sasin J, Bottini N, Friedberg I, Friedberg I, Osterman A, et al. Protein tyrosine phosphatases in the human genome. Cell 2004;117(6):699–711.
[4] Mustelin T. A brief introduction to the protein phosphatase families. Methods Mol Biol 2007;365:9–22.
[5] Begley MJ, Taylor GS, Kim SA, Veine DM, Dixon JE, Stuckey JA. Crystal structure of a phosphoinositide phosphatase, MTMR2: insights into myotubular myopathy and Charcot-Marie-Tooth syndrome. Mol Cell 2003;12(6):1391–402.
[6] Lee JO, Yang H, Georgescu MM, Di Cristofano A, Maehama T, Shi Y, et al. Crystal structure of the PTEN tumor suppressor: implications for its phosphoinositide phosphatase activity and membrane association. Cell 1999;99(3):323–34.
[7] Kamenski T, Heilmeier S, Meinhart A, Cramer P. Structure and mechanism of RNA polymerase II CTD phosphatases. Mol Cell 2004;15(3):399–407.
[8] Barford D, Das AK, Egloff MP. The structure and mechanism of protein phosphatases: insights into catalysis and regulation. Annu Rev Biophys Biomol Struct 1998;27:133–64.
[9] Gallego M, Virshup DM. Protein serine/threonine phosphatases: life, death, and sleeping. Curr Opin Cell Biol 2005;17(2):197–202.
[10] Janssens V, Goris J. Protein phosphatase 2A: a highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling. Biochem J 2001;353(Pt 3):417–39.
[11] Bryant JC, Westphal RS, Wadzinski BE. Methylated C-terminal leucine residue of PP2A catalytic subunit is important for binding of regulatory Balpha subunit. Biochem J 1999;339 (Pt 2):241–6.
[12] Schweighofer A, Hirt H, Meskiene I. Plant PP2C phosphatases: emerging functions in stress signaling. Trends Plant Sci 2004;9(5):236–43.
[13] Parameswara VK, Sule AJ, Esser V. Have we overlooked the importance of serine/threonine protein phosphatases in pancreatic beta-cells? Role played by protein phosphatase 2A in insulin secretion. JOP 2005;6(4):303–15.
[14] Fiedler B, Wollert KC. Targeting calcineurin and associated pathways in cardiac hypertrophy and failure. Expert Opin Ther Targets 2005;9(5):963–73.
[15] Tian Q, Wang J. Role of serine/threonine protein phosphatase in Alzheimer's disease. Neurosignals 2002;11(5):262–9.
[16] Cohen PT. Protein phosphatase 1 – targeted in many directions. J Cell Sci 2002;115 (Pt 2):241–56.
[17] Green AA, Cori GT, Oncley WanbJL. CRYSTALLINE MUSCLE PHOSPHORYLASE: I. PREPARATION, PROPERTIES, AND MOLECULAR WEIGHT. Journal of Biological Chemistry 1943;151(1):21–9.

[18] Goldberg J, Huang HB, Kwon YG, Greengard P, Nairn AC, Kuriyan J. Three-dimensional structure of the catalytic subunit of protein serine/threonine phosphatase-1. Nature 1995;376(6543):745–53.
[19] Egloff MP, Cohen PT, Reinemer P, Barford D. Crystal structure of the catalytic subunit of human protein phosphatase 1 and its complex with tungstate. J Mol Biol 1995;254(5):942–59.
[20] Hendrickx A, Beullens M, Ceulemans H, Den Abt T, Van Eynde A, Nicolaescu E, et al. Docking motif-guided mapping of the interactome of protein phosphatase-1. Chem Biol 2009; 16(4):365–71.
[21] Walaas SI, Greengard P. Protein phosphorylation and neuronal function. Pharmacol Rev 1991;43(3):299–349.
[22] Desdouits F, Cheetham JJ, Huang HB, Kwon YG, da Cruz e Silva EF, Denefle P, et al. Mechanism of inhibition of protein phosphatase 1 by DARPP-32: studies with recombinant DARPP-32 and synthetic peptides. Biochem Biophys Res Commun 1995;206(2):652–8.
[23] Serra D, Asins G, Hegardt FG. Purification and characterization of a protein inhibitor from rat liver that inhibits type 1 protein phosphatase when 3-hydroxy-3-methylglutaryl CoA reductase is the substrate. Journal of lipid research 1990;31(5):919–26.
[24] Alemany S, Pelech S, Brierley CH, Cohen P. The protein phosphatases involved in cellular regulation. Evidence that dephosphorylation of glycogen phosphorylase and glycogen synthase in the glycogen and microsomal fractions of rat liver are catalysed by the same enzyme: protein phosphatase-1. Eur J Biochem 1986;156(1):101–10.
[25] Wang QM, Guan KL, Roach PJ, DePaoli-Roach AA. Phosphorylation and activation of the ATP-Mg-dependent protein phosphatase by the mitogen-activated protein kinase. J Biol Chem 1995;270(31):18352–8.
[26] Ingebritsen TS, Cohen P. The protein phosphatases involved in cellular regulation. 1. Classification and substrate specificities. Eur J Biochem 1983;132(2):255–61.
[27] Li M, Guo H, Damuni Z. Purification and characterization of two potent heat-stable protein inhibitors of protein phosphatase 2A from bovine kidney. Biochemistry 1995; 34(6):1988–96.
[28] Li M, Makkinje A, Damuni Z. Molecular identification of I1PP2A, a novel potent heat-stable inhibitor protein of protein phosphatase 2A. Biochemistry 1996;35(22):6998–7002.
[29] Li M, Makkinje A, Damuni Z. The myeloid leukemia-associated protein SET is a potent inhibitor of protein phosphatase 2A. J Biol Chem 1996;271(19):11059–62.
[30] Fischer EH, Pocker A, Saari JC. The structure, function and control of glycogen phosphorylase. Essays Biochem 1970;6:23–68.
[31] Egloff MP, Johnson DF, Moorhead G, Cohen PT, Cohen P, Barford D. Structural basis for the recognition of regulatory subunits by the catalytic subunit of protein phosphatase 1. EMBO J 1997;16(8):1876–87.
[32] Meiselbach H, Sticht H, Enz R. Structural analysis of the protein phosphatase 1 docking motif: molecular description of binding specificities identifies interacting proteins. Chem Biol 2006;13(1):49–59.
[33] Hubbard MJ, Cohen P. On target with a new mechanism for the regulation of protein phosphorylation. Trends Biochem Sci 1993;18(5):172–7.
[34] Terrak M, Kerff F, Langsetmo K, Tao T, Dominguez R. Structural basis of protein phosphatase 1 regulation. Nature 2004;429(6993):780–4.
[35] Hirano K, Phan BC, Hartshorne DJ. Interactions of the subunits of smooth muscle myosin phosphatase. J Biol Chem 1997;272(6):3683–8.
[36] Dohadwala M, da Cruz e Silva EF, Hall FL, Williams RT, Carbonaro-Hall DA, Nairn AC, et al. Phosphorylation and inactivation of protein phosphatase 1 by cyclin-dependent kinases. Proc Natl Acad Sci USA 1994;91(14):6408–12.

[37] Eto M, Brautigan DL. Endogenous inhibitor proteins that connect Ser/Thr kinases and phosphatases in cell signaling. IUBMB Life 2012;64(9):732–9.
[38] Janssens V, Goris J, Van Hoof C. PP2A: the expected tumor suppressor. Curr Opin Genet Dev 2005;15(1):34–41.
[39] Arino J, Woon CW, Brautigan DL, Miller TB, Jr., Johnson GL. Human liver phosphatase 2A: cDNA and amino acid sequence of two catalytic subunit isotypes. Proc Natl Acad Sci USA 1988; 85(12):4252–6.
[40] Stone SR, Hofsteenge J, Hemmings BA. Molecular cloning of cDNAs encoding two isoforms of the catalytic subunit of protein phosphatase 2A. Biochemistry 1987;26(23):7215–20.
[41] Kremmer E, Ohst K, Kiefer J, Brewis N, Walter G. Separation of PP2A core enzyme and holoenzyme with monoclonal antibodies against the regulatory A subunit: abundant expression of both forms in cells. Mol Cell Biol 1997;17(3):1692–701.
[42] Mumby M. PP2A: unveiling a reluctant tumor suppressor. Cell 2007;130(1):21–4.
[43] Xu Y, Xing Y, Chen Y, Chao Y, Lin Z, Fan E, et al. Structure of the protein phosphatase 2A holoenzyme. Cell 2006;127(6):1239–51.
[44] Drewes G, Mandelkow EM, Baumann K, Goris J, Merlevede W, Mandelkow E. Dephosphorylation of tau protein and Alzheimer paired helical filaments by calcineurin and phosphatase-2A. FEBS Lett 1993;336(3):425–32.
[45] Gong CX, Grundke-Iqbal I, Iqbal K. Dephosphorylation of Alzheimer's disease abnormally phosphorylated tau by protein phosphatase-2A. Neuroscience 1994;61(4):765–72.
[46] Kitajima TS, Sakuno T, Ishiguro K, Iemura S, Natsume T, Kawashima SA, et al. Shugoshin collaborates with protein phosphatase 2A to protect cohesin. Nature 2006;441(7089):46–52.
[47] Tang Z, Shu H, Qi W, Mahmood NA, Mumby MC, Yu H. PP2A is required for centromeric localization of Sgo1 and proper chromosome segregation. Dev Cell 2006;10(5):575–85.
[48] Tolstykh T, Lee J, Vafai S, Stock JB. Carboxyl methylation regulates phosphoprotein phosphatase 2A by controlling the association of regulatory B subunits. EMBO J 2000; 19(21):5682–91.
[49] Wu J, Tolstykh T, Lee J, Boyd K, Stock JB, Broach JR. Carboxyl methylation of the phosphoprotein phosphatase 2A catalytic subunit promotes its functional association with regulatory subunits in vivo. EMBO J 2000;19(21):5672–81.
[50] Ogris E, Gibson DM, Pallas DC. Protein phosphatase 2A subunit assembly: the catalytic subunit carboxy terminus is important for binding cellular B subunit but not polyomavirus middle tumor antigen. Oncogene 1997;15(8):911–7.
[51] Sontag E, Hladik C, Montgomery L, Luangpirom A, Mudrak I, Ogris E, et al. Downregulation of protein phosphatase 2A carboxyl methylation and methyltransferase may contribute to Alzheimer disease pathogenesis. J Neuropathol Exp Neurol 2004;63(10):1080–91.
[52] Turowski P, Fernandez A, Favre B, Lamb NJ, Hemmings BA. Differential methylation and altered conformation of cytoplasmic and nuclear forms of protein phosphatase 2A during cell cycle progression. J Cell Biol 1995;129(2):397–410.
[53] Xing Y, Xu Y, Chen Y, Jeffrey PD, Chao Y, Lin Z, et al. Structure of protein phosphatase 2A core enzyme bound to tumor-inducing toxins. Cell 2006;127(2):341–53.
[54] Ruediger R, Ruiz J, Walter G. Human cancer-associated mutations in the Aα subunit of protein phosphatase 2A increase lung cancer incidence in Aα knock-in and knockout mice. Mol Cell Biol 2011;31(18):3832–44.
[55] Ruediger R, Pham HT, Walter G. Disruption of protein phosphatase 2A subunit interaction in human cancers with mutations in the A alpha subunit gene. Oncogene 2001;20(1):10–5.
[56] Ruediger R, Pham HT, Walter G. Alterations in protein phosphatase 2A subunit interaction in human carcinomas of the lung and colon with mutations in the A beta subunit gene. Oncogene 2001;20(15):1892–9.

[57] Groves MR, Hanlon N, Turowski P, Hemmings BA, Barford D. The structure of the protein phosphatase 2A PR65/A subunit reveals the conformation of its 15 tandemly repeated HEAT motifs. Cell 1999;96(1):99–110.
[58] Xing Y, Li Z, Chen Y, Stock JB, Jeffrey PD, Shi Y. Structural mechanism of demethylation and inactivation of protein phosphatase 2A. Cell 2008;133(1):154–63.
[59] Shi Y. Assembly and structure of protein phosphatase 2A. Sci China C Life Sci 2009;52(2):135–46.
[60] Xu Y, Chen Y, Zhang P, Jeffrey PD, Shi Y. Structure of a protein phosphatase 2A holoenzyme: insights into B55-mediated Tau dephosphorylation. Mol Cell 2008;31(6):873–85.
[61] Wall MA, Coleman DE, Lee E, Iniguez-Lluhi JA, Posner BA, Gilman AG, et al. The structure of the G protein heterotrimer Gi alpha 1 beta 1 gamma 2. Cell 1995;83(6):1047–58.
[62] Wang J, Wang Z, Yu T, Yang H, Virshup DM, Kops GJ, et al. Crystal structure of a PP2A B56-BubR1 complex and its implications for PP2A substrate recruitment and localization. Protein Cell 2016;7(7):516–26.
[63] Chen J, Martin BL, Brautigan DL. Regulation of protein serine-threonine phosphatase type-2A by tyrosine phosphorylation. Science 1992;257(5074):1261–4.
[64] Letourneux C, Rocher G, Porteu F. B56-containing PP2A dephosphorylate ERK and their activity is controlled by the early gene IEX-1 and ERK. EMBO J 2006;25(4):727–38.
[65] Xu Z, Cetin B, Anger M, Cho US, Helmhart W, Nasmyth K, et al. Structure and function of the PP2A-shugoshin interaction. Mol Cell 2009;35(4):426–41.
[66] Rusnak F, Mertz P. Calcineurin: form and function. Physiol Rev 2000;80(4):1483–521.
[67] Griffith JP, Kim JL, Kim EE, Sintchak MD, Thomson JA, Fitzgibbon MJ, et al. X-ray structure of calcineurin inhibited by the immunophilin-immunosuppressant FKBP12-FK506 complex. Cell 1995;82(3):507–22.
[68] Kissinger CR, Parge HE, Knighton DR, Lewis CT, Pelletier LA, Tempczyk A, et al. Crystal structures of human calcineurin and the human FKBP12-FK506-calcineurin complex. Nature 1995;378(6557):641–4.
[69] Ye Q, Wang H, Zheng J, Wei Q, Jia Z. The complex structure of calmodulin bound to a calcineurin peptide. Proteins 2008;73(1):19–27.
[70] Li H, Rao A, Hogan PG. Structural delineation of the calcineurin-NFAT interaction and its parallels to PP1 targeting interactions. J Mol Biol 2004;342(5):1659–74.
[71] Roy J, Li H, Hogan PG, Cyert MS. A conserved docking site modulates substrate affinity for calcineurin, signaling output, and in vivo function. Mol Cell 2007;25(6):889–901.
[72] Li H, Zhang L, Rao A, Harrison SC, Hogan PG. Structure of calcineurin in complex with PVIVIT peptide: portrait of a low-affinity signalling interaction. J Mol Biol 2007;369(5):1296–306.
[73] Chen MX, Cohen PT. Activation of protein phosphatase 5 by limited proteolysis or the binding of polyunsaturated fatty acids to the TPR domain. FEBS Lett 1997;400(1):136–40.
[74] Hinds TD, Jr., Sanchez ER. Protein phosphatase 5. Int J Biochem Cell Biol 2008;40(11):2358–62.
[75] Golden T, Swingle M, Honkanen RE. The role of serine/threonine protein phosphatase type 5 (PP5) in the regulation of stress-induced signaling networks and cancer. Cancer Metastasis Rev 2008;27(2):169–78.
[76] Zeke T, Morrice N, Vazquez-Martin C, Cohen PT. Human protein phosphatase 5 dissociates from heat-shock proteins and is proteolytically activated in response to arachidonic acid and the microtubule-depolymerizing drug nocodazole. Biochem J 2005;385(Pt 1):45–56.
[77] Becker W, Kentrup H, Klumpp S, Schultz JE, Joost HG. Molecular cloning of a protein serine/threonine phosphatase containing a putative regulatory tetratricopeptide repeat domain. J Biol Chem 1994;269(36):22586–92.
[78] Chen MX, McPartlin AE, Brown L, Chen YH, Barker HM, Cohen PT. A novel human protein serine/threonine phosphatase, which possesses four tetratricopeptide repeat motifs and localizes to the nucleus. EMBO J 1994;13(18):4278–90.

[79] Das AK, Cohen PW, Barford D. The structure of the tetratricopeptide repeats of protein phosphatase 5: implications for TPR-mediated protein-protein interactions. EMBO J 1998;17(5):1192–9.
[80] Kang H, Sayner SL, Gross KL, Russell LC, Chinkers M. Identification of amino acids in the tetratricopeptide repeat and C-terminal domains of protein phosphatase 5 involved in autoinhibition and lipid activation. Biochemistry 2001;40(35):10485–90.
[81] Yang J, Roe SM, Cliff MJ, Williams MA, Ladbury JE, Cohen PT, et al. Molecular basis for TPR domain-mediated regulation of protein phosphatase 5. EMBO J 2005;24(1):1–10.
[82] Cliff MJ, Harris R, Barford D, Ladbury JE, Williams MA. Conformational diversity in the TPR domain-mediated interaction of protein phosphatase 5 with Hsp90. Structure 2006; 14(3):415–26.
[83] Yamaguchi Y, Katoh H, Mori K, Negishi M. Galpha(12) and Galpha(13) interact with Ser/Thr protein phosphatase type 5 and stimulate its phosphatase activity. Curr Biol 2002;12(15):1353–8.
[84] Gentile S, Darden T, Erxleben C, Romeo C, Russo A, Martin N, et al. Rac GTPase signaling through the PP5 protein phosphatase. Proc Natl Acad Sci USA 2006;103(13):5202–6.
[85] Skinner J, Sinclair C, Romeo C, Armstrong D, Charbonneau H, Rossie S. Purification of a fatty acid-stimulated protein-serine/threonine phosphatase from bovine brain and its identification as a homolog of protein phosphatase 5. J Biol Chem 1997;272(36):22464–71.
[86] Botes DP, Tuinman AA, Wessels PL, Viljoen CC, Kruger Hn, Williams DH, et al. The structure of cyanoginosin-LA, a cyclic heptapeptide toxin from the cyanobacterium Microcystis aeruginosa. J Chem Soc, Perkin Trans 1 1984:2311.
[87] Honkanen RE, Zwiller J, Moore RE, Daily SL, Khatra BS, Dukelow M, et al. Characterization of microcystin-LR, a potent inhibitor of type 1 and type 2A protein phosphatases. J Biol Chem 1990;265(32):19401–4.
[88] Yoshizawa S, Matsushima R, Watanabe MF, Harada K, Ichihara A, Carmichael WW, et al. Inhibition of protein phosphatases by microcystins and nodularin associated with hepatotoxicity. J Cancer Res Clin Oncol 1990;116(6):609–14.
[89] Zhang Z, Zhao S, Deans-Zirattu S, Bai G, Lee EYC. Mutagenesis of the catalytic subunit of rabbit muscle protein phosphatase-1. In: Khandelwal RL, Wang JH, editors. Reversible protein phosphorylation in cell regulation. Boston: Springer US, 1993:113–9.
[90] MacKintosh RW, Dalby KN, Campbell DG, Cohen PT, Cohen P, MacKintosh C. The cyanobacterial toxin microcystin binds covalently to cysteine-273 on protein phosphatase 1. FEBS Lett 1995;371(3):236–40.
[91] Tachibana K, Scheuer PJ, Tsukitani Y, Kikuchi H, Van Engen D, Clardy J, et al. Okadaic acid, a cytotoxic polyether from two marine sponges of the genus Halichondria. Journal of the American Chemical Society 1981;103(9):2469–71.
[92] Bialojan C, Takai A. Inhibitory effect of a marine-sponge toxin, okadaic acid, on protein phosphatases. Specificity and kinetics. Biochem J 1988;256(1):283–90.
[93] Maynes JT, Bateman KS, Cherney MM, Das AK, Luu HA, Holmes CF, et al. Crystal structure of the tumor-promoter okadaic acid bound to protein phosphatase-1. J Biol Chem 2001; 276(47):44078–82.
[94] Zhang L, Zhang Z, Long F, Lee EY. Tyrosine-272 is involved in the inhibition of protein phosphatase-1 by multiple toxins. Biochemistry 1996;35(5):1606–11.
[95] Hiraga A, Kikuchi K, Tamura S, Tsuiki S. Purification and characterization of Mg2+-dependent glycogen synthase phosphatase (phosphoprotein phosphatase IA) from rat liver. Eur J Biochem 1981;119(3):503–10.
[96] Lammers T, Lavi S. Role of type 2C protein phosphatases in growth regulation and in cellular stress signaling. Crit Rev Biochem Mol Biol 2007;42(6):437–61.
[97] Tamura S, Lynch KR, Larner J, Fox J, Yasui A, Kikuchi K, et al. Molecular cloning of rat type 2C (IA) protein phosphatase mRNA. Proc Natl Acad Sci USA 1989;86(6):1796–800.

[98] Das AK, Helps NR, Cohen PT, Barford D. Crystal structure of the protein serine/threonine phosphatase 2C at 2.0 A resolution. EMBO J 1996;15(24):6798–809.
[99] Kriventseva EV, Koch I, Apweiler R, Vingron M, Bork P, Gelfand MS, et al. Increase of functional diversity by alternative splicing. Trends Genet 2003;19(3):124–8.
[100] Xue T, Wang D, Zhang S, Ehlting J, Ni F, Jakab S, et al. Genome-wide and expression analysis of protein phosphatase 2C in rice and Arabidopsis. BMC Genomics 2008;9:550.
[101] Rantanen MK, Lehtio L, Rajagopal L, Rubens CE, Goldman A. Structure of Streptococcus agalactiae serine/threonine phosphatase. The subdomain conformation is coupled to the binding of a third metal ion. FEBS J 2007;274(12):3128–37.
[102] Wehenkel A, Bellinzoni M, Schaeffer F, Villarino A, Alzari PM. Structural and binding studies of the three-metal center in two mycobacterial PPM Ser/Thr protein phosphatases. J Mol Biol 2007;374(4):890–8.
[103] Brautigan DL, Brown M, Grindrod S, Chinigo G, Kruszewski A, Lukasik SM, et al. Allosteric activation of protein phosphatase 2C by D-chiro-inositol-galactosamine, a putative mediator mimetic of insulin action. Biochemistry 2005;44(33):11067–73.
[104] Ma Y, Szostkiewicz I, Korte A, Moes D, Yang Y, Christmann A, et al. Regulators of PP2C phosphatase activity function as abscisic acid sensors. Science 2009;324(5930):1064–8.
[105] Takekawa M, Adachi M, Nakahata A, Nakayama I, Itoh F, Tsukuda H, et al. p53-inducible wip1 phosphatase mediates a negative feedback regulation of p38 MAPK-p53 signaling in response to UV radiation. EMBO J 2000;19(23):6517–26.
[106] Ofek P, Ben-Meir D, Kariv-Inbal Z, Oren M, Lavi S. Cell cycle regulation and p53 activation by protein phosphatase 2C alpha. J Biol Chem 2003;278(16):14299–305.
[107] Shreeram S, Demidov ON, Hee WK, Yamaguchi H, Onishi N, Kek C, et al. Wip1 phosphatase modulates ATM-dependent signaling pathways. Mol Cell 2006;23(5):757–64.
[108] Allemand E, Hastings ML, Murray MV, Myers MP, Krainer AR. Alternative splicing regulation by interaction of phosphatase PP2Cgamma with nucleic acid-binding protein YB-1. Nat Struct Mol Biol 2007;14(7):630–8.
[109] Gao T, Furnari F, Newton AC. PHLPP: a phosphatase that directly dephosphorylates Akt, promotes apoptosis, and suppresses tumor growth. Mol Cell 2005;18(1):13–24.
[110] Qiao M, Wang Y, Xu X, Lu J, Dong Y, Tao W, et al. Mst1 is an interacting protein that mediates PHLPPs' induced apoptosis. Mol Cell 2010;38(4):512–23.
[111] Vogt A, Tamewitz A, Skoko J, Sikorski RP, Giuliano KA, Lazo JS. The benzo[c]phenanthridine alkaloid, sanguinarine, is a selective, cell-active inhibitor of mitogen-activated protein kinase phosphatase-1. J Biol Chem 2005;280(19):19078–86.
[112] Sierecki E, Sinko W, McCammon JA, Newton AC. Discovery of small molecule inhibitors of the PH domain leucine-rich repeat protein phosphatase (PHLPP) by chemical and virtual screening. J Med Chem.2010;53(19):6899–911.
[113] Rogers JP, Beuscher AEt, Flajolet M, McAvoy T, Nairn AC, Olson AJ, et al. Discovery of protein phosphatase 2C inhibitors by virtual screening. J Med Chem 2006;49(5):1658–67.
[114] Swierczek K, Pandey AS, Peters JW, Hengge AC. A comparison of phosphonothioic acids with phosphonic acids as phosphatase inhibitors. J Med Chem 2003;46(17):3703–8.
[115] Aburai N, Yoshida M, Ohnishi M, Kimura K. Sanguinarine as a potent and specific inhibitor of protein phosphatase 2C in vitro and induces apoptosis via phosphorylation of p38 in HL60 cells. Biosci Biotechnol Biochem 2010;74(3):548–52.
[116] Li J, Yang Y, Peng Y, Austin RJ, van Eyndhoven WG, Nguyen KC, et al. Oncogenic properties of PPM1D located within a breast cancer amplification epicenter at 17q23. Nat Genet 2002; 31(2):133–4.
[117] Tan DS, Lambros MB, Rayter S, Natrajan R, Vatcheva R, Gao Q, et al. PPM1D is a potential therapeutic target in ovarian clear cell carcinomas. Clin Cancer Res 2009;15(7):2269–80.

[118] Le Guezennec X, Bulavin DV. WIP1 phosphatase at the crossroads of cancer and aging. Trends Biochem Sci 2010;35(2):10–14.
[119] Bulavin DV, Phillips C, Nannenga B, Timofeev O, Donehower LA, Anderson CW, et al. Inactivation of the Wip1 phosphatase inhibits mammary tumorigenesis through p38 MAPK-mediated activation of the p16(Ink4a)-p19(Arf) pathway. Nat Genet 2004;36(4):343–50.
[120] Belova GI, Demidov ON, Fornace AJ, Jr., Bulavin DV. Chemical inhibition of Wip1 phosphatase contributes to suppression of tumorigenesis. Cancer Biol Ther 2005;4(10):1154–8.
[121] Yamaguchi H, Durell SR, Feng H, Bai Y, Anderson CW, Appella E. Development of a substrate-based cyclic phosphopeptide inhibitor of protein phosphatase 2Cdelta, Wip1. Biochemistry 2006;45(44):13193–202.
[122] Lin YX, Xu WN, Liang LR, Pang BS, Nie XH, Zhang J, et al. The cross-sectional and longitudinal association of the BODE index with quality of life in patients with chronic obstructive pulmonary disease. Chin Med J (Engl) 2009;122(24):2939–44.
[123] Allen KN, Dunaway-Mariano D. Phosphoryl group transfer: evolution of a catalytic scaffold. Trends Biochem Sci 2004;29(9):495–503.
[124] Zhang M, Liu J, Kim Y, Dixon JE, Pfaff SL, Gill GN, et al. Structural and functional analysis of the phosphoryl transfer reaction mediated by the human small C-terminal domain phosphatase, Scp1. Protein Sci 2010;19(5):974–86.
[125] Archambault J, Chambers RS, Kobor MS, Ho Y, Cartier M, Bolotin D, et al. An essential component of a C-terminal domain phosphatase that interacts with transcription factor IIF in Saccharomyces cerevisiae. Proc Natl Acad Sci USA 1997;94(26):14300–5.
[126] Buratowski S. The CTD code. Nat Struct Biol 2003;10(9):679–80.
[127] Glover-Cutter K, Kim S, Espinosa J, Bentley DL. RNA polymerase II pauses and associates with pre-mRNA processing factors at both ends of genes. Nat Struct Mol Biol 2008;15(1):71–8.
[128] Fabrega C, Shen V, Shuman S, Lima CD. Structure of an mRNA capping enzyme bound to the phosphorylated carboxy-terminal domain of RNA polymerase II. Mol Cell 2003; 11(6):1549–61.
[129] Cho EJ, Kobor MS, Kim M, Greenblatt J, Buratowski S. Opposing effects of Ctk1 kinase and Fcp1 phosphatase at Ser 2 of the RNA polymerase II C-terminal domain. Genes Dev 2001; 15(24):3319–29.
[130] Hausmann S, Erdjument-Bromage H, Shuman S. Schizosaccharomyces pombe carboxyl-terminal domain (CTD) phosphatase Fcp1: distributive mechanism, minimal CTD substrate, and active site mapping. J Biol Chem 2004;279(12):10892–900.
[131] Zhang Y, Kim Y, Genoud N, Gao J, Kelly JW, Pfaff SL, et al. Determinants for dephosphorylation of the RNA polymerase II C-terminal domain by Scp1. Mol Cell 2006;24(5):759–70.
[132] Zhang M, Cho EJ, Burstein G, Siegel D, Zhang Y. Selective inactivation of a human neuronal silencing phosphatase by a small molecule inhibitor. ACS Chem Biol 2011;6(5):511–9.
[133] Ghosh A, Shuman S, Lima CD. The structure of Fcp1, an essential RNA polymerase II CTD phosphatase. Mol Cell 2008;32(4):478–90.
[134] Sapkota G, Knockaert M, Alarcon C, Montalvo E, Brivanlou AH, Massague J. Dephosphorylation of the linker regions of Smad1 and Smad2/3 by small C-terminal domain phosphatases has distinct outcomes for bone morphogenetic protein and transforming growth factor-beta pathways. J Biol Chem 2006;281(52):40412–9.
[135] Yeo M, Lee SK, Lee B, Ruiz EC, Pfaff SL, Gill GN. Small CTD phosphatases function in silencing neuronal gene expression. Science 2005;307(5709):596–600.
[136] Visvanathan J, Lee S, Lee B, Lee JW, Lee SK. The microRNA miR-124 antagonizes the anti-neural REST/SCP1 pathway during embryonic CNS development. Genes Dev 2007;21(7):744–9.
[137] Xue Y, Ouyang K, Huang J, Zhou Y, Ouyang H, Li H, et al. Direct conversion of fibroblasts to neurons by reprogramming PTB-regulated microRNA circuits. Cell 2013;152(1–2):82–96.

[138] Gohla A, Birkenfeld J, Bokoch GM. Chronophin, a novel HAD-type serine protein phosphatase, regulates cofilin-dependent actin dynamics. Nat Cell Biol 2005;7(1):21–9.
[139] Russell P, Nurse P. cdc25+ functions as an inducer in the mitotic control of fission yeast. Cell 1986;45(1):145–53.
[140] Allegrini S, Scaloni A, Careddu MG, Cuccu G, D'Ambrosio C, Pesi R, et al. Mechanistic studies on bovine cytosolic 5′-nucleotidase II, an enzyme belonging to the HAD superfamily. Eur J Biochem 2004;271(23–24):4881–91.
[141] Denu JM, Dixon JE. A catalytic mechanism for the dual-specific phosphatases. Proc Natl Acad Sci USA 1995;92(13):5910–14.
[142] Denu JM, Dixon JE. Protein tyrosine phosphatases: mechanisms of catalysis and regulation. Curr Opin Chem Biol 1998;2(5):633–41.
[143] Guan KL, Broyles SS, Dixon JE. A Tyr/Ser protein phosphatase encoded by vaccinia virus. Nature 1991;350(6316):359–62.
[144] Derrien M, Punjabi A, Khanna M, Grubisha O, Traktman P. Tyrosine phosphorylation of A17 during vaccinia virus infection: involvement of the H1 phosphatase and the F10 kinase. J Virol 1999;73(9):7287–96.
[145] Liu K, Lemon B, Traktman P. The dual-specificity phosphatase encoded by vaccinia virus, VH1, is essential for viral transcription in vivo and in vitro. J Virol 1995;69(12):7823–34.
[146] Najarro P, Traktman P, Lewis JA. Vaccinia virus blocks gamma interferon signal transduction: viral VH1 phosphatase reverses Stat1 activation. J Virol 2001;75(7):3185–96.
[147] Ishibashi T, Bottaro DP, Chan A, Miki T, Aaronson SA. Expression cloning of a human dual-specificity phosphatase. Proc Natl Acad Sci USA 1992;89(24):12170–74.
[148] Denu JM, Zhou G, Wu L, Zhao R, Yuvaniyama J, Saper MA, et al. The purification and characterization of a human dual-specific protein tyrosine phosphatase. J Biol Chem 1995; 270(8):3796–803.
[149] Todd JL, Tanner KG, Denu JM. Extracellular regulated kinases (ERK) 1 and ERK2 are authentic substrates for the dual-specificity protein-tyrosine phosphatase VHR. A novel role in down-regulating the ERK pathway. J Biol Chem 1999;274(19):13271–80.
[150] Guan KL, Dixon JE. Evidence for protein-tyrosine-phosphatase catalysis proceeding via a cysteine-phosphate intermediate. J Biol Chem 1991;266(26):17026–30.
[151] Denu JM, Zhou G, Guo Y, Dixon JE. The catalytic role of aspartic acid-92 in a human dual-specific protein-tyrosine-phosphatase. Biochemistry 1995;34(10):3396–403.
[152] Denu JM, Stuckey JA, Saper MA, Dixon JE. Form and function in protein dephosphorylation. Cell 1996;87(3):361–64.
[153] Zhou G, Denu JM, Wu L, Dixon JE. The catalytic role of Cys124 in the dual specificity phosphatase VHR. J Biol Chem 1994;269(45):28084–90.
[154] Kamata H, Honda S, Maeda S, Chang L, Hirata H, Karin M. Reactive oxygen species promote TNFalpha-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. Cell 2005;120(5):649–61.
[155] Chiarugi P, Cirri P. Redox regulation of protein tyrosine phosphatases during receptor tyrosine kinase signal transduction. Trends Biochem Sci 2003;28(9):509–14.
[156] Hinton SD, Myers MP, Roggero VR, Allison LA, Tonks NK. The pseudophosphatase MK-STYX interacts with G3BP and decreases stress granule formation. Biochem J 2010;427(3):349–57.
[157] Ekerot M, Stavridis MP, Delavaine L, Mitchell MP, Staples C, Owens DM, et al. Negative-feedback regulation of FGF signalling by DUSP6/MKP-3 is driven by ERK1/2 and mediated by Ets factor binding to a conserved site within the DUSP6/MKP-3 gene promoter. Biochem J 2008;412(2):287–98.
[158] Brondello JM, Pouyssegur J, McKenzie FR. Reduced MAP kinase phosphatase-1 degradation after p42/p44MAPK-dependent phosphorylation. Science 1999;286(5449):2514–17.

[159] Wu Q, Huang S, Sun Y, Gu S, Lu F, Dai J, et al. Dual specificity phosphotase 18, interacting with SAPK, dephosphorylates SAPK and inhibits SAPK/JNK signal pathway in vivo. Front Biosci 2006;11:2714–24.
[160] Camps M, Nichols A, Gillieron C, Antonsson B, Muda M, Chabert C, et al. Catalytic activation of the phosphatase MKP-3 by ERK2 mitogen-activated protein kinase. Science 1998; 280(5367):1262–65.
[161] Stewart AE, Dowd S, Keyse SM, McDonald NQ. Crystal structure of the MAPK phosphatase Pyst1 catalytic domain and implications for regulated activation. Nature Structural Biology 1999;6:74.
[162] Katagiri C, Masuda K, Urano T, Yamashita K, Araki Y, Kikuchi K, et al. Phosphorylation of Ser-446 determines stability of MKP-7. J Biol Chem 2005;280(15):14716–22.
[163] Xu S, Furukawa T, Kanai N, Sunamura M, Horii A. Abrogation of DUSP6 by hypermethylation in human pancreatic cancer. J Hum Genet 2005;50(4):159–67.
[164] Chi H, Flavell RA. Acetylation of MKP-1 and the control of inflammation. Sci Signal 2008; 1(41):pe44.
[165] Liu Y, Shepherd EG, Nelin LD. MAPK phosphatases – regulating the immune response. Nat Rev Immunol 2007;7(3):202–12.
[166] Jeffrey KL, Camps M, Rommel C, Mackay CR. Targeting dual-specificity phosphatases: manipulating MAP kinase signalling and immune responses. Nat Rev Drug Discovery 2007;6(5): 391–403.
[167] Chi H, Barry SP, Roth RJ, Wu JJ, Jones EA, Bennett AM, et al. Dynamic regulation of pro- and anti-inflammatory cytokines by MAPK phosphatase 1 (MKP-1) in innate immune responses. Proc Natl Acad Sci USA 2006;103(7):2274–79.
[168] Zhao Q, Wang X, Nelin LD, Yao Y, Matta R, Manson ME, et al. MAP kinase phosphatase 1 controls innate immune responses and suppresses endotoxic shock. J Exp Med 2006;203(1):131–40.
[169] Hammer M, Mages J, Dietrich H, Schmitz F, Striebel F, Murray PJ, et al. Control of dual-specificity phosphatase-1 expression in activated macrophages by IL-10. Eur J Immunol 2005;35(10):2991–3001.
[170] Chu Y, Solski PA, Khosravi-Far R, Der CJ, Kelly K. The mitogen-activated protein kinase phosphatases PAC1, MKP-1, and MKP-2 have unique substrate specificities and reduced activity in vivo toward the ERK2 sevenmaker mutation. J Biol Chem 1996;271(11):6497–501.
[171] Jeffrey KL, Brummer T, Rolph MS, Liu SM, Callejas NA, Grumont RJ, et al. Positive regulation of immune cell function and inflammatory responses by phosphatase PAC-1. Nat Immunol 2006;7 (3):274–83.
[172] Zhang Y, Blattman JN, Kennedy NJ, Duong J, Nguyen T, Wang Y, et al. Regulation of innate and adaptive immune responses by MAP kinase phosphatase 5. Nature 2004;430(7001):793–97.
[173] Furukawa T, Sunamura M, Motoi F, Matsuno S, Horii A. Potential tumor suppressive pathway involving DUSP6/MKP-3 in pancreatic cancer. Am J Pathol 2003;162(6):1807–15.
[174] Nunes-Xavier C, Roma-Mateo C, Rios P, Tarrega C, Cejudo-Marin R, Tabernero L, et al. Dual-specificity MAP kinase phosphatases as targets of cancer treatment. Anticancer Agents Med Chem 2011;11(1):109–32.
[175] Hoornaert I, Marynen P, Goris J, Sciot R, Baens M. MAPK phosphatase DUSP16/MKP-7, a candidate tumor suppressor for chromosome region 12p12-13, reduces BCR-ABL-induced transformation. Oncogene 2003;22(49):7728–36.
[176] Brown MR, Chuaqui R, Vocke CD, Berchuck A, Middleton LP, Emmert-Buck MR, et al. Allelic loss on chromosome arm 8p: analysis of sporadic epithelial ovarian tumors. Gynecol Oncol 1999; 74(1):98–102.
[177] Sato M, Vaughan MB, Girard L, Peyton M, Lee W, Shames DS, et al. Multiple oncogenic changes (K-RAS(V12), p53 knockdown, mutant EGFRs, p16 bypass, telomerase) are not sufficient to

confer a full malignant phenotype on human bronchial epithelial cells. Cancer Res 2006; 66(4):2116–28.
[178] Srikanth S, Franklin CC, Duke RC, Kraft RS. Human DU145 prostate cancer cells overexpressing mitogen-activated protein kinase phosphatase-1 are resistant to Fas ligand-induced mitochondrial perturbations and cellular apoptosis. Mol Cell Biochem 1999;199(1–2):169–78.
[179] Weber RG, Rieger J, Naumann U, Lichter P, Weller M. Chromosomal imbalances associated with response to chemotherapy and cytotoxic cytokines in human malignant glioma cell lines. Int J Cancer 2001;91(2):213–18.
[180] Bang YJ, Kwon JH, Kang SH, Kim JW, Yang YC. Increased MAPK activity and MKP-1 overexpression in human gastric adenocarcinoma. Biochem Biophys Res Commun 1998;250(1):43–47.
[181] Murty VV, Reuter VE, Bosl GJ, Chaganti RS. Deletion mapping identifies loss of heterozygosity at 5p15.1-15.2, 5q11 and 5q34-35 in human male germ cell tumors. Oncogene 1996;12(12):2719–23.
[182] Peng HQ, Liu L, Goss PE, Bailey D, Hogg D. Chromosomal deletions occur in restricted regions of 5q in testicular germ cell cancer. Oncogene 1999;18(21):3277–83.
[183] Sanchez-Perez I, Martinez-Gomariz M, Williams D, Keyse SM, Perona R. CL100/MKP-1 modulates JNK activation and apoptosis in response to cisplatin. Oncogene 2000;19(45):5142–52.
[184] Niwa R, Nagata-Ohashi K, Takeichi M, Mizuno K, Uemura T. Control of actin reorganization by Slingshot, a family of phosphatases that dephosphorylate ADF/cofilin. Cell 2002;108(2): 233–46.
[185] Ohta Y, Kousaka K, Nagata-Ohashi K, Ohashi K, Muramoto A, Shima Y, et al. Differential activities, subcellular distribution and tissue expression patterns of three members of Slingshot family phosphatases that dephosphorylate cofilin. Genes Cells 2003;8(10):811–24.
[186] Kaji N, Ohashi K, Shuin M, Niwa R, Uemura T, Mizuno K. Cell cycle-associated changes in Slingshot phosphatase activity and roles in cytokinesis in animal cells. J Biol Chem 2003;278 (35):33450–55.
[187] Huang TY, DerMardirossian C, Bokoch GM. Cofilin phosphatases and regulation of actin dynamics. Curr Opin Cell Biol 2006;18(1):26–31.
[188] Bessette DC, Qiu D, Pallen CJ. PRL PTPs: mediators and markers of cancer progression. Cancer Metastasis Rev 2008;27(2):231–52.
[189] Stephens BJ, Han H, Gokhale V, Von Hoff DD. PRL phosphatases as potential molecular targets in cancer. Mol Cancer Ther 2005;4(11):1653–61.
[190] Wang J, Kirby CE, Herbst R. The tyrosine phosphatase PRL-1 localizes to the endoplasmic reticulum and the mitotic spindle and is required for normal mitosis. J Biol Chem 2002;277 (48):46659–68.
[191] Zeng Q, Si X, Horstmann H, Xu Y, Hong W, Pallen CJ. Prenylation-dependent association of protein-tyrosine phosphatases PRL-1, -2, and -3 with the plasma membrane and the early endosome. J Biol Chem 2000;275(28):21444–52.
[192] Kozlov G, Cheng J, Ziomek E, Banville D, Gehring K, Ekiel I. Structural insights into molecular function of the metastasis-associated phosphatase PRL-3. J Biol Chem 2004;279(12): 11882–89.
[193] Jeong DG, Kim SJ, Kim JH, Son JH, Park MR, Lim SM, et al. Trimeric structure of PRL-1 phosphatase reveals an active enzyme conformation and regulation mechanisms. J Mol Biol 2005;345(2):401–13.
[194] Kim KA, Song JS, Jee J, Sheen MR, Lee C, Lee TG, et al. Structure of human PRL-3, the phosphatase associated with cancer metastasis. FEBS Lett 2004;565(1–3):181–87.
[195] Achiwa H, Lazo JS. PRL-1 tyrosine phosphatase regulates c-Src levels, adherence, and invasion in human lung cancer cells. Cancer Res 2007;67(2):643–50.
[196] Luo Y, Liang F, Zhang ZY. PRL1 promotes cell migration and invasion by increasing MMP2 and MMP9 expression through Src and ERK1/2 pathways. Biochemistry 2009;48(8):1838–46.

[197] Fiordalisi JJ, Keller PJ, Cox AD. PRL tyrosine phosphatases regulate rho family GTPases to promote invasion and motility. Cancer Res 2006;66(6):3153–61.
[198] Wang H, Quah SY, Dong JM, Manser E, Tang JP, Zeng Q. PRL-3 down-regulates PTEN expression and signals through PI3K to promote epithelial-mesenchymal transition. Cancer Res 2007; 67(7):2922–26.
[199] Forte E, Orsatti L, Talamo F, Barbato G, De Francesco R, Tomei L. Ezrin is a specific and direct target of protein tyrosine phosphatase PRL-3. Biochim Biophys Acta 2008;1783(2):334–44.
[200] Diamond RH, Cressman DE, Laz TM, Abrams CS, Taub R. PRL-1, a unique nuclear protein tyrosine phosphatase, affects cell growth. Mol Cell Biol 1994;14(6):3752–62.
[201] Zeng Q, Dong JM, Guo K, Li J, Tan HX, Koh V, et al. PRL-3 and PRL-1 promote cell migration, invasion, and metastasis. Cancer Res 2003;63(11):2716–22.
[202] Wang L, Peng L, Dong B, Kong L, Meng L, Yan L, et al. Overexpression of phosphatase of regenerating liver-3 in breast cancer: association with a poor clinical outcome. Ann Oncol 2006;17(10):1517–22.
[203] Miskad UA, Semba S, Kato H, Yokozaki H. Expression of PRL-3 phosphatase in human gastric carcinomas: close correlation with invasion and metastasis. Pathobiology 2004;71(4): 176–84.
[204] Zhao WB, Li Y, Liu X, Zhang LY, Wang X. Evaluation of PRL-3 expression, and its correlation with angiogenesis and invasion in hepatocellular carcinoma. Int J Mol Med 2008;22(2): 187–92.
[205] Dai N, Lu AP, Shou CC, Li JY. Expression of phosphatase regenerating liver 3 is an independent prognostic indicator for gastric cancer. World J Gastroenterol 2009;15(12):1499–505.
[206] Visintin R, Craig K, Hwang ES, Prinz S, Tyers M, Amon A. The phosphatase Cdc14 triggers mitotic exit by reversal of Cdk-dependent phosphorylation. Mol Cell 1998;2(6):709–18.
[207] Gray CH, Good VM, Tonks NK, Barford D. The structure of the cell cycle protein Cdc14 reveals a proline-directed protein phosphatase. EMBO J 2003;22(14):3524–35.
[208] Songyang Z, Blechner S, Hoagland N, Hoekstra MF, Piwnica-Worms H, Cantley LC. Use of an oriented peptide library to determine the optimal substrates of protein kinases. Curr Biol 1994;4(11):973–82.
[209] Lu KP, Liou YC, Zhou XZ. Pinning down proline-directed phosphorylation signaling. Trends Cell Biol 2002;12(4):164–72.
[210] Trautmann S, McCollum D. Cell cycle: new functions for Cdc14 family phosphatases. Curr Biol 2002;12(21):R733–35.
[211] Gallo O, Pinto S, Boccuzzi S, Dilaghi M, Gallina E, Attanasio M, et al. Monocyte tumor necrosis factor production in head and neck squamous cell carcinoma. Laryngoscope 1992;102(4): 447–50.
[212] Trinkle-Mulcahy L, Lamond AI. Mitotic phosphatases: no longer silent partners. Curr Opin Cell Biol 2006;18(6):623–31.
[213] Mailand N, Lukas C, Kaiser BK, Jackson PK, Bartek J, Lukas J. Deregulated human Cdc14A phosphatase disrupts centrosome separation and chromosome segregation. Nat Cell Biol 2002;4(4):317–22.
[214] Clemente-Blanco A, Mayan-Santos M, Schneider DA, Machin F, Jarmuz A, Tschochner H, et al. Cdc14 inhibits transcription by RNA polymerase I during anaphase. Nature 2009; 458(7235):219–22.
[215] Esteban V, Vazquez-Novelle MD, Calvo E, Bueno A, Sacristan MP. Human Cdc14A reverses CDK1 phosphorylation of Cdc25A on serines 115 and 320. Cell Cycle 2006;5(24):2894–98.
[216] Paulsen MT, Starks AM, Derheimer FA, Hanasoge S, Li L, Dixon JE, et al. The p53-targeting human phosphatase hCdc14A interacts with the Cdk1/cyclin B complex and is differentially expressed in human cancers. Mol Cancer 2006;5:25.

[217] Lanzetti L, Margaria V, Melander F, Virgili L, Lee MH, Bartek J, et al. Regulation of the Rab5 GTPase-activating protein RN-tre by the dual specificity phosphatase Cdc14A in human cells. J Biol Chem 2007;282(20):15258–70.
[218] Steck PA, Pershouse MA, Jasser SA, Yung WK, Lin H, Ligon AH, et al. Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat Genet 1997;15(4):356–362.
[219] Naguib A, Bencze G, Cho H, Zheng W, Tocilj A, Elkayam E, et al. PTEN functions by recruitment to cytoplasmic vesicles. Mol Cell 2015;58(2):255–68.
[220] Gil A, Rodriguez-Escudero I, Stumpf M, Molina M, Cid VJ, Pulido R. A functional dissection of PTEN N-terminus: implications in PTEN subcellular targeting and tumor suppressor activity. PLoS One 2015;10(4):e0119287.
[221] Iijima M, Huang YE, Luo HR, Vazquez F, Devreotes PN. Novel mechanism of PTEN regulation by its phosphatidylinositol 4,5-bisphosphate binding motif is critical for chemotaxis. J Biol Chem 2004;279(16):16606–13.
[222] Hopkins BD, Fine B, Steinbach N, Dendy M, Rapp Z, Shaw J, et al. A secreted PTEN phosphatase that enters cells to alter signaling and survival. Science 2013;341(6144):399–402.
[223] Masson GR, Perisic O, Burke JE, Williams RL. The intrinsically disordered tails of PTEN and PTEN-L have distinct roles in regulating substrate specificity and membrane activity. Biochem J 2016;473(2):135–44.
[224] Shi Y, Wang J, Chandarlapaty S, Cross J, Thompson C, Rosen N, et al. PTEN is a protein tyrosine phosphatase for IRS1. Nat Struct Mol Biol 2014;21(6):522–27.
[225] Shnitsar I, Bashkurov M, Masson GR, Ogunjimi AA, Mosessian S, Cabeza EA, et al. PTEN regulates cilia through Dishevelled. Nat Commun 2015;6:8388.
[226] Chen Z, Trotman LC, Shaffer D, Lin HK, Dotan ZA, Niki M, et al. Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. Nature 2005;436 (7051):725–30.
[227] Pilarski R, Eng C. Will the real Cowden syndrome please stand up (again)? Expanding mutational and clinical spectra of the PTEN hamartoma tumour syndrome. J Med Genet 2004;41(5):323–26.
[228] Napoli E, Ross-Inta C, Wong S, Hung C, Fujisawa Y, Sakaguchi D, et al. Mitochondrial dysfunction in Pten haplo-insufficient mice with social deficits and repetitive behavior: interplay between Pten and p53. PLoS One 2012;7(8):e42504.
[229] Wishart MJ, Dixon JE. PTEN and myotubularin phosphatases: from 3-phosphoinositide dephosphorylation to disease. Trends Cell Biol 2002;12(12):579–85.
[230] Robinson FL, Dixon JE. Myotubularin phosphatases: policing 3-phosphoinositides. Trends Cell Biol 2006;16(8):403–12.
[231] Laporte J, Liaubet L, Blondeau F, Tronchere H, Mandel JL, Payrastre B. Functional redundancy in the myotubularin family. Biochem Biophys Res Commun 2002;291(2):305–12.
[232] Laporte J, Blondeau F, Buj-Bello A, Mandel JL. The myotubularin family: from genetic disease to phosphoinositide metabolism. Trends Genet 2001;17(4):221–28.
[233] Kim SA, Taylor GS, Torgersen KM, Dixon JE. Myotubularin and MTMR2, phosphatidylinositol 3-phosphatases mutated in myotubular myopathy and type 4B Charcot-Marie-Tooth disease. J Biol Chem 2002;277(6):4526–31.
[234] Aoki N, Aoyama K, Nagata M, Matsuda T. A growing family of dual specificity phosphatases with low molecular masses. J Biochem 2001;130(1):133–40.
[235] Yuvaniyama J, Denu JM, Dixon JE, Saper MA. Crystal structure of the dual specificity protein phosphatase VHR. Science 1996;272(5266):1328–31.
[236] Schumacher MA, Todd JL, Rice AE, Tanner KG, Denu JM. Structural basis for the recognition of a bisphosphorylated MAP kinase peptide by human VHR protein phosphatase. Biochemistry 2002;41(9):3009–17.

[237] Pavic K, Duan G, Kohn M. VHR/DUSP3 phosphatase: structure, function and regulation. FEBS J 2015;282(10):1871–90.
[238] Wang JY, Yeh CL, Chou HC, Yang CH, Fu YN, Chen YT, et al. Vaccinia H1-related phosphatase is a phosphatase of ErbB receptors and is down-regulated in non-small cell lung cancer. J Biol Chem 2011;286(12):10177–84.
[239] Alonso A, Rahmouni S, Williams S, van Stipdonk M, Jaroszewski L, Godzik A, et al. Tyrosine phosphorylation of VHR phosphatase by ZAP-70. Nat Immunol 2003;4(1):44–48.
[240] Pavic K, Rios P, Dzeyk K, Koehler C, Lemke EA, Kohn M. Unnatural amino acid mutagenesis reveals dimerization as a negative regulatory mechanism of VHR's phosphatase activity. ACS Chem Biol 2014;9(7):1451–59.
[241] Henkens R, Delvenne P, Arafa M, Moutschen M, Zeddou M, Tautz L, et al. Cervix carcinoma is associated with an up-regulation and nuclear localization of the dual-specificity protein phosphatase VHR. BMC Cancer 2008;8:147.
[242] Arnoldussen YJ, Lorenzo PI, Pretorius ME, Waehre H, Risberg B, Maelandsmo GM, et al. The mitogen-activated protein kinase phosphatase vaccinia H1-related protein inhibits apoptosis in prostate cancer cells and is overexpressed in prostate cancer. Cancer Res 2008; 68(22):9255–64.
[243] Alonso A, Burkhalter S, Sasin J, Tautz L, Bogetz J, Huynh H, et al. The minimal essential core of a cysteine-based protein-tyrosine phosphatase revealed by a novel 16-kDa VH1-like phosphatase, VHZ. J Biol Chem 2004;279(34):35768–74.
[244] Tang JP, Tan CP, Li J, Siddique MM, Guo K, Chan SW, et al. VHZ is a novel centrosomal phosphatase associated with cell growth and human primary cancers. Mol Cancer 2010;9:128.
[245] Lin FY, Chang CW, Cheong ML, Chen HC, Lee DY, Chang GD, et al. Dual-specificity phosphatase 23 mediates GCM1 dephosphorylation and activation. Nucleic Acids Res 2011;39(3):848–61.
[246] Takagaki K, Satoh T, Tanuma N, Masuda K, Takekawa M, Shima H, et al. Characterization of a novel low-molecular-mass dual-specificity phosphatase-3 (LDP-3) that enhances activation of JNK and p38. Biochem J 2004;383(Pt. 3):447–55.
[247] Chen HH, Luche R, Wei B, Tonks NK. Characterization of two distinct dual specificity phosphatases encoded in alternative open reading frames of a single gene located on human chromosome 10q22.2. J Biol Chem 2004;279(40):41404–13.
[248] Nakamura K, Shima H, Watanabe M, Haneji T, Kikuchi K. Molecular cloning and characterization of a novel dual-specificity protein phosphatase possibly involved in spermatogenesis. Biochem J 1999;344 Pt 3:819–25.
[249] Katagiri C, Masuda K, Nomura M, Tanoue K, Fujita S, Yamashita Y, et al. DUSP13B/TMDP inhibits stress-activated MAPKs and suppresses AP-1-dependent gene expression. Mol Cell Biochem 2011;352(1–2):155–62.
[250] Takagaki K, Shima H, Tanuma N, Nomura M, Satoh T, Watanabe M, et al. Characterization of a novel low-molecular-mass dual specificity phosphatase-4 (LDP-4) expressed in brain. Mol Cell Biochem 2007;296(1–2):177–84.
[251] Yu W, Imoto I, Inoue J, Onda M, Emi M, Inazawa J. A novel amplification target, DUSP26, promotes anaplastic thyroid cancer cell growth by inhibiting p38 MAPK activity. Oncogene 2007;26(8):1178–87.
[252] Tanuma N, Nomura M, Ikeda M, Kasugai I, Tsubaki Y, Takagaki K, et al. Protein phosphatase Dusp26 associates with KIF3 motor and promotes N-cadherin-mediated cell-cell adhesion. Oncogene 2009;28(5):752–61.
[253] Shang X, Vasudevan SA, Yu Y, Ge N, Ludwig AD, Wesson CL, et al. Dual-specificity phosphatase 26 is a novel p53 phosphatase and inhibits p53 tumor suppressor functions in human neuroblastoma. Oncogene 2010;29(35):4938–46.

[254] Friedberg I, Nika K, Tautz L, Saito K, Cerignoli F, Friedberg I, et al. Identification and characterization of DUSP27, a novel dual-specific protein phosphatase. FEBS Lett 2007;581(13): 2527–33.
[255] Lountos GT, Tropea JE, Waugh DS. Structure of human dual-specificity phosphatase 27 at 2.38 A resolution. Acta Crystallogr D Biol Crystallogr 2011;67(Pt 5):471–79.
[256] Wishart MJ, Denu JM, Williams JA, Dixon JE. A single mutation converts a novel phosphotyrosine binding domain into a dual-specificity phosphatase. J Biol Chem 1995;270(45):26782–85.
[257] Wishart MJ, Dixon JE. Gathering STYX: phosphatase-like form predicts functions for unique protein-interaction domains. Trends Biochem Sci 1998;23(8):301–06.
[258] Reiterer V, Fey D, Kolch W, Kholodenko BN, Farhan H. Pseudophosphatase STYX modulates cell-fate decisions and cell migration by spatiotemporal regulation of ERK1/2. Proc Natl Acad Sci USA 2013;110(31):E2934–43.
[259] Wishart MJ, Dixon JE. The archetype STYX/dead-phosphatase complexes with a spermatid mRNA-binding protein and is essential for normal sperm production. Proc Natl Acad Sci USA 2002;99(4):2112–17.
[260] Munoz-Alonso MJ, Guillemain G, Kassis N, Girard J, Burnol AF, Leturque A. A novel cytosolic dual specificity phosphatase, interacting with glucokinase, increases glucose phosphorylation rate. J Biol Chem 2000;275(42):32406–12.
[261] Bonham CA, Vacratsis PO. Redox regulation of the human dual specificity phosphatase YVH1 through disulfide bond formation. J Biol Chem 2009;284(34):22853–64.
[262] Beeser AE, Cooper TG. The dual-specificity protein phosphatase Yvh1p regulates sporulation, growth, and glycogen accumulation independently of catalytic activity in Saccharomyces cerevisiae via the cyclic AMP-dependent protein kinase cascade. J Bacteriol 2000; 182(12):3517–28.
[263] Cain EL, Braun SE, Beeser A. Characterization of a human cell line stably over-expressing the candidate oncogene, dual specificity phosphatase 12. PLoS One 2011;6(4):e18677.
[264] Sharda PR, Bonham CA, Mucaki EJ, Butt Z, Vacratsis PO. The dual-specificity phosphatase hYVH1 interacts with Hsp70 and prevents heat-shock-induced cell death. Biochem J 2009; 418(2):391–401.
[265] Choi HR, Kim WK, Park A, Jung H, Han BS, Lee SC, et al. Protein tyrosine phosphatase profiling studies during brown adipogenic differentiation of mouse primary brown preadipocytes. BMB Rep 2013;46(11):539–43.
[266] Das SK, Chu WS, Hale TC, Wang X, Craig RL, Wang H, et al. Polymorphisms in the glucokinase-associated, dual-specificity phosphatase 12 (DUSP12) gene under chromosome 1q21 linkage peak are associated with type 2 diabetes. Diabetes 2006;55(9):2631–39.
[267] Alonso A, Narisawa S, Bogetz J, Tautz L, Hadzic R, Huynh H, et al. VHY, a novel myristoylated testis-restricted dual specificity protein phosphatase related to VHX. J Biol Chem 2004;279 (31):32586–91.
[268] Schmidt F, van den Eijnden M, Pescini Gobert R, Saborio GP, Carboni S, Alliod C, et al. Identification of VHY/Dusp15 as a regulator of oligodendrocyte differentiation through a systematic genomics approach. PLoS One 2012;7(7):e40457.
[269] Li JP, Fu YN, Chen YR, Tan TH. JNK pathway-associated phosphatase dephosphorylates focal adhesion kinase and suppresses cell migration. J Biol Chem 2010;285(8): 5472–78.
[270] Sekine Y, Ikeda O, Hayakawa Y, Tsuji S, Imoto S, Aoki N, et al. DUSP22/LMW-DSP2 regulates estrogen receptor-alpha-mediated signaling through dephosphorylation of Ser-118. Oncogene 2007;26(41):6038–49.
[271] Sekine Y, Tsuji S, Ikeda O, Sato N, Aoki N, Aoyama K, et al. Regulation of STAT3-mediated signaling by LMW-DSP2. Oncogene 2006;25(42):5801–06.

[272] Wu Q, Gu S, Dai J, Dai J, Wang L, Li Y, et al. Molecular cloning and characterization of a novel dual-specificity phosphatase 18 gene from human fetal brain. Biochim Biophys Acta 2003; 1625(3):296–304.
[273] Zama T, Aoki R, Kamimoto T, Inoue K, Ikeda Y, Hagiwara M. A novel dual specificity phosphatase SKRP1 interacts with the MAPK kinase MKK7 and inactivates the JNK MAPK pathway. Implication for the precise regulation of the particular MAPK pathway. J Biol Chem 2002; 277(26):23909–18.
[274] Zama T, Aoki R, Kamimoto T, Inoue K, Ikeda Y, Hagiwara M. Scaffold role of a mitogen-activated protein kinase phosphatase, SKRP1, for the JNK signaling pathway. J Biol Chem 2002; 277(26):23919–26.
[275] Zheng H, Li Q, Chen R, Zhang J, Ran Y, He X, et al. The dual-specificity phosphatase DUSP14 negatively regulates tumor necrosis factor- and interleukin-1-induced nuclear factor-kappa B activation by dephosphorylating the protein kinase TAK1. J Biol Chem 2013;288(2):819–25.
[276] Deng Q, Li KY, Chen H, Dai JH, Zhai YY, Wang Q, et al. RNA interference against cancer/testis genes identifies dual specificity phosphatase 21 as a potential therapeutic target in human hepatocellular carcinoma. Hepatology 2014;59(2):518–30.
[277] Deshpande T, Takagi T, Hao L, Buratowski S, Charbonneau H. Human PIR1 of the protein-tyrosine phosphatase superfamily has RNA 5′-triphosphatase and diphosphatase activities. J Biol Chem 1999;274(23):16590–94.
[278] Lima CD, Wang LK, Shuman S. Structure and mechanism of yeast RNA triphosphatase: an essential component of the mRNA capping apparatus. Cell 1999;99(5):533–43.
[279] Ganesh S, Tsurutani N, Suzuki T, Hoshii Y, Ishihara T, Delgado-Escueta AV, et al. The carbohydrate-binding domain of Lafora disease protein targets Lafora polyglucosan bodies. Biochem Biophys Res Commun 2004;313(4):1101–09.
[280] Wang J, Stuckey JA, Wishart MJ, Dixon JE. A unique carbohydrate binding domain targets the lafora disease phosphatase to glycogen. J Biol Chem 2002;277(4):2377–80.
[281] Gentry MS, Dowen RH 3rd, Worby CA, Mattoo S, Ecker JR, Dixon JE. The phosphatase laforin crosses evolutionary boundaries and links carbohydrate metabolism to neuronal disease. J Cell Biol 2007;178(3):477–88.
[282] Gentry MS, Roma-Mateo C, Sanz P. Laforin, a protein with many faces: glucan phosphatase, adapter protein, et al. FEBS J 2013;280(2):525–537.
[283] Lohi H, Ianzano L, Zhao XC, Chan EM, Turnbull J, Scherer SW, et al. Novel glycogen synthase kinase 3 and ubiquitination pathways in progressive myoclonus epilepsy. Hum Mol Genet 2005;14(18):2727–36.
[284] Pagliarini DJ, Wiley SE, Kimple ME, Dixon JR, Kelly P, Worby CA, et al. Involvement of a mitochondrial phosphatase in the regulation of ATP production and insulin secretion in pancreatic beta cells. Mol Cell 2005;19(2):197–207.
[285] Pagliarini DJ, Worby CA, Dixon JE. A PTEN-like phosphatase with a novel substrate specificity. J Biol Chem 2004;279(37):38590–96.
[286] Zhang J, Guan Z, Murphy AN, Wiley SE, Perkins GA, Worby CA, et al. Mitochondrial phosphatase PTPMT1 is essential for cardiolipin biosynthesis. Cell Metab 2011;13(6):690–700.
[287] Ramponi G, Manao G, Camici G, Cappugi G, Ruggiero M, Bottaro DP. The 18 kDa cytosolic acid phosphatase from bovine live has phosphotyrosine phosphatase activity on the autophosphorylated epidermal growth factor receptor. FEBS Lett 1989;250(2):469–73.
[288] Wo YY, McCormack AL, Shabanowitz J, Hunt DF, Davis JP, Mitchell GL, et al. Sequencing, cloning, and expression of human red cell-type acid phosphatase, a cytoplasmic phosphotyrosyl protein phosphatase. J Biol Chem 1992;267(15):10856–65.
[289] Hopkinson DA, Spencer N, Harris H. Red cell acid phosphatase variants: a new human polymorphism. Nature 1963;199:969–71.

[290] Lawrence GL, van Etten RL. The low-molecular-weight acid phosphatase from bovine liver: isolation, amino acid composition, and chemical modification studies. Arch Biochem Biophys 1981;206(1):122–31.
[291] de Araujo PS, Mies V, Miranda O. Subcellular distribution of low- and high-molecular-weight acid phosphatases. Biochim Biophys Acta 1976;452(1):121–30.
[292] Ramponi G, Stefani M. Structure and function of the low Mr phosphotyrosine protein phosphatases. Biochim Biophys Acta 1997;1341(2):137–56.
[293] Zhang ZY, Van Etten RL. Purification and characterization of a low-molecular-weight acid phosphatase – a phosphotyrosyl-protein phosphatase from bovine heart. Arch Biochem Biophys 1990;282(1):39–49.
[294] Raugei G, Ramponi G, Chiarugi P. Low molecular weight protein tyrosine phosphatases: small, but smart. Cell Mol Life Sci 2002;59(6):941–49.
[295] Cirri P, Fiaschi T, Chiarugi P, Camici G, Manao G, Raugei G, et al. The molecular basis of the differing kinetic behavior of the two low molecular mass phosphotyrosine protein phosphatase isoforms. J Biol Chem 1996;271(5):2604–07.
[296] Ramponi G, Stefani M. Structural, catalytic, and functional properties of low M(r), phosphotyrosine protein phosphatases. Evidence of a long evolutionary history. Int J Biochem Cell Biol 1997;29(2):279–92.
[297] Modesti A, Marzocchini R, Raugei G, Chiti F, Sereni A, Magherini F, et al. Cloning, expression and characterisation of a new human low Mr phosphotyrosine protein phosphatase originating by alternative splicing. FEBS Lett 1998;431(1):111–15.
[298] Tailor P, Gilman J, Williams S, Mustelin T. A novel isoform of the low molecular weight phosphotyrosine phosphatase, LMPTP-C, arising from alternative mRNA splicing. Eur J Biochem 1999;262(2):277–82.
[299] Zhang M, Van Etten RL, Stauffacher CV. Crystal structure of bovine heart phosphotyrosyl phosphatase at 2.2-A resolution. Biochemistry 1994;33(37):11097–105.
[300] Su XD, Taddei N, Stefani M, Ramponi G, Nordlund P. The crystal structure of a low-molecular-weight phosphotyrosine protein phosphatase. Nature 1994;370(6490):575–78.
[301] Zhang Z, Harms E, Van Etten RL. Asp129 of low molecular weight protein tyrosine phosphatase is involved in leaving group protonation. J Biol Chem 1994;269(42):25947–50.
[302] Chiarugi P, Cirri P, Taddei L, Giannoni E, Camici G, Manao G, et al. The low M(r) protein-tyrosine phosphatase is involved in Rho-mediated cytoskeleton rearrangement after integrin and platelet-derived growth factor stimulation. J Biol Chem 2000;275(7): 4640–46.
[303] Huang L, Sankar S, Lin C, Kontos CD, Schroff AD, Cha EH, et al. HCPTPA, a protein tyrosine phosphatase that regulates vascular endothelial growth factor receptor-mediated signal transduction and biological activity. J Biol Chem 1999;274(53):38183–88.
[304] Park EK, Warner N, Mood K, Pawson T, Daar IO. Low-molecular-weight protein tyrosine phosphatase is a positive component of the fibroblast growth factor receptor signaling pathway. Mol Cell Biol 2002;22(10):3404–14.
[305] Bottini E, Bergamaschi A, Magrini A, Spina C, Ammendola L, Grassi S, et al. Allergy and ACP1 genetic polymorphism. Allergy Asthma Proc 2007;28(1):87–92.
[306] Bottini N, Bottini E, Gloria-Bottini F, Mustelin T. Low-molecular-weight protein tyrosine phosphatase and human disease: in search of biochemical mechanisms. Arch Immunol Ther Exp (Warsz) 2002;50(2):95–104.
[307] Bottini N, Gloria-Bottini F, Lucarini N, Ronchetti PG, Fontana L. Inflammatory bowel disease: Are there gender differences in the genetics of signal transduction? A preliminary study of cytosolic low molecular weight protein tyrosine phosphatase. Dis Markers 2000; 16(3–4):163–66.

[308] Shimohama S, Fujimoto S, Chachin M, Taniguchi T, Perry G, Whitehouse PJ, et al. Alterations of low molecular weight acid phosphatase protein level in Alzheimer's disease. Brain Res 1995;699(1):125–29.
[309] Chiarugi P, Taddei ML, Schiavone N, Papucci L, Giannoni E, Fiaschi T, et al. LMW-PTP is a positive regulator of tumor onset and growth. Oncogene 2004;23(22):3905–14.
[310] Kikawa KD, Vidale DR, Van Etten RL, Kinch MS. Regulation of the EphA2 kinase by the low molecular weight tyrosine phosphatase induces transformation. J Biol Chem 2002; 277(42):39274–79.
[311] Homan KT, Balasubramaniam D, Zabell AP, Wiest O, Helquist P, Stauffacher CV. Identification of novel inhibitors for a low molecular weight protein tyrosine phosphatase via virtual screening. Bioorg Med Chem 2010;18(14):5449–56.
[312] Mascarello A, Chiaradia LD, Vernal J, Villarino A, Guido RV, Perizzolo P, et al. Inhibition of Mycobacterium tuberculosis tyrosine phosphatase PtpA by synthetic chalcones: kinetics, molecular modeling, toxicity and effect on growth. Bioorg Med Chem 2010;18(11):3783–89.
[313] Seiler CL, Richards KA, Jakubowski HV, McIntee EJ. Identification of new inhibitors for low molecular weight protein tyrosine phosphatase isoform B. Bioorg Med Chem Lett 2013; 23(21):5912–14.
[314] Forghieri M, Laggner C, Paoli P, Langer T, Manao G, Camici G, et al. Synthesis, activity and molecular modeling of a new series of chromones as low molecular weight protein tyrosine phosphatase inhibitors. Bioorg Med Chem 2009;17(7):2658–72.
[315] Maccari R, Ottana R, Ciurleo R, Paoli P, Manao G, Camici G, et al. Structure-based optimization of benzoic acids as inhibitors of protein tyrosine phosphatase 1B and low molecular weight protein tyrosine phosphatase. ChemMedChem 2009;4(6):957–62.
[316] Boutros R, Lobjois V, Ducommun B. CDC25 phosphatases in cancer cells: key players? Good targets? Nat Rev Cancer 2007;7(7):495–507.
[317] Rudolph J. Cdc25 phosphatases: structure, specificity, and mechanism. Biochemistry 2007; 46(12):3595–604.
[318] Galaktionov K, Beach D. Specific activation of cdc25 tyrosine phosphatases by B-type cyclins: evidence for multiple roles of mitotic cyclins. Cell 1991;67(6):1181–94.
[319] Krek W, Nigg EA. Mutations of p34cdc2 phosphorylation sites induce premature mitotic events in HeLa cells: evidence for a double block to p34cdc2 kinase activation in vertebrates. EMBO J 1991;10(11):3331–41.
[320] Honda R, Ohba Y, Nagata A, Okayama H, Yasuda H. Dephosphorylation of human p34cdc2 kinase on both Thr-14 and Tyr-15 by human cdc25B phosphatase. FEBS Lett 1993;318(3):331–34.
[321] Bordo D, Bork P. The rhodanese/Cdc25 phosphatase superfamily. Sequence-structure-function relations. EMBO Rep 2002;3(8):741–46.
[322] Cipollone R, Ascenzi P, Tomao P, Imperi F, Visca P. Enzymatic detoxification of cyanide: clues from Pseudomonas aeruginosa Rhodanese. J Mol Microbiol Biotechnol 2008;15(2–3):199–211.
[323] Conklin DS, Galaktionov K, Beach D. 14-3-3 proteins associate with cdc25 phosphatases. Proc Natl Acad Sci USA 1995;92(17):7892–96.
[324] Chen RA, Michaeli T, Van Aelst L, Ballester R. A role for the noncatalytic N terminus in the function of Cdc25, a Saccharomyces cerevisiae Ras-guanine nucleotide exchange factor. Genetics 2000;154(4):1473–84.
[325] Bernardi R, Liebermann DA, Hoffman B. Cdc25A stability is controlled by the ubiquitin-proteasome pathway during cell cycle progression and terminal differentiation. Oncogene 2000;19(20):2447–54.
[326] Gabrielli BG, Clark JM, McCormack AK, Ellem KA. Hyperphosphorylation of the N-terminal domain of Cdc25 regulates activity toward cyclin B1/Cdc2 but not cyclin A/Cdk2. J Biol Chem 1997;272(45):28607–14.

[327] Fauman EB, Cogswell JP, Lovejoy B, Rocque WJ, Holmes W, Montana VG, et al. Crystal structure of the catalytic domain of the human cell cycle control phosphatase, Cdc25A. Cell 1998; 93(4):617–25.
[328] Reynolds RA, Yem AW, Wolfe CL, Deibel MR, Jr., Chidester CG, Watenpaugh KD. Crystal structure of the catalytic subunit of Cdc25B required for G2/M phase transition of the cell cycle. J Mol Biol 1999;293(3):559–68.
[329] Kolmodin K, Aqvist J. Prediction of a ligand-induced conformational change in the catalytic core of Cdc25A. FEBS Lett 2000;465(1):8–11.
[330] Kristjansdottir K, Rudolph J. Cdc25 phosphatases and cancer. Chem Biol 2004;11(8): 1043–51.
[331] Karlsson-Rosenthal C, Millar JB. Cdc25: mechanisms of checkpoint inhibition and recovery. Trends Cell Biol 2006;16(6):285–92.
[332] Lazo JS, Wipf P. Is Cdc25 a druggable target? Anticancer Agents Med Chem 2008;8(8):837–42.
[333] Rayapureddi JP, Kattamuri C, Steinmetz BD, Frankfort BJ, Ostrin EJ, Mardon G, et al. Eyes absent represents a class of protein tyrosine phosphatases. Nature 2003;426(6964):295–98.
[334] Li X, Oghi KA, Zhang J, Krones A, Bush KT, Glass CK, et al. Eya protein phosphatase activity regulates Six1-Dach-Eya transcriptional effects in mammalian organogenesis. Nature 2003;426(6964):247–54.
[335] Jemc J, Rebay I. The eyes absent family of phosphotyrosine phosphatases: properties and roles in developmental regulation of transcription. Ann Rev Biochem 2007;76:513–38.
[336] Tootle TL, Silver SJ, Davies EL, Newman V, Latek RR, Mills IA, et al. The transcription factor Eyes absent is a protein tyrosine phosphatase. Nature 2003;426(6964):299–302.
[337] Rebay I, Silver SJ, Tootle TL. New vision from Eyes absent: transcription factors as enzymes. Trends Genet 2005;21(3):163–71.
[338] Reichenberger KJ, Coletta RD, Schulte AP, Varella-Garcia M, Ford HL. Gene amplification is a mechanism of Six1 overexpression in breast cancer. Cancer Res 2005;65(7):2668–75.
[339] Pandey RN, Rani R, Yeo EJ, Spencer M, Hu S, Lang RA, et al. The Eyes Absent phosphatase-transactivator proteins promote proliferation, transformation, migration, and invasion of tumor cells. Oncogene 2010;29(25):3715–22.
[340] Zhang L, Yang N, Huang J, Buckanovich RJ, Liang S, Barchetti A, et al. Transcriptional coactivator Drosophila eyes absent homologue 2 is up-regulated in epithelial ovarian cancer and promotes tumor growth. Cancer Res 2005;65(3):925–32.
[341] Seifried A, Schultz J, Gohla A. Human HAD phosphatases: structure, mechanism, and roles in health and disease. FEBS J 2013;280(2):549–71.
[342] Aravind L, Galperin MY, Koonin EV. The catalytic domain of the P-type ATPase has the haloacid dehalogenase fold. Trends Biochem Sci 1998;23(4):127–29.
[343] Jung SK, Jeong DG, Chung SJ, Kim JH, Park BC, Tonks NK, et al. Crystal structure of ED-Eya2: insight into dual roles as a protein tyrosine phosphatase and a transcription factor. FASEB J 2010;24(2):560–69.
[344] Xiao A, Li H, Shechter D, Ahn SH, Fabrizio LA, Erdjument-Bromage H, et al. WSTF regulates the H2A.X DNA damage response via a novel tyrosine kinase activity. Nature 2009; 457(7225):57–62.
[345] Cook PJ, Ju BG, Telese F, Wang X, Glass CK, Rosenfeld MG. Tyrosine dephosphorylation of H2AX modulates apoptosis and survival decisions. Nature 2009;458(7238):591–96.
[346] Krishnan N, Jeong DG, Jung SK, Ryu SE, Xiao A, Allis CD, et al. Dephosphorylation of the C-terminal tyrosyl residue of the DNA damage-related histone H2A.X is mediated by the protein phosphatase eyes absent. J Biol Chem 2009;284(24):16066–70.
[347] Okabe Y, Sano T, Nagata S. Regulation of the innate immune response by threonine-phosphatase of Eyes absent. Nature 2009;460(7254):520–24.

[348] Wayne S, Robertson NG, DeClau F, Chen N, Verhoeven K, Prasad S, et al. Mutations in the transcriptional activator EYA4 cause late-onset deafness at the DFNA10 locus. Hum Mol Genet 2001;10(3):195–200.
[349] Schonberger J, Wang L, Shin JT, Kim SD, Depreux FF, Zhu H, et al. Mutation in the transcriptional coactivator EYA4 causes dilated cardiomyopathy and sensorineural hearing loss. Nat Genet 2005;37(4):418–22.
[350] Patrick AN, Cabrera JH, Smith AL, Chen XS, Ford HL, Zhao R. Structure-function analyses of the human SIX1-EYA2 complex reveal insights into metastasis and BOR syndrome. Nat Struct Mol Biol 2013;20(4):447–53.

3 Protein tyrosine phosphatases: Molecular structure and mechanism

3.1 The protein tyrosine phosphatases

The importance of tyrosine-based phosphorylation in cell signaling is eminent in the various human diseases that rise due to erroneous activities of various protein tyrosine kinases and/or protein tyrosine phosphatases (PTPs) [1–3]. Overall, there are about ~107 PTPs for about ~95 protein tyrosine kinases to maintain a balance of tyrosine-based phosphorylation in the cell [4]. Historically, the PTPs have been undermined and were looked merely as "housekeeping" genes that allowed for keeping a basal level of tyrosine phosphorylation. In the present times, the role of PTPs is more recognized as their exquisite regulation in both space and time is being appreciated [4]. This chapter discusses their domain organization with a specific emphasis on the molecular structure of their catalytic domain.

The PTPs are the largest superfamily of protein phosphatase genes with about ~107 members. These genes are defined by their active site motif that CxxxxxR where the cysteine residue is a must for its catalytic function. On the basis of the sequence of their domains, the PTPs can be divided into three main classes: class I PTPs, class II PTPs and class III PTPs [5] (Table 3.1). The class I PTPs include the majority of the human PTP genes. They are further divided into the classical PTPs and the dual-specificity DUSP protein phosphatases. The classical PTPs are also of two types: the membrane-bound receptor protein tyrosine phosphatases (RPTPs) and the cytosolic non-receptor protein tyrosine phosphatases (NRPTPs). All the class I PTPs are speculated to have a common structural ancestor that has provided them a distinct molecular fold [6]. The DUSP subfamily is the most diverse and includes about ~61 human genes. Members of this family include the phosphothreonine/phosphotyrosine-specific mitogen-activated protein (MAP) phosphatases; the phosphoserine-specific slingshot phosphatases; phosphotyrosine-specific phosphatases of the liver (PRLs). This family also includes the mRNA-specific atypical DSPs; the phosphoserine/phosphothreonine-specific Cdc14 phosphatases; the phosphatidylinositol-(3,4,5)-triphosphate utilizing PTENs and the myotubularins that can use both phosphatidylinositol-3-phosphate and phosphatidylinositol-(3,5)-bisphosphate as their substrates. The class II PTPs are represented by a lone member – the phosphotyrosine-specific low molecular weight protein tyrosine phosphatase (LMPTP). The LMPTP is evolutionarily more ancient than the class I PTPs and shows resemblance to the bacterial arsenate reductases [7]. The class III PTPs are related to the bacterial rhodanese-like enzymes. These include the phosphothreonine/phosphotyrosine-specific Cdc25 phosphatases [6].

The PTP domain is a catalytic machine of about ~280 residues. However, other than the catalytic domain, various noncatalytic domains occur alongside the PTPs in various family members of the PTP family. These noncatalytic domains play their own

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Table 3.1: PTP proteins in the human genome.

Class	PTP category	Gene name (protein name and synonyms) [UniProt]
Class I	RPTPs	PTPRA (RPTPα) [P18433], PTPRB (RPTPβ) [B2RU80], PTPRC (CD45, LCA) [P08575], PTPRD (RPTPδ) [P23468], PTPRE (RPTPε) [P23469], PTPRF (LAR) [P10586], PTPRG (RPTPγ) [P23470], PTPRH (SAP1) [Q9HD43], PTPRJ (DEP1, CD148, RPTPη) [Q12913], PTPRK (RPTPκ) [Q15262], PTPRM (RPTPμ) [P28827], PTPRN (IA-2, Islet cell antigen 512) [Q16849], PTPRN2 (PTPRP, RPTPπ,IA-2β, phogrin) [Q92932], PTPRO (GLEPP1, PTP-U2, PTPROτ isoforms A/B/C) [Q16827], PTPRQ (PTPS31) [Q9UMZ3], PTPRR (PTP-SL, PCPTP, PTPBR7, PC12-PTP1) [Q15256], PTPRS (RPTPσ) [Q13332], PTPRT (RPTPρ) [Q99M80], PTPRU (PTPJ/PTP-U1/PTPRomicron isoforms 1/2/3) [Q92729], PTPRZ (RPTPζ) [P23471]
Class I	NRPTPs	PTPN1 (PTP1B) [P18031], PTPN2 (TCPTP, MPTP, PTP-S) [P17706], PTPN3 (PTPH1) [P26045], PTPN4 (PTP-MEG1, TEP) [P29074], PTPN5[a] (STEP) [P54829], PTPN6 (SHP1, PTP1C,SH-PTP1, HCP) [P29350], PTPN7 (HePTP, LCPTP) [P35236], PTPN9 (PTP-MEG2) [P43378], PTPN11 (SHP2, SH-PTP2,Syp, PTP1D,PTP2C, SH-PTP3) [Q06124], PTPN12 (PTP-PEST,PTP-P19, PTPG1) [Q05209], PTPN13 (PTP-BAS, FAP-1,PTP1E, RIP,PTPL1, PTP-BL) [Q12923], PTPN14 (PTP36, PEZ, PTPD2) [Q15678], PTPN18 (PTP-HSCF, PTP20,BDP) [Q99952], PTPN20 (TypPTP) [Q4JDL3], PTPN21 (PTPD1, PTP2E, PTP-RL10) [Q16825], PTPN22 (LYP) [Q9Y2R2], PTPN23 (HD-PTP, HDPTP, PTP-TD14, KIAA1471) [Q9H3S7]
Class I	Map kinase phosphatases (MKPs)	DUSP1 (MKP-1, 3CH134, PTPN10, erp,CL100/HVH1) [P28562], DUSP2 (PAC-1) [Q05923], DUSP4 (MKP-2, hVH2/TYP1) [Q13115], DUSP5 (hVH3/B23) [Q16690], DUSP6 (MKP-3,PYST1) [Q16828], DUSP7 (MKP-X, PYST2, B59) [Q16829], DUSP8 (hVH5, M3/6, HB5) [Q13202], DUSP9 (MKP-4, Pyst3) [Q99956], DUSP10 (MKP-5) [Q9Y6W6], DUSP16 (MKP-7, MKP-M) [Q9BY84], DUSP24 (MK-STYX, MKSTYX,DUSP24, STYXL1) [Q9Y6J8]
Class I	Atypical dualspe-cificityphospha-tases	DUSP3 (VHR, T-DSP11) [P51452], DUSP11 (PIR1) [O75319], DUSP12 (HYVH1, GKAP, LMW-DSP4) [Q9UNI6], DUSP13A[b] (BEDP) [Q6B8I1], DUSP13B[b] (TMDP, TS-DSP6) [Q9UII6], DUSP14 (MKP6, MKP-L) [O95147], DUSP15 (VHY, Q9H1R2) [Q9H1R2], DUSP18 (DUSP20, LMW-DSP20) [Q8NEJ0], DUSP19 (DUSP17, SKRP1,LDP-2, TS-DSP1) [Q8WTR2], DUSP21 (LMW-DSP21,BJ-HCC-26 tumor antigen) [Q9H596], DUSP22 (VHX, MKPX, JSP1,LMW-DSP2,TS-DSP2, JKAP) [Q9NRW4], DUSP23 (VHZ, DUSP25,FLJ20442,LMW-DSP3) [Q9BVJ7], DUSP26 (MKP-8, DUSP24, LDP4, NATA1, SKRP3) [Q9BV47], PTPMT1 (MOSP, PLIP) [Q8WUK0], DUSP27[c] (FMDSP, DUPD1) [Q5VZP5], DUSP28 (VHP, DUSP26) [Q4G0W2], EPM2A (Laforin) [O95278], RNGTT (mRNA cap-ping enzyme) [O60942], STYX (STYX) [Q8WUJ0]
Class I	Slingshots	SSH1 (SSH1, slingshot 1) [Q8WYL5], SSH2 (SSH2, slingshot 2) [Q76I76], SSH3 (SSH3,slingshot 3) [Q8TE77]

Table 3.1: (*continued*)

Class	PTP category	Gene name (protein name and synonyms) [UniProt]
Class I	PRLs	PTP4A1 (PRL-1) [Q93096], PTP4A2 (PRL-2, OV-1) [Q12974], PTP4A3 (PRL-3) [O75365]
Class I	CDC14s	CDC14A (CDC14A) [Q9UNH5], CDC14B (CDC14B) [O60729], CDKN3 (KAP) [Q16667], PTPDC (PTPDC, PTP9Q22) [A2A3K4]
Class I	PTENs	PTEN (PTEN, MMAC1, TEP1) [P60484], TPIP (TPIPα, TPTE) [Q6XPS3], TPTE (PTEN-like,PTEN2) [P56180], TNS1 (Tensin-1, TNS) [Q9HBL0], TENC1 (C1-TEN, TENC1, KIAA1075, TNS2) [Q63HR2], TNS3 (Tensin 3) [Q68CZ2]
Class I	Myotubularins	MTM1 (Myotubularin) [Q13496], MTMR1 (MTMR1) [Q13613], MTMR2 (MTMR2) [Q13614], MTMR3 (MTMR3, FYVE-DSP1) [Q13615], MTMR4 (MTMR4, FYVE-DSP2) [Q9NYA4], MTMR5 (MTMR5, SBF1) [O95248], MTMR6 (MTMR6) [Q9Y217], MTMR7 (MTMR7) [Q9Y216], MTMR8 (MTMR8) [Q96EF0], MTMR9 (MTMR9, LIP-STYX) [Q96QG7], MTMR10 (MTMR10) [Q9NXD2], MTMR11 (MTMP11, CRA α/β) [A4FU01], MTMR12 (MTMR12, 3-PAP) [Q9C0I1], MTMR13 (MTMR13, SBF2,CMT4B2) [Q86WG5], MTMR14[d] (hJumpy, FLJ22075, hEDTP) [Q8NCE2]
Class I	Inositol 4-phosphatases	INPP4A (Inositol 4-phosphatase type I) [Q96PE3], INPP4B (Inositol 4-phosphatase type II) [O15327]
Class II	Class II PTPs	ACP1 (LMPTP, low Mr PTP,LMWPTP, BHPTP) [P24666]
Class III	Class III PTPs	CDC25A (CDC25A) [P30304], CDC25B (CDC25B) [P30305], CDC25C (CDC25C) [P30307]
Class IV	Class IV PTPs	EYA1[Q99502], EYA2 [O00167], EYA3 [Q99504], EYA4 [O95677]

Table adapted from Ref [5].
a STEP has two alternately spliced forms, $STEP_{46}$ and $STEP_{61}$.
b Human DUSP13 gene has two DSP coding regions: DUSP13A (3 exons) and DUSP13B (3 exons). These are separated by two noncoding exons.
c Formerly DUPD1.
d Lacks typical myotubularin domain characteristics, but has phosphatidylinositol-(3,5)-bisphosphate phosphatase activity.

roles relevant to signal transduction and needs of the cell. Many of these domains or motifs allow for compartmentalization of PTPs in varied locations in the cell. For example, DUSP1 (MKP-1), DUSP2 (PAC-1), DUSP4 (MKP-2), DUSP6 (MKP-6) and DUSP16 (MKP-7) contain a nuclear localization signal that allows for their movement into the nucleus. Similarly, PTPN3 (PTPH1), PTPN4 (PTP-MEG1) and PTPN13 (PTP-BAS) have PDZ domains that allow for their targeting and functioning on the cell cytoskeleton. Specific interaction motifs are required by the PTPs to mediate interactions with unique substrates. For example, a proline-rich motif in PTPN22 (LYP) binds to the SH3 domain of Src kinase and directs its substrate preference [8]. Similarly, both PTPN5

(STEP) and PTPN7 (hematopoietic PTP [HePTP]) have a kinase interaction motif (KIM) that allows them to access MAP kinase substrates. Some noncatalytic domains also allow for the regulation of PTPs. In the case of PTPN6 (SHP1) and PTPN11 (SHP2), both contain two tandem SH2 domains that they use for recognizing target phosphotyrosine residues. However, in the resting state, these SH2 domain fold over the SHP1 and SHP2 to lock them in an autoinhibited states. Only upon stimulation, these inhibited states are released for appropriate signaling activity [9]. The membrane-bound receptor PTPs have diverse extracellular domains including Fibronectin type III repeats, MAM (meprin, A-5 protein, and receptor protein-tyrosine phosphatase mu) domains, immunoglobulin domains and carbonic anhydrase domains that they employ to interact with the extracellular matrix and ligands. Many members of these RPTPs contain a second PTP domain that functions as a pseudophosphatase and cannot catalyze dephosphorylation. This silent second domain is used for the modulation of activity of the active PTP domain [10].

3.2 The PTP catalytic domain

PTPs have been extensively characterized for the molecular details of their catalytic domains. PTPN1 (PTP1B), the first PTP to be purified and sequenced, was also the first to be crystallized and structurally explored [11–14]. Since 1994, PTP1B serves as the prototype of the PTP superfamily of proteins and is used as the reference. The structure of the domain is a well-conserved central twisted β-sheet flanked by an array of α-helices (Figure 3.1). The active site is highly conserved wherein essential

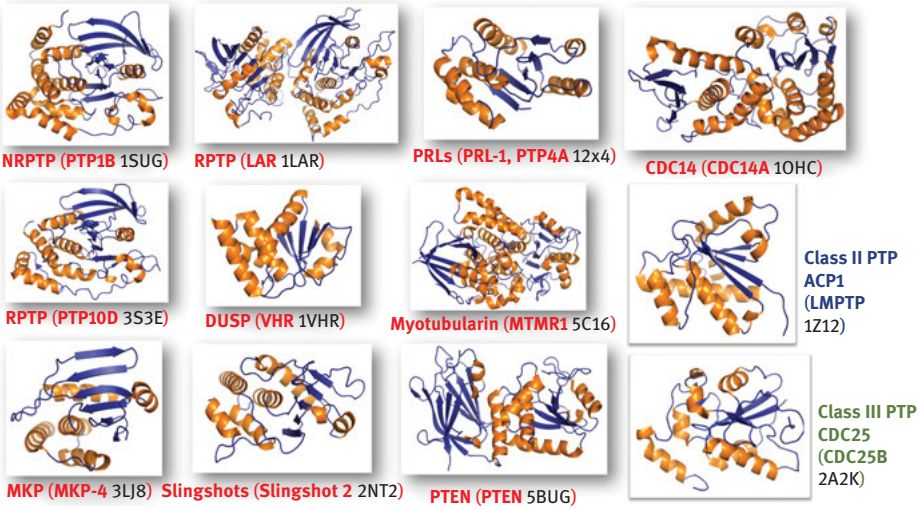

Figure 3.1: Catalytic domains of representative cysteine-based PTPs.

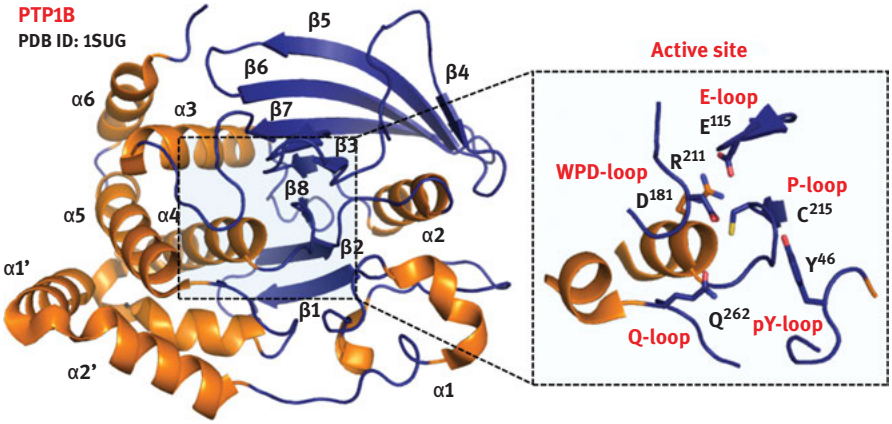

Figure 3.2: Structural elements of the conserved PTP domain. The classic PTP domain of Class I PTPs is shown.

loops orchestrate the functioning of the conserved PTP active site (Figure 3.2); (a) the phosphate-binding loop (P-loop) has the conserved active site cysteine alongside an invariant arginine; (b) the WPD-loop has the conserved aspartate that behaves as the acid-base in the catalysis; (c) the Q-loop has the invariant glutamine residues that activate water molecules in the active site to break the cysteinyl–phosphate intermediate and (d) the E-loop that coordinates the dynamics and working of the active site. Various subclasses show some peculiarities in the loops, but the P-loop is the uniform signature of the PTP domain. In the classical PTPs, the WPD-loop is flexible and forms a lid over the active site [15]. However in the DUSPs and the PTENs, this loop is short and relatively much less flexible [16]. In contrast, the myotubularins and the Cdc25 phosphatases have displaced or missing WPD-loop, and use an alternate mechanism of catalysis that does not require the WPD-loop aspartate [17]. In the classical class I PTPs, there is also a phosphotyrosine recognition loop that contains a conserved phenylalanine or tyrosine to identify and mediate the entry of the phosphotyrosine into the PTP-active site. This specific loop lines the active-site cleft to allow for the specific entry of phosphotyrosine substrates over phosphothreonine/phosphoserine [18]. Understandably, this loop is absent in DUSPs, which allow for the entry of phosphothreonine/phosphoserine substrates into their shallower active sites [19].

An evolutionary analysis of the class I classical PTPs has allowed for a ten-motif-based description of their ~280 amino acid catalytic domain (Figure 3.3) [6]. This exclusive description of the classical PTP domain is based on an analysis of multiple sequence alignments that allow for defining a motif to be a stretch of at least three residues, wherein two of the three amino acids are 80% conserved throughout the sequence alignment of the family members [6]. In addition, a low-resolution homology modeling called the Cα-regiovariation score analysis [20] was used to analyze these

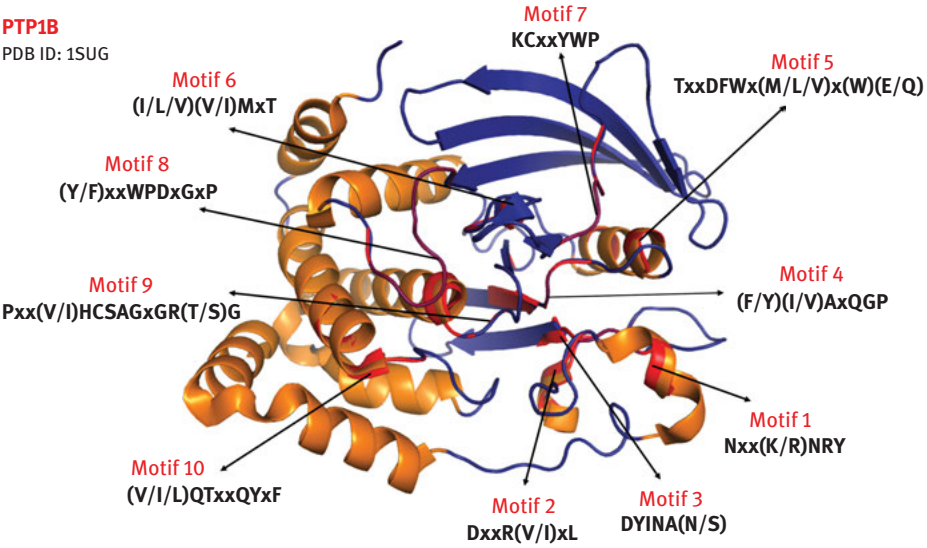

Figure 3.3: The ten motifs defining the core of the PTPs.

sequences on a tertiary structure template described by highly conserved PTP fold of ~37 nonredundant PTP catalytic domains known at that time. Using a sphere of influence of 7.0Å per residue, the average degree of conservation was calculated for each residue in the conserved PTP tertiary structure template. An amalgamation of these sequence-based and low-resolution homology modeling studies has allowed for defining these ten motifs that are the signature of the classical PTP domain (Table 3.2). On the basis of their contributions to the working of the PTP domain, these motifs can be understood as functional (motifs 1, 8, 9 and 10) or structural motifs (motifs 2, 3, 4, 5, 6 and 7). The functional motif-9 VHCSXGXGR(T/S)G along with the structural motif-4 (F/Y)IAxQGP are most conserved in all PTP domains. The motif 9 includes the nucleophilic cysteine and the CxxxxxR sequence at the active site and motif 4 forms the hydrophobic core of the PTP domain. Understandably, the various loops that form the active site of the PTP domain find an obvious place in these ten motifs. Motif-8 (Y/F)xxWPDxGxP includes the WPD-loop; motif-1 Nxx(K/R)NRY contains phosphotyrosine recognition loop and motif-10 (V/I/L)QTxxQYxF harbors the Q-loop in its sequence. The highly conserved motif-4 (F/Y)IAxQGP packs together with motif-3 DYINA(N/S) in a parallel–antiparallel β-sheet arrangement at the core of the PTP domain. The dense hydrophobic PTP domain core also includes motif-2 DxxR(V/I)xL, motif-5 TxxDFWx(M/L/V)x(W)(E/Q), motif-6 (I/L/V)(V/I)MxT and motif-7 KCxxYWP. Aromatic residues form motif 5 and motif 7 engage in T-stacking [21] of their aromatic rings (Phe95, Trp96, Tyr124 and Trp125 in PTP1B) to stabilize the core of the PTP domain. As the hydrophobic packing of any protein domain contributes to its thermodynamic stability, aberrations in these key structural motifs are linked to temperature sensitivity and structural fragility of mutant PTPs [22, 23].

Table 3.2: Ten motifs define the conserved class I classical PTP catalytic domain.

Motif (residues PTP1B)	Motif Sequence	Function
Motif 1(40–46)	Nxx(K/R)NRY	*pTyr-recognition loop* Asn 44: Coordinates Asn68 that links Arg257 Arg 45: Putative substrate binding site, electrostatic attraction of ligand Tyr 46: Hydrophobic packing with phosphotyrosine residue of substrate
Motif 2(53–59)	DxxR(V/I)xL	*Conserved secondary structure (β1 sheet), surface exposed* Arg 56: H-bonds to Asp65Ile 57: Hydrophobic core cluster (residues 57, 67, 69, 82, 98) Leu 59: Hydrophobic core
Motif 3(65–70)	DYINA(N/S)	*Core structure* Tyr 66: Coordinates Asn44 through hydrogen bondingIle 67: Hydrophobic core cluster (residues 57, 67, 69, 82, 98) Asn 68: H-bonds with Arg257 Ala 69: Hydrophobic core cluster (residues 57, 67, 69, 82, 98)
Motif 4(81–87)	(F/Y)(I/V) AxQGP	*Core structure surrounding the PTP-loop*Ile 82: Hydrophobic core cluster (residues 57, 67, 69, 82, 98) Ala 83: Packs/surrounds the PTP-loop Gln 85: H-bonds with highly buried water molecule Gly 86: Packs/surrounds the PTP-loop Pro 87: Packs/surrounds the PTP-loop
Motif 5(91–101)	TxxDFWx(M/L/V)x(W)(E/Q)	*Conserved secondary structure (α2 helix)* Asp 94: Contributes to conserved subdomain at the "back side" Phe 95: Energetically favored T-stacking arrangement with invariant Trp96 Trp 96: H-bonds to backbone of invariant Tyr124 Met 98: Hydrophobic core cluster (residues 57, 67, 69, 82, 98) Trp 100: Contributes to conserved subdomain at the "back side"
Motif 6(107–111)	(I/L/V)(V/I) MxT	*Hydrophobic core structure* Ile 107: Hydrophobic core structure packs with invariant Trp96 Val108: Hydrophobic core structure packs with invariant Trp96 Met 109: Packs with invariant Trp125 Thr 111: Packs with PTP-loop
Motif 7(120–126)	KCxxYWP	*Hydrophobic core structure* Lys 120: Interacts with Asp181 (ligand induced) Tyr 124: H-bonds with His214, stabilizing T-stacking arrangement with Trp125

(*continued*)

Table 3.2: (*continued*)

Motif (residues PTP1B)	Motif Sequence	Function
		Trp 125: Favored T-arrangement of aromatic ring system with Tyr124
Motif 8(176–185)	(Y/F) xxWPDxGxP	*WPD-loop, surface-exposed loop containing general acid* Trp 179: Center of movable WPD-loop, mediating motion of loop Pro 180: H-bonds to NH2 of Arg221, mediating motion of loop Asp 181: General acid catalyst Gly 183: Energetically favorable in loop motion (acts as hinge) Pro 185: Energetically favorable in loop movement (no backbone H-bonding)
Motif 9(210–223)	Pxx(V/I) Pxx(V/I) (T/S)G	*P-loop containing the active site Cys* Pro 210: Structural hydrophobic core His 214 : Lowers p*K*a of Cys215 Cys 215: Nucleophile Ser 216: H-bonds with Tyr46 stabilizing its interaction with substrate Ala 217: Phosphotyrosine binding, nonpolar interaction with substrate phenyl Gly 218: Phosphotyrosine binding Gly 220: Phosphotyrosine binding Arg 221: H-bonds with phosphate oxygens (transition-state stabilization) Thr 222: Lowers p*K*a of Cys215
Motif 10(261–269)	(V/I/L) QTxxQYxF	*Q-loop: Interaction with active site water molecule* Gln 262: H-bonds with scissile oxygen and active site water molecule Gln 266: H-bonds with active site water molecule Tyr 267: Defines α6′ helix structure Phe 269: Defines α6′ helix structure

Table adapted from Ref [6].

3.2.1 The P-loop

The base of the PTP-active site is the P-loop that contains the active site cysteine in the conserved CxxxxxR motif. This cysteine transiently accepts the incoming phosphate group to form a cysteinyl–phosphate intermediate that is directly stabilized by the arginine of this motif [24]. The P-loop pocket is lined by residues that provide a high positive charge to the active site to attract the incoming phosphotyrosine. This microenvironment of the high positive charge allows for an altered p*K*a of the active site cysteine and stabilizes its thiolate anion (Cys-S^-)

form. The p*K*a of a cysteine side chain sulfhydryl is about ~8.18 under regular conditions [25]. However, this high p*K*a is ineffective for using the cysteine as an effective nucleophile in the phosphatase catalytic cycle. The positively charged active of the PTPs and the conserved arginine of the CxxxxxR motif ensure that the structural p*K*a of the PTP-active site cysteine drops to about ~4.5–5.0 pH units [26]. This allows the thiolate form the active site cysteine to be stable at physiological pH and function as an effective nucleophile. The low p*K*a of the active site cysteine also allows for its regulation by reversible oxidation by reactive oxygen or nitrogen species [27, 28].

In the class I classical PTPs, the P-loop is a part of motif-9 VHCSXGXGR(T/S)G that includes the CxxxxxR motif in itself. Importantly, a conserved glutamate reside from the E-loop (Glu115 in PTP1B) makes a salt-bridge with the CxxxxxR arginine (Arg 221 in PTP1B). The conserved nature of this interaction across all PTP domains defines the architecture and functioning of the P-loop. The serine residue adjacent to this arginine (Ser 222 in PTP1B) is also known to stabilize the active site cysteine and contribute to the kinetics of the PTP domain [29]. Another conserved arginine that is placed in between motif 9 and motif 10 (Arg257 in PTP1B) also contributes to the electrostatic potentials of the active site [30]. Mutation of the active site cysteine to serine silences the phosphatase activity of PTP domain but leaves the positive charge of the active site undisturbed. This cysteine to serine mutants hence retain their ability to bind substrates and are also seen to naturally occur as silent tandem domains in many bi-domain receptor PTPs [10]. This property of these mutant domains has been used to study the various interaction of PTPs with their substrate peptides [31, 32].

3.2.2 The WPD-loop

The WPD is present in all PTP domains with the exception of Cdc25 phosphatases and myotubularins. The loop contains the conserved residues tryptophan–proline–aspartate (WPD) and is found in about approximately 30–40 upstream of the active site P-loop in phosphotyrosine-specific PTPs and DUSPs. In the class II LMPTP, the WPD-loop is located about ~120 residues downstream of the P-loop. This is unique to the LMPTPs where the P-loop itself is located toward the N-terminus of the protein [33] . The tryptophan of the WPD-loop forms the hinge required for loop flexibility during catalysis [34]. Biochemical studies have validated that mutations in this conserved tryptophan abrogate the phosphatase activity of PTPs [34, 35]. The aspartate residue of the WPD-loop forms the general acid/base required for the activity of the PTP domain. Mutation of this aspartate to alanine creates a “substrate-trap” wherein the substrates enter the active site of the PTP domain but are trapped inside as the second step of donation of proton to the leaving phenolate group is compromised [35]. In addition, the mutation of aspartate to alanine reduces the possible

electrostatic repulsion between the WPD-loop and the incoming phosphotyrosine. In this way, these aspartate to alanine mutants bind the substrate even more efficiently than the P-loop mutants that use the cysteine to serine mutation. This strategy has been successfully used for the identification of various PTP substrates both *in vitro* and *in vivo* [36–38]. PTPs with natural variations in the aspartate of the WPD-loop are either inactive or have minuscule phosphatase activity. For example, this residue is naturally a glutamate in the silent tandem domains of bidomain receptor PTPs like LAR, PTPδ and PTPα [10]. The non-receptor HD-PTP also has the same aspartate to glutamate mutation and is inactive [39]. The only exception here is PTPD1 that also has the same glutamate mutation but has been shown to dephosphorylate the pTyr 527 or Src protein kinase [40].

The WPD-loop essentially acts as "gate" to the active site of the PTP domain and its flexibility allows it to operate as a regulatory switch. In the absence of the substrate, in the apo-enzyme, dynamics of the WPD-loop allow it to sample all possible conformations between the "open" and "closed" states [41]. A substrate can access the active site when the WPD-loop is "open." The loop then closes upon the formation of the enzyme – substrate complex and places the general acid/base aspartate at its required position for catalysis. In the class I classical PTPs, the WPD-loop is a part of motif-8 (Y/F)xxWPDxGxP that moves several angstroms during the catalytic cycle. Consequently, the WPD-loop has been characterized in various "open" and "closed" conformations (Figure 3.4) [42]. The two prolines and the central glycine are critical for this dynamic motion of the WPD-loop [43]. Mutation of the WPD-loop proline to tyrosine occurs naturally in IA-2β (PTPR N2) and makes IA-2β a

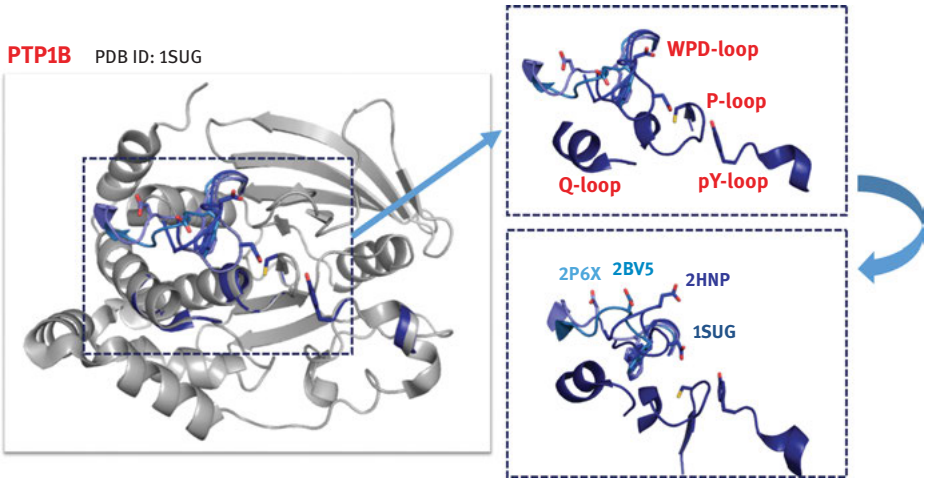

Figure 3.4: Concerted movement to the WPD-loop with the P-loop at the active site allows for proper positioning of the general acid/base aspartate at the active site during the course of the catalytic cycle.

silent PTP [40]. The crucial dynamics of this WPD-loop make it an attractive target for the design of various allosteric inhibitors that compromise its flexibility and allow for modulation of PTP function [44, 45].

3.2.3 The Q-loop

The Q-loop is a characteristic feature of all classical PTPs and is named after the 97% conserved glutamine residue that appears in it. A similar positioned loop is also present in the DUSPs, albeit lacking the glutamine residue [46]. In the classical class I PTPs, this loop forms a part of motif-10 (V/I/L)QTxxQYxF that shows two conserved glutamines. The Q-loop also functions to maintain hydrogen interactions with the P-loop and also combines with the aspartate of the WPD-loop to maintain the required active site confirmation that is optimal for catalysis. The first glutamine of the motif 10 (Gln 262 in PTP1B) serves to activate a water molecule at the active site that serves to hydrolyze the cysteinyl–phosphate intermediate in the second step of PTP catalysis [47]. Most importantly, the two glutamines (Gln262 and Gln266 in PTP1B) restrict the activity of classical PTPs to work as a hydrolase that transfers phosphate only onto a water molecule [48]. Mutations in these glutamines are enough to modulate the PTPs from being hydrolases to phosphotransferases. Intriguingly, DUSPs (that lack the glutamines) and LMPTPs (that lack the Q-loop), are both able to catalyze the transfer of the phosphate moiety from the cysteinyl-phosphate intermediate onto alcohols as the nucleophilic acceptors [48] .

3.2.4 The pY-recognition loop

The phosphotyrosine recognition loop is also referred to as the substrate-binding loop in literature. This loop that is exclusive to the classical PTPs as a part of motif-1 Nxx(K/R)NRY defines the depth of the PTP-active site. The loop contains a 84% conserved tyrosine or phenylalanine residue that acts as a causeway to the active site, providing it a depth of about ~9.0 Å [49]. The aromatic ring of the tyrosine/phenylalanine of the loop makes π–π stacking interactions with the incoming phosphotyrosine and facilitates its entry into the PTP-active site (Figure 3.5) [18]. In this way it allows the PTP to exclusively choose a phosphotyrosine as its substrate as opposed to the much shorter phosphoserine/phosphothreonine. Mutation of this aromatic tyrosine or phenylalanine causes a major decrease in the kinetic parameters and efficiency of the PTP domain [18].

The highly conserved arginine residue of the loop is essential to maintain the loop conformation on the surface of the active site. In addition, this arginine also provides a positive charge that can attract the negatively charged phosphotyrosine to the active site. An asparagine or aspartate residue found two residue

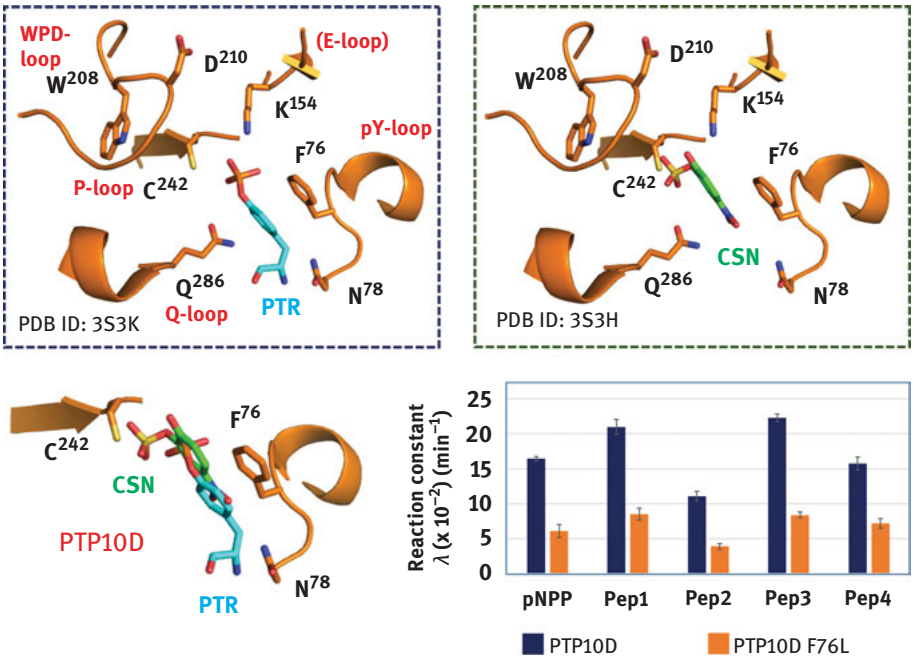

Figure 3.5: Role of the phosphotyrosine-recognition loop in mediating proper substrate entry into the PTP-active site.

C-terminal to the pY-loop forms bipartite hydrogen bounds with the incoming substrate and contributes to its binding at the active site surface [50]. The pY-loop is naturally mutated at its tyrosine/phenylalanine residue in a few PTPs. The most prominent occurrence of this alteration occurs in the silent domains of bi-domain PTPs including LAR, PTPα, PTPσ and PTPε, where their WPD-loop aspartate is also replaced by a glutamic acid [10]. These PTP domains hence lack any detectable phosphatase activity despite a well-formed P-loop and the presence of a functional active site cysteine. Sequence analysis-based computational studies have been used to assess coevolution of these mutations in the pY- and WPD-loops to understand this specific set of inactive PTP domains [10, 51]. Interestingly, just two-point mutations in these PTP domains to recreate the conserved pY-loop and WPD-loop are enough to activate these domains into functional phosphatases [52].

3.2.5 The E-loop

The E-loop is also a signature of the class I classical PTPs, where it contains an absolutely conserved glutamate residue that makes bipartite hydrogen bonds with

the arginine of the CxxxxxR motif of the P-loop. This loop is not conserved in the DUSP or other family members. This glutamate allows for the proper positioning of the P-loop that is optimal for catalysis. The E-loop tends to adopt a β-hairpin structure and also contains two lysine residues (about 85–90% conserved) that also play important roles in structuring of the PTP-active site. The lysine adjacent to the E-loop glutamate makes salt-bridging interactions with the aspartate of the WPD-loop in the closed conformation. In this way, it allows for stabilization of the substrate-bound PTP domain. Mutation of this lysine to alanine results in a decrease in catalytic efficiency of HePTP [53]. The second lysine lies directly in-line with the center of the active site and provides the active site a positive charge to stabilize the incoming phosphotyrosine and also reduce the p*K*a of the active site cysteine as described earlier. The E-loop has been observed in multiple conformations in various crystal structures of PTPs and its conformation ranges from a β-hairpin to a fully disordered loop (Figure 3.6) [54–56]. However, in all the structures with regard to the interaction between the CxxxxxR arginine and the glutamate, the role of the lysine residues is conserved and consistent.

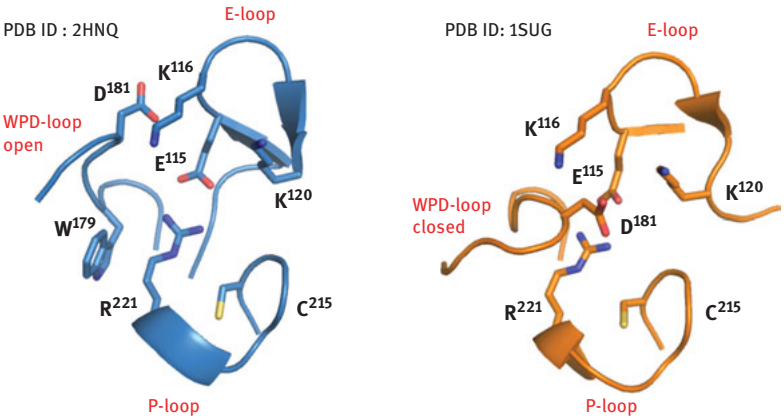

Figure 3.6: The E-loop works in coordination with the WPD-loop and the P-loop. The open and closed conformation of the WPD-loop are shown.

3.3 Catalytic domains of different PTP classes

All the PTPs are analyzed to have evolved from a common ancestral gene that coded for the conserved CxxxxxR motif. This ancestral oxyanion-binding protein then diversified into various other enzyme classes. The structural resemblance and relation of class II PTPs to bacterial arsenate reductase and class III PTPs to bacterial rhodanases suggests an early divergence of these three classes of PTPs. Structurally, class II PTPs closely resemble the class I PTP domains. Both consist of a central twisted β-sheet that is surrounded by α-helices (Figure 3.1). However, the arrangement of these secondary

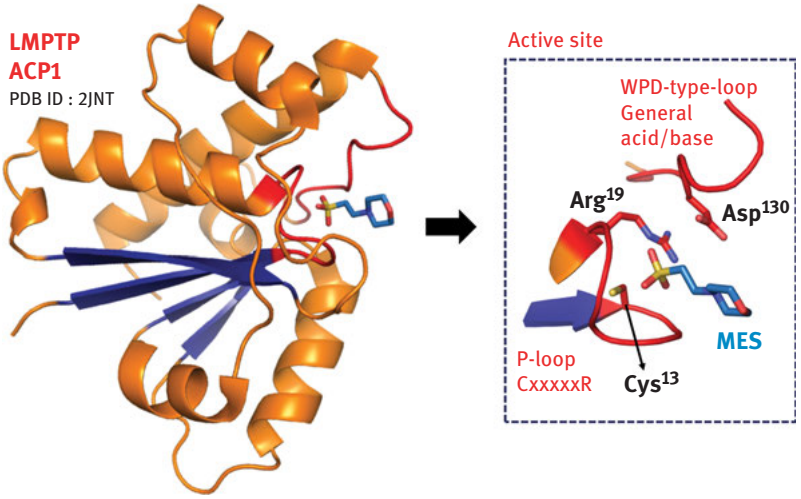

Figure 3.7: The catalytic domain and active site of class II PTPs/LMPTP.

structure elements is distinct in the two classes, making their domains non-superimposable (Figure 3.7). The class II PTP catalytic domain is much smaller (~18 kDa) as compared to the class I PTP domain (30 kDa). Peculiarly, the phosphate-binding P-loop is present in the extreme N-terminus of the class II PTP catalytic domains. Class II PTPs also have the well-characterized WPD-loop with the conserved aspartate that serves as the general acid/base in the catalytic reaction. Although distinct from the phosphotyrosine recognition loop, the class II PTPs have a similar loop defining its depth and substrate recruitment [57]. In the human genome, there is only a single member of the class II PTPs – LMPTP, coded by the *ACP1* gene [7]. The protein has three main splice variants: LMPTP isoform A (exon 3 spliced), LMPTP isoform B (exon 4 spliced) and LMPTP isoform C (both exon 3 and 4 spliced) [58, 59]. These two exons encode the loop analogous to the phosphotyrosine binding loop and the substrate binding loop in classical PTPs. Nonetheless, LMPTP isoforms A and B both show detectable phosphatase activity and are crucial role players in human cell physiology [7]. LMPTP isoform C and a fourth splice variant SV4 are small proteins (12–15 kDa) that are catalytically deficient [60].

The class III PTPs in the human genome are composed of three members: Cdc25A, Cdcd25B and Cdc25C. Just like the class II member, these three proteins also have isoforms formed by alternate splicing of their mRNA transcripts [61]. Just like the class II PTPs, the class III PTPs are much smaller in size than the class I members owing to their narrower β-sheet surrounded by smaller helices. The architecture of the class III PTPs is most similar to prokaryotic sulfur transport protein [62]. Their catalytic domain can be distinguished into two regions: the N-terminal region and the C-terminal region. In contrast to the class II PTPs that contain the CxxxxxR motif in their extreme N-terminus, the class III PTPs have their active site in their C-terminal region (Figure 3.8) [61]. Unlike

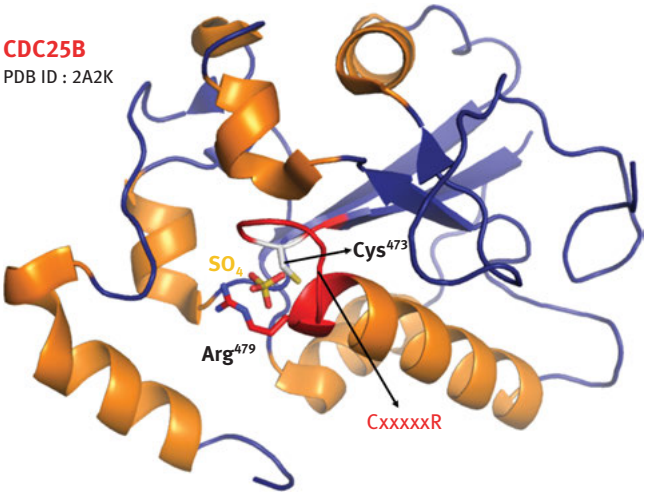

Figure 3.8: The catalytic domain and active site of Class III PTPs/Cdc25 phosphatases.

the class I PTPs, the class III PTP catalytic domains do not contain any equivalents of the WPD-loop, phosphotyrosine binding loop or the Q-loop. Their shallow active site contains the CxxxxxR motif and accepts both phosphotyrosine and phosphothreonine as substrates. This C-terminal region that harbors the active site is the most conserved region of the domain. The N-terminal region of class III PTPs allows for their posttranslational modification, ubiquitination, protein–protein interaction with various partners and contributes the regulation of their catalytic activity [63–65].

3.4 The catalytic mechanism

The catalytic mechanism of PTPs essentially is made of two chemical steps [66, 67]. In the first step, the active site cysteine of the P-loop (that is present in the thiolate form) acts as a nucleophile and initiates the breaking of the phosphorus–oxygen bond of the substrate phosphotyrosine (Figure 3.9). In this step, the aspartate of the WPD-loop acts as the general acid and donates a proton to the leaving hydroxyl group. The product of this step is a released tyrosine moiety on the substrate peptide and the formation of the cysteinyl–phosphate intermediate at the PTP-active site. In the second catalytic step, the WPD-loop aspartate acts as a general base that extracts a proton from an activated water molecule. This activation and splitting of water is assisted by the glutamine residue from the Q-loop. Activated water attacks the phosphorus–sulfur bond of the cysteinyl–phosphate intermediate to release an inorganic phosphate and replenish the thiolate form of the active site cysteine [68].

The aforementioned mechanism of PTP-based dephosphorylation of phosphotyrosine has been extensively characterized both biochemically and structurally

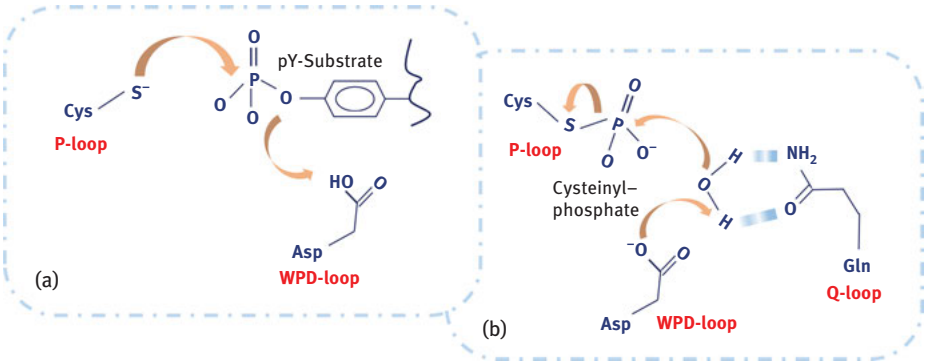

Figure 3.9: The two-step catalytic mechanism of cysteine-based PTPs. In the first step (a) the nucleophilic cysteine attacks the incoming substrate and a conserved aspartate acts as a general acid. The second step (b) is the hydrolysis of the cysteinyl–phosphate intermediate by activated water and the conserved aspartate acting as a general base.

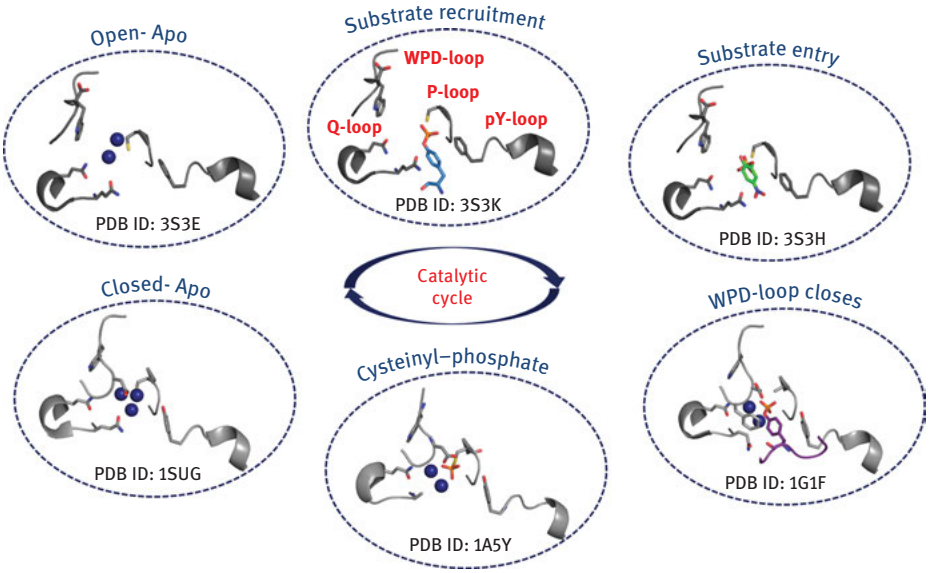

Figure 3.10: Various stages of the PTP catalytic cycle as visualized by X-ray crystallographic studies.

(Figure 3.10). Various residues have been identified that allow for orchestrating the catalytic steps. Substrate recruitment is assisted by the tyrosine/phenylalanine of the phosphotyrosine binding loop [18]. Substrate entry and optimal positioning is mediated by the phosphotyrosine recognition loop and the E-loop lysine. As the substrate enters the active site, the WPD-loop closes over the substrate. A semiconserved phenylalanine (adjacent to the acid/base aspartate) of WPD-loop makes π–π stacking interactions with

the substrate phosphotyrosine, placing it directly in-line to the active site thiolate cysteine for an optimal nucleophilic attack. The cysteinyl–phosphate intermediate is also stabilized by various amino acid residues in the active site, including the conserved arginine from the P-loop. Mutants that lack this arginine show decreased residual catalytic activity [68]. Mutants of conserved glutamine required for the second step of catalysis have been used visualize the cysteinyl–pshopahte intermediate [69, 70]. Mutants of the WPD-loop aspartate have been used to make "substrate trap" mutants of PTP to fish-out their cellular target proteins [37, 71]. Active site cysteine mutations have been used to visualize the PTP–substrate peptide complexes [72, 73].

3.5 Differences in the mechanics of class I, II and III PTPs

The PTPs use the mechanics of a conserved nucleophilic cysteine in concert with key motifs present in the various loops surrounding the active site. The class I and II PTPs use their general acid/base aspartate residue in the WPD-loop (or DPYY-loop) in the mechanism detailed earlier. This loop is, however, missing in the class III Cdc25 PTPs. Hence, the role of the general acid/base in the mechanics of the CDC25 catalytic cycle has been controversial. Early studies on Cdc25 phosphatases suggested an aspartate located in a nonconserved region to serve as the general acid/base [74]. However, structural studies on the catalytic domain of Cdc25A did not corroborate the speculating and suggested only as a structural role for this residue [75]. Consequent structural studies on Cdc25B prompted speculations about two glutamates present in the X_5-segment of the P-loop to be the plausible general acid/base [76]. Although molecular dynamics simulation studies have provided some support for this interpretation [77], the exact mechanism of Cdc25 phosphatase catalytic cycle remains controversial. Alternate explanations have also been provided for the working of Cdc25 phosphatases. These include the suggestion that the general acid/base may in fact be provided by the incoming substrate itself [78]. Another explanation eliminates the requirement of a general acid/base in the Cdc25 phosphatase catalytic mechanism, suggesting pH changes at the Cdc25 phosphatase-active site during the course of the catalytic cycle to compensate for lack of a committed acidic amino acid proton donor. The glutamates of the X_5-segment of the P-loop are suggested to be involved in the activation of water for the hydrolysis of the cysteinyl–phosphate intermediate [61, 79].

3.6 Crystallographic strategies to study PTP structure

The PTP catalytic domain has been extensively characterized for its structural determinants [18]. Most common form is the *Apo*-form that is used form foundational structural analysis [80]. The most common product-trap complexes of PTPs include the structures with phosphate or phosphate mimetics like sulfate, vanadate or tungstate groups in the

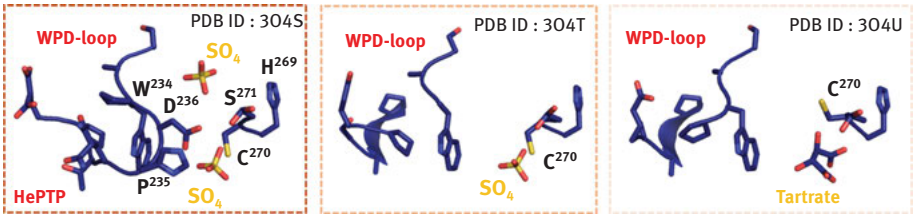

Figure 3.11: Dynamic movement of the WPD-loop at the HePTP-active site.

active site [18, 53]. In the case of the HePTP, crystal forms are available that show movements in the WPD-loop dynamics in direct correlation with the occupancy of the sulfate ion at the active site (Figure 3.11) [53, 81]. In addition to these small ions bound to the PTP-active site, crystal structures have also been obtained with PTPs bound to small molecules like *p*-nitrophenylphosphate (*p*NPP) [82] and *p*-nitrocatecholsulfate [18]. Several strategies have been employed to inactivate PTPs to aid formation of stable PTP–phosphopeptide complexes. As mentioned earlier, essentially three positions are targeted in the PTP structure: the P-loop cysteine, the WPD-loop aspartate and the Q-loop glutamine [37]. The active site cysteine is either mutated to an alanine [73, 82] or a less intrusive serine residue [83, 84]. The WPD-loop aspartate has been mutated to an alanine [37, 85], which has also been used as a double modification with the Q-loop glutamine to alanine mutation [86]. These combinations of mutations have allowed for a thorough investigation of the PTP catalytic mechanism and also made way for efficient analysis of their substrate proteins. Many structures of PTPs have been solved as peptide complexes from their target substrate proteins [32, 49, 84, 87].

3.7 Active site determinants

While the active site of classical PTPs is extremely well conserved, subtle differences on the surface are enough to provide for variability. This has been studied using a generic substrate *p*NPP, a small molecule that resembles the phosphotyrosine. This molecule has slower rate of entry into the PTP-active site owing to the lack of a peptide backbone that allows for substrate anchoring on the PTP surface [88]. Nonetheless, *p*NPP provides the simplest method to access the PTP-active site. This simple substrate has been handy in measuring the differences the catalytic efficiencies of various PTP catalytic domains. It is surprising how a conserved active site provides for a broad range of catalytic prowess. For example, the human HePTP is almost tenfold less efficient than the placental PTP1B [89]. In contrast, the parasitic *Yersinia* PTP YopH is about tenfold more efficient than PTP1B [90]. One of these variations is provided by the microenvironment around the WPD-loop whose dynamics can directly affect catalytic activity [45, 91]. Hence, subtleties in the microenvironment of the active site are sufficient for variability. In the case of myotubularins, a sequence motif WDR

(tryptophan–aspartate–arginine) is embedded in its CxxxxxR motif to form the CxxxWDR-active site motif. This sequence makes the myotubularins specific to phosphatidylinositol-3-phosphate and phosphatidylinositol-(3,5)-bisphosphate substrates. Mutation of the WDR aspartate is enough to inactivate the enzyme [92].

Another determinant of substrate specificity and catalytic efficiency is contained in the phosphotyrosine-binding loop. As explained before (Section 3.2.4) the phosphotyrosine binding loop (or motif-10 Nxx(K/R)NRY in classical class I PTPs) uses its tyrosine/phenylalanine residue for defining the depth of the active site, phosphotyrosine specificity and recruitment. The residue present two places downstream of this tyrosine/phenylalanine is usually an aspartate or asparagine for optimal substrate utilization [93]. In the KIM family of PTPs (HePTP, STEP), this residue is a threonine and negatively influences recruitment of phosphotyrosine substrates [94]. Mutation of this threonine to aspartate or asparagine allows for a 10- to 70-fold increase in catalytic turnover rates and efficient utilization of Erk2-based phosphopeptides.

Alongside these subtle microenvironment determinants of substrate recruitment, protein–protein interactions are critical to functional efficiency of PTP catalytic domains. For example, the DUSP MAP kinase phosphatase (MKP-3) is over a million-fold more efficient in dephosphorylating the Erk1/2 protein as compared to utilizing its activation loop-based phosphopeptide [95]. Both structural and biochemical studies explain a significant conformational change of the active site loop may be achieved for MKP-3 upon Erk1/2 binding. This explains how the use of phosphopeptides may perhaps be a limiting strategy in understanding the mechanics of the PTP domain.

3.8 Accessory substrate binding determinants

A combination of sequence analysis and low-resolution homology modeling has allowed for the characterization of both conserved and variable pockets around the PTP-active site that could serve as secondary spots of substrate binding. These pockets provide for a novel perspective for structure-based drug design and are being explored by various research groups [80]. In the case of PTP1B, a secondary phosphotyrosine binding site has been located that lies just proximal to the active site [6]. The architecture of this site is crucial for PTP1B's ability to bind the phosphorylated insulin receptor kinase activation loop that contains two phosphotyrosine residues placed adjacent to each other [73].

This pocket is defined as the "second site" and is defined by the residues of the helices α2′ and the loop that connect α2′ to α1. Two arginine residues, Arg24 and Arg254, provide the positive charge required for binding the second phosphotyrosine (Figure 3.12). The base of the pocket is formed by a small glycine (Gly259) that serves as a "gateway" residue. This glycine is a key determinant in deciding substrate recognition by PTPs. PTP domains like in LAR and PTPα show a limited substrate

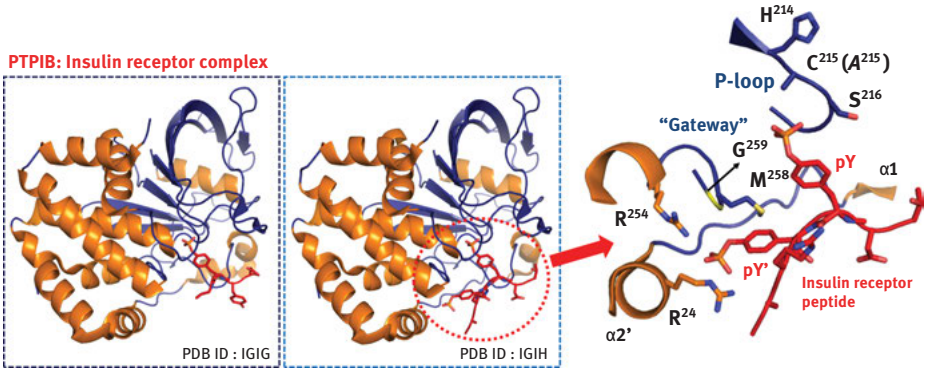

Figure 3.12: The second phosphotyrosine-binding site of PTP1B.

preference. Mutation of the corresponding gateway residue of PTPα to a glycine switches its substrate preference to be more like that of PTP1B [96]. An effective paradigm for inhibitor design utilizes accessing both the active site and this second site by a bidentate ligand [13]. This approach has allowed for designing of PTP1B-specific inhibitors (e.g. compound 901; K_i = 76 nM) that are over five-fold more selective for PTP1B over its closely related TCPTP [97]. Moreover, peptides containing two adjacent nonhydrolysable phosphotyrosine mimetics have been shown to be effective inhibitors of PTP1B [98].

The second site in PTP1B has been explored in molecular detail in other known PTP structures [80]. Using the architecture of the second site (as defined by the Arg24 residue and the gateway Gly259), Alastair J. Barr and coworkers have grouped the known structurally characterized PTP domains into five categories (Table 3.3 and Figure 3.13). These workers define the second site by an additional gateway residue (Met258 in PTP1B) alongside Gly259 at the base of the pocket. They also define the α2′-α1 loop as a "second site loop" that shows extreme conformations (ranging from closed in HePTP to open in PTPγ). The first category includes PTP1B, TCPTP, SHP1, SHP2, BDP1, DEP1, PTPBAS, LYP, PTPγ, PTPβ and PEST catalytic domains. All of these have a secondary site similar to that in PTP1B that has a basic residue in the Arg24 position and the second site loop is open. PTPγ and PTPβ have bulkier aromatic residues as at the gateway and are hence slightly different from the other category members.

The second category includes the pseudophosphatases 1A2 and 1A2β that have a cysteine residue at Arg24 position, proline and glycine at the gateway positions and the second site loop in an open conformation. The third category has receptor PTPs: LAR, PTPσ and PTPδ (which also have similar composition of their extracellular domains). These PTP catalytic domains have a restricted second site, with an acidic residue at the Arg24 position, and bulkier residues at the gateway positions. Their second site loop is open. The fourth category includes the catalytic domains of PTPH1, MEG1, PTPD1, PTPD2, CD45, PTPμ, PTPκ, PTPρ, PTPε and PTPα. Their second

Table 3.3: Five categories of PTP domains based on the "second site".

Category	PTP	Gateway residues	Second-site loop residue	Second-site loop conformation
I	PTP1B	Met^{258} Gly^{259}	Arg^{24}	Open
	TCPTP	Met Gly	Arg	Open
	MEG2	Ala Phe	Arg	Open
	BAS	His Gly	Gln	Open
	TYP	Ser Gly	Gln	n/a
	HDPTP	Lys His	Gln	n/a
	PTPβ	Val His	Arg	Open
	DEP1	Pro Leu	Arg	Open
	GLEPP1	Met Ser	Arg	Open
	SAP1	Val Leu	Ser	n/a
	PTPS31	Met Cys	Pro	n/a
	SHP1	Ser Gly	Gln	Open
	SHP2	Ser Gly	Gln	Open
	BDP1	Pro Ala	Gln	Open
	LYP	Pro Ser	Lys	Open
	PTPγ	Asn Tyr	Gln	Open
	PEST	His Ser	Arg	n/a
	PTPζ	Asn Tyr	Gln	Open
II	IA2	Pro Gly	Cys	Open
	1A2β	Pro Gly	Cys	Open
III	PTPδ	Asn Tyr	Asp	Open
	LAR	Asn Tyr	Asp	Open
	PTPσ	Asn Tyr	Asp	Open
IV	PTPκ	Ile Asn	Phe	Closed
	PTPμ	Val Asn	Phe	Closed
	PTP τ	Val Asn	Pro	Closed
	PTPλ	Val Asn	Phe	n/a
	PTPα	Cys Gln	Pro	Closed
	PTPε	Pro Gln	Pro	Closed
	CD45	Cys Leu	Pro	Closed
	MEG1	Ala Met	Tyr	Closed
	PTPH1	Ala Met	Tyr	Closed
	PTPD1	Met Met	Leu	n/a
	PTPD2	Met Phe	Pro	Closed
V	PCPT1	Gly Gly	Pro	Closed
	STEP	Gly Gly	Pro	Closed
	HEPTP	Gly Gly	Pro	Closed

Table adapted from Ref [74].

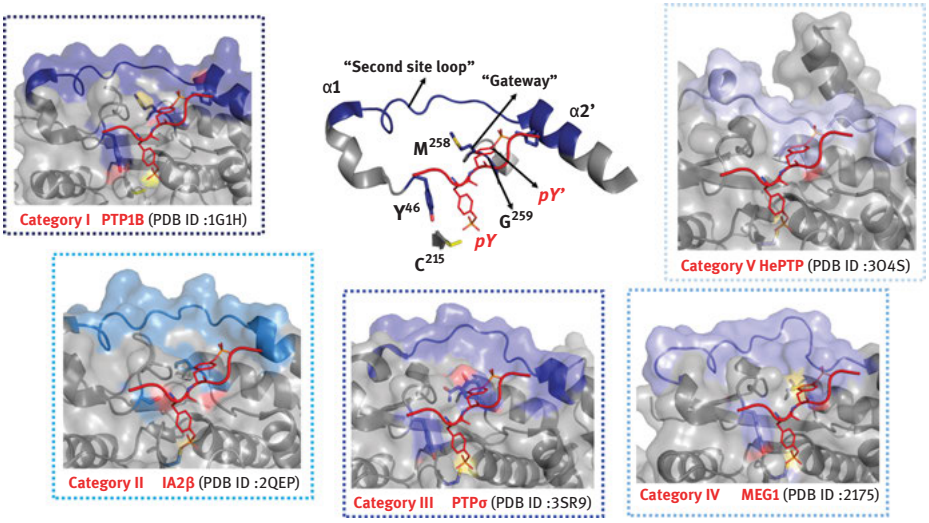

Figure 3.13: The second site and gateway residue allow for the classification of classic PTP catalytic domains into five categories.

site is completely inaccessible: an aromatic residue or a proline fills the Arg24 position, aliphatic residues occupy the gateway positions and second site loop is in a twisted closed conformation. The fifth category includes PCPTP, STEP and HePTP catalytic domain. In this case, the gateway positions are both glycine residues, but the Arg24 position is blocked by aromatic residue or a proline making the site inaccessible. In addition, their second site loop is in the closed conformation.

3.9 The unique class IV PTPs

The class IV PTPs contain four paralogs Eya1, Eya2, Eya3 and Eya4. These are evolutionarily distinct from the class I, II and III PTPs. The Eya proteins are structurally and mechanistically a part of the haloacid dehalogenase (HAD) superfamily of enzymes. The HAD superfamily mainly includes phosphotransferases (including phosphatases) that are diverse in sequence but conserved in their active site arrangement and mechanism [4, 99]. All members of the HAD superfamily share a conserved active site machinery positioned in a modified Rossman fold [100, 101]. The central sheet of the Rossman fold consists of at least five β-strands in a 54123 arrangement; displaying the connecting loops that contain the core catalytic residues (Figure 3.14). The HAD phosphatases also have three additional structural elements known as the squiggle, flap and cap domains [101, 102]. These elements allow for conformational flexibility in these phosphatases to promote substrate recognition and recruitment.

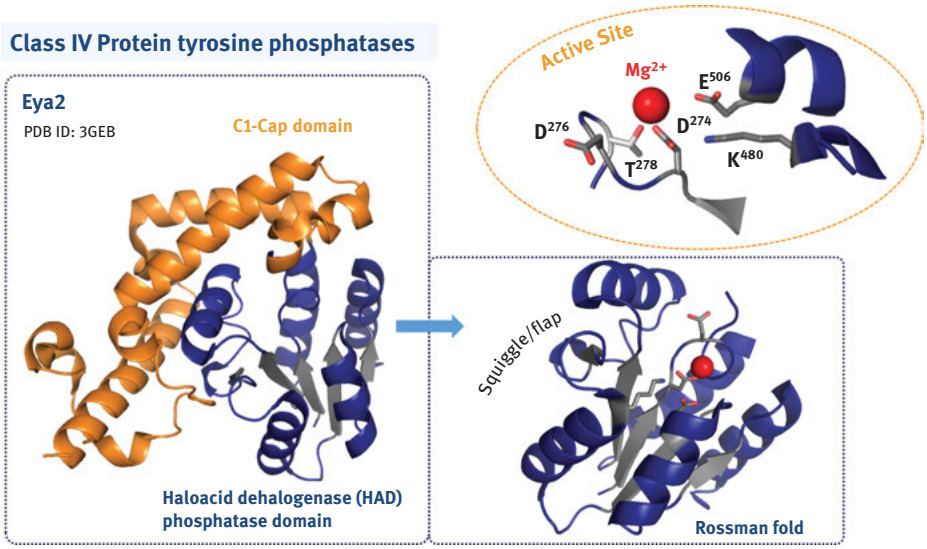

Figure 3.14: The catalytic domain and active site of class IV PTPs.

The squiggle and the flap elements are located next to the β1 strand of the core Rossman fold. Squiggle is a small stretch of amino acids (about six residues) that forms a single helical turn that connects to the flap that adopts a β-hairpin turn. The squiggle can wind its helix to trigger a movement of the flap located next to active site [102]. The concerted movement of the squiggle and flap elements allows for regulating solvent access and exclusion from the active site during the course of the catalytic cycle. The cap domain functions in concert with the squiggle and flap elements to shield the active site cavity. These cap domains are reported to be of three types, named as C0, C1 and C2. These cap domains are reported to contain determinants of substrate specificity and also oligomerization. The Eya proteins contain a unique C1 cap domain that has a helical bundle comprising of seven α-helices (residues 283–423 in Eya2; Figure 3.14) [103].

The conserved catalytic core of the HAD phosphatases is defined by four signature motifs that are distinct from the ten motifs defining the classic PTPs [104]. Motif I contains the active site nucleophilic aspartate in a conserved ϕϕϕDxDx(T/V)(L/V)ϕ sequence, where ϕ is a hydrophobic amino acid. The two aspartate residues (Asp274 and Asp276 in Eya2) of the motif coordinate an Mg^{2+} ion that is conserved at the HAD phosphatase-active site (Figure 3.14) [105]. Motif II contains a serine or threonineresidue in the sequence ϕϕϕϕϕϕ(S/T) (Thr278 in Eya2). This serine/threonine makes hydrogen binds with the incoming substrate and promotes its proper configuration at the active site. Motif III is less conserved and is essentially a critical lysine residue (Lys480 in Eya2) that is positioned about 18–20 amino acids upstream of motif IV. This lysine stabilizes the aspartyl–

phosphate intermediate at the active site. Motif IV is the conserved (G/S)(D/S)xxxx (D/E)ϕϕϕϕ or a (G/S)DDϕϕϕϕ sequence [106]. The aspartate (and/or glutamate) (Asp502 in Eya2) from motif IV works with the dual-aspartates of motif I to assist in the chelation of the Mg^{2+} ion at the active site.

3.10 An alternate mechanism for the Class IV PTPs

The class IV PTPs use an aspartyl–phosphate intermediate dependent catalytic mechanism. Much like the catalytic mechanism of the class I–III PTPs, it is suggested to take place in two sequential steps (Figure 3.15). In the first step, the catalytic aspartate of motif I (Asp274 in Eya2) initiates a nucleophilic attack on the incoming substrate to form the aspartyl–phosphate intermediate. The Mg^{2+} ion coordinated at the phosphatase active site aids the optimal positioning of the substrate relative to the two aspartates of motif I. Residues from other motifs and loop, along with the Mg^{2+} ion, stabilize the intermediate [107]. The Asp + two residue of motif I (Asp276 in Eya2) functions as a general acid/base to protonate the leaving group. In the second step of the reaction, the same aspartate (Asp276 in Eya2) functions as a general base to deprotonate water and allow for the hydrolysis of the aspartyl–phosphate intermediate. Structural studies using BeF_3 and AlF_3 as the transition-state mimetics of the phosphoenzyme of Eya2 are available (Figure 3.15) [103]. The BeF_3-bound Eya2 shows the extensive interactions of key residues Asp274, Asp276, Lys480, Thr447 and Thr448 with the transition state mimetic. The

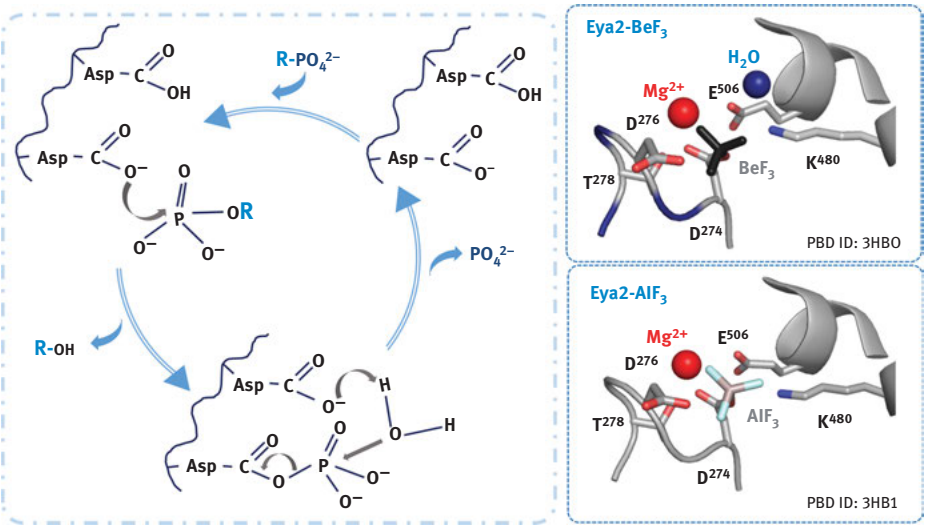

Figure 3.15: The catalytic mechanism of aspartate-based class IV PTPs. Transition state mimetics bound to the active of Eya2 allow for a structural understanding of its mechanics.

structural shift in the orientation of Asp276 is correlated with the positioning of an ordered water molecule between Asp276 and Glu277. Glu277 is hence suggested to function like the Q-loop glutamine residues of the classic PTPs to activate water molecules at the Eya2-active site. Mutagenesis of Glu277 of Eya2 is reported to decrease its activity by 100-fold, thereby providing support for the structural analysis [68]. In contrast, no ordered water molecule is seen in the required position in the AlF_3-bound structure thereby suggesting a hitherto unknown mechanism of catalytic cycle regulation of Eya2.

References

[1] Cohen P. Protein kinases – the major drug targets of the twenty-first century? Nat Rev Drug Discovery 2002;1(4):309–15.

[2] Xu Y, Fisher GJ. Receptor type protein tyrosine phosphatases (RPTPs) – roles in signal transduction and human disease. J Cell Commun Signal 2012;6(3):125-38.

[3] Tautz L, Pellecchia M, Mustelin T. Targeting the PTPome in human disease. Expert Opin Ther Targets 2006;10(1):157–77.

[4] Tonks NK. Protein tyrosine phosphatases: from genes, to function, to disease. Nat Rev Mol Cell Biol 2006;7(11):833–46.

[5] Tautz L, Critton DA, Grotegut S. Protein tyrosine phosphatases: structure, function, and implication in human disease. Methods Mol Biol 2013;1053:179–221.

[6] Andersen JN, Mortensen OH, Peters GH, Drake PG, Iversen LF, Olsen OH, et al. Structural and evolutionary relationships among protein tyrosine phosphatase domains. Mol Cell Biol 2001;21(21):7117–36.

[7] Bottini N, Bottini E, Gloria-Bottini F, Mustelin T. Low-molecular-weight protein tyrosine phosphatase and human disease: in search of biochemical mechanisms. Arch Immunol Ther Exp (Warsz) 2002;50(2):95–104.

[8] Vang T, Liu WH, Delacroix L, Wu S, Vasile S, Dahl R, et al. LYP inhibits T-cell activation when dissociated from CSK. Nat Chem Biol 2012;8(5):437–46.

[9] Lorenz U. SHP-1 and SHP-2 in T cells: two phosphatases functioning at many levels. Immunol Rev 2009;228(1):342–59.

[10] Ahuja LG, Gopal B. Bi-domain protein tyrosine phosphatases reveal an evolutionary adaptation to optimize signal transduction. Antioxid Redox Signal 2014;20(14):2141–59.

[11] Tonks NK, Diltz CD, Fischer EH. Purification of the major protein-tyrosine-phosphatases of human placenta. J Biol Chem 1988;263(14):6722–30.

[12] Charbonneau H, Tonks NK, Kumar S, Diltz CD, Harrylock M, Cool DE, et al. Human placenta protein-tyrosine-phosphatase: amino acid sequence and relationship to a family of receptor-like proteins. Proc Natl Acad Sci USA 1989;86(14):5252–6.

[13] Puius YA, Zhao Y, Sullivan M, Lawrence DS, Almo SC, Zhang ZY. Identification of a second aryl phosphate-binding site in protein-tyrosine phosphatase 1B: a paradigm for inhibitor design. Proc Natl Acad Sci U S A 1997;94(25):13420–5.

[14] Barford D, Flint AJ, Tonks NK. Crystal structure of human protein tyrosine phosphatase 1B. Science 1994;263(5152):1397–404.

[15] Stuckey JA, Schubert HL, Fauman EB, Zhang Z-Y, Dixon JE, Saper MA. Crystal structure of Yersinia protein tyrosine phosphatase at 2.5 A and the complex with tungstate. Nature 1994;370(6490):571–5.

[16] Yuvaniyama J, Denu JM, Dixon JE, Saper MA. Crystal structure of the dual specificity protein phosphatase VHR. Science 1996;272(5266):1328–31.
[17] Begley MJ, Taylor GS, Kim S-A, Veine DM, Dixon JE, Stuckey JA. Crystal structure of a phosphoinositide phosphatase, MTMR2. Mol Cell 2003;12(6):1391–402.
[18] Madan LL, Gopal B. Conformational basis for substrate recruitment in protein tyrosine phosphatase 10D. Biochemistry 2011;50(46):10114–25.
[19] Caunt CJ, Keyse SM. Dual-specificity MAP kinase phosphatases (MKPs): Shaping the outcome of MAP kinase signalling. FEBS J 2013;280(2):489–504.
[20] Cardle L, Dufton M. Identification of important functional environs in protein tertiary structures from the analysis of residue variation in 3-D: application to cytochromes c and carboxypeptidases A and B. Protein Eng Des Sel 1994;7(12):1423–31.
[21] McGaughey GB, Gagne M, Rappe AK. pi-Stacking interactions. Alive and well in proteins. J Biol Chem 1998;273(25):15458–63.
[22] Muise ES, Vrielink A, Ennis MA, Lemieux NH, Tremblay ML. Thermosensitive mutants of the MPTP and hPTP1B protein tyrosine phosphatases: isolation and structural analysis. Protein Sci 1996;5(4):604–13.
[23] Tsai A, Itoh M, Streuli M, Thai T, Saito H. Isolation and characterization of temperature-sensitive and thermostable mutants of the human receptor-like protein tyrosine phosphatase LAR. J Biol Chem 1991;266(16):10534–43.
[24] Zhang Z-Y, Wang Y, Wu L, Fauman EB, Stuckey JA, Schubert HL, et al. The Cys (X) 5Arg catalytic motif in phosphoester hydrolysis. Biochemistry 1994;33(51):15266–70.
[25] Eberlein TH. Essentials of organic chemistry: for students of pharmacy, medicinal chemistry, and biological chemistry (Paul M. Dewick). J Chem Educ 2008;85(2):204.
[26] Zhang ZY, Dixon JE. Active site labeling of the Yersinia protein tyrosine phosphatase: the determination of the pKa of the active site cysteine and the function of the conserved histidine 402. Biochemistry 1993;32(36):9340–5.
[27] Ostman A, Frijhoff J, Sandin A, Bohmer FD. Regulation of protein tyrosine phosphatases by reversible oxidation. J Biochem 2011;150(4):345–56.
[28] Frijhoff J, Dagnell M, Godfrey R, Ostman A. Regulation of protein tyrosine phosphatase oxidation in cell adhesion and migration. Antioxid Redox Signal 2014;20(13):1994–2010.
[29] Zhang Z-Y, Palfey BA, Wu L, Zhao Y. Catalytic function of the conserved hydroxyl group in the protein tyrosine phosphatase signature motif. Biochemistry 1995;34(50):16389–96.
[30] Peters GH, Frimurer TM, Olsen OH. Electrostatic Evaluation of the Signature Motif (H/V) CX5R (S/T) in Protein– Tyrosine Phosphatases. Biochemistry 1998;37(16):5383–93.
[31] Salmeen A, Andersen JN, Myers MP, Tonks NK, Barford D. Molecular basis for the dephosphorylation of the activation segment of the insulin receptor by protein tyrosine phosphatase 1B. Mol Cell 2000;6(6):1401–12.
[32] Sarmiento M, Puius YA, Vetter SW, Keng Y-F, Wu L, Zhao Y, et al. Structural basis of plasticity in protein tyrosine phosphatase 1B substrate recognition. Biochemistry 2000;39(28):8171–9.
[33] Tailor P, Gilman J, Williams S, Mustelin T. A novel isoform of the low molecular weight phosphotyrosine phosphatase, LMPTP-C, arising from alternative mRNA splicing. FEBS J 1999;262(2):277–82.
[34] Keng YF, Wu L, Zhang ZY. Probing the function of the conserved tryptophan in the flexible loop of the Yersinia protein-tyrosine phosphatase. FEBS J 1999;259(3):809–14.
[35] Zhang Z-Y. Protein-tyrosine phosphatases: biological function, structural characteristics, and mechanism of catalysis. Crit Rev Biochem Mol Biol 1998;33(1):1–52.
[36] Wälchli S, Curchod M-L, Gobert RP, Arkinstall S, van Huijsduijnen RH. Identification of tyrosine phosphatases that dephosphorylate the insulin receptor. A brute force approach based on “substrate-trapping” mutants. J Biol Chem 2000;275(13):9792–6.

[37] Flint AJ, Tiganis T, Barford D, Tonks NK. Development of "substrate-trapping" mutants to identify physiological substrates of protein tyrosine phosphatases. Proc Natl Acad Sci 1997; 94(5):1680–5.
[38] Garton AJ, Flint AJ, Tonks NK. Identification of p130 (cas) as a substrate for the cytosolic protein tyrosine phosphatase PTP-PEST. Mol Cell Biol 1996;16(11):6408–18.
[39] Gingras M-C, Zhang YL, Kharitidi D, Barr AJ, Knapp S, Tremblay ML, et al. HD-PTP is a catalytically inactive tyrosine phosphatase due to a conserved divergence in its phosphatase domain. PloS One 2009;4(4):e5105.
[40] Cardone L, Carlucci A, Affaitati A, Livigni A, Garbi C, Varrone S, et al. Mitochondrial AKAP121 binds and targets protein tyrosine phosphatase D1, a novel positive regulator of src signaling. Mol Cell Biol 2004;24(11):4613–26.
[41] Kurkcuoglu Z, Bakan A, Kocaman D, Bahar I, Doruker P. Coupling between catalytic loop motions and enzyme global dynamics. PLoS Comput Biol 2012;8(9):e1002705.
[42] Barr AJ, Ugochukwu E, Lee WH, King ON, Filippakopoulos P, Alfano I, et al. Large-scale structural analysis of the classical human protein tyrosine phosphatome. Cell 2009; 136(2):352–63.
[43] Peters GH, Frimurer TM, Andersen JN, Olsen OH. Molecular dynamics simulations of protein-tyrosine phosphatase 1B. II. Substrate-enzyme interactions and dynamics. Biophys J 2000; 78(5):2191–200.
[44] Wiesmann C, Barr KJ, Kung J, Zhu J, Erlanson DA, Wang S, et al. Allosteric inhibition of protein tyrosine phosphatase 1B. Nat Struct Mol Biol 2004;11(8):730.
[45] Kamerlin SCL, Rucker R, Boresch S. A molecular dynamics study of WPD-loop flexibility in PTP1B. Biochem Biophys Res Commun 2007;356(4):1011–6.
[46] Martell KJ, Angelotti T, Ullrich A. The "VH1-like" dual-specificity protein tyrosine phosphatases. Mol Cells 1998;8(1):2–11.
[47] Pannifer AD, Flint AJ, Tonks NK, Barford D. Visualization of the cysteinyl-phosphate intermediate of a protein-tyrosine phosphatase by X-ray crystallography. J Biol Chem 1998; 273(17):10454–62.
[48] Zhao Y, Wu L, Noh SJ, Guan K-L, Zhang Z-Y. Altering the nucleophile specificity of a protein-tyrosine phosphatase-catalyzed reaction probing the function of the invariant glutamine residues. J Biol Chem 1998;273(10):5484–92.
[49] Jia Z, Barford D, Flint AJ, Tonks NK. Structural basis for phosphotyrosine peptide recognition by protein tyrosine phosphatase 1B. Science 1995;268(5218):1754.
[50] Peti W, Page R. Strategies to make protein serine/threonine (PP1, calcineurin) and tyrosine phosphatases (PTP1B) druggable: achieving specificity by targeting substrate and regulatory protein interaction sites. Bioorg Med Chem 2015;23(12):2781–5.
[51] Pils B, Schultz Jr. Evolution of the multifunctional protein tyrosine phosphatase family. Mol Biol Evol 2004;21(4):625–31.
[52] Lim KL, Kolatkar PR, Ng KP, Ng CH, Pallen CJ. Interconversion of the kinetic identities of the tandem catalytic domains of receptor-like protein-tyrosine phosphatase PTPα by two point mutations is synergistic and substrate-dependent. J Biol Chem 1998;273(44): 28986–93.
[53] Critton DA, Tautz L, Page R. Visualizing active-site dynamics in single crystals of HePTP: opening of the WPD loop involves coordinated movement of the E loop. J Mol Biol 2011;405(3):619–29.
[54] Eswaran J, von Kries JP, Marsden B, Longman E, Debreczeni JÉ, Ugochukwu E, et al. Crystal structures and inhibitor identification for PTPN5, PTPRR and PTPN7: a family of human MAPK-specific protein tyrosine phosphatases. Biochem J 2006;395(3):483–91.
[55] Iversen LF, Møller KB, Pedersen AK, Peters GH, Petersen AS, Andersen HS, et al. Structure determination of T cell protein-tyrosine phosphatase. J Biol Chem 2002;277(22):19982–90.

[56] Asante-Appiah E, Patel S, Desponts C, Taylor JM, Lau C, Dufresne C, et al. Conformation-assisted inhibition of protein-tyrosine phosphatase-1B elicits inhibitor selectivity over T-cell protein-tyrosine phosphatase. J Biol Chem 2006;281(12):8010–5.
[57] Zabell AP, Schroff AD, Jr., Bain BE, Van Etten RL, Wiest O, Stauffacher CV. Crystal structure of the human B-form low molecular weight phosphotyrosyl phosphatase at 1.6-A resolution. J Biol Chem 2006;281(10):6520–7.
[58] Bryson GL, Massa H, Trask BJ, Van Etten RL. Gene structure, sequence, and chromosomal localization of the human red cell-type low-molecular-weight acid phosphotyrosyl phosphatase gene, ACP1. Genomics 1995;30(2):133–40.
[59] Tailor P, Gilman J, Williams S, Mustelin T. A novel isoform of the low molecular weight phosphotyrosine phosphatase, LMPTP-C, arising from alternative mRNA splicing. Eur J Biochem 1999;262(2):277–82.
[60] Ramponi G, Manao G, Camici G, Cappugi G, Ruggiero M, Bottaro DP. The 18 kDa cytosolic acid phosphatase from bovine live has phosphotyrosine phosphatase activity on the autophosphorylated epidermal growth factor receptor. FEBS Lett 1989;250(2):469–73.
[61] Rudolph J. Cdc25 phosphatases: structure, specificity, and mechanism. Biochemistry 2007; 46(12):3595–604.
[62] Hofmann K, Bucher P, Kajava AV. A model of Cdc25 phosphatase catalytic domain and Cdk-interaction surface based on the presence of a rhodanese homology domain. J Mol Biol 1998;282(1):195–208.
[63] Donzelli M, Squatrito M, Ganoth D, Hershko A, Pagano M, Draetta GF. Dual mode of degradation of Cdc25 A phosphatase. EMBO J 2002;21(18):4875–84.
[64] Hoffmann I, Draetta G, Karsenti E. Activation of the phosphatase activity of human cdc25A by a cdk2-cyclin E dependent phosphorylation at the G1/S transition. EMBO J 1994;13(18): 4302–10.
[65] Giles N, Forrest A, Gabrielli B. 14-3-3 acts as an intramolecular bridge to regulate cdc25B localization and activity. J Biol Chem 2003;278(31):28580–7.
[66] Denu JM, Dixon JE. Protein tyrosine phosphatases: mechanisms of catalysis and regulation. Curr Opin Chem Biol 1998;2(5):633–41.
[67] Zhang ZY, Wang Y, Dixon JE. Dissecting the catalytic mechanism of protein-tyrosine phosphatases. Proc Natl Acad Sci USA 1994;91(5):1624–7.
[68] Zhang ZY. Protein tyrosine phosphatases: structure and function, substrate specificity, and inhibitor development. Ann Rev Pharmacol Toxicol 2002;42:209–34.
[69] Denu JM, Lohse DL, Vijayalakshmi J, Saper MA, Dixon JE. Visualization of intermediate and transition-state structures in protein-tyrosine phosphatase catalysis. Proc Natl Acad Sci USA 1996;93(6):2493–8.
[70] Pannifer AD, Flint AJ, Tonks NK, Barford D. Visualization of the cysteinyl-phosphate intermediate of a protein-tyrosine phosphatase by x-ray crystallography. J Biol Chem 1998; 273(17):10454–62.
[71] Blanchetot C, Chagnon M, Dube N, Halle M, Tremblay ML. Substrate-trapping techniques in the identification of cellular PTP targets. Methods 2005;35(1):44–53.
[72] Jia Z, Barford D, Flint AJ, Tonks NK. Structural basis for phosphotyrosine peptide recognition by protein tyrosine phosphatase 1B. Science 1995;268(5218):1754–8.
[73] Salmeen A, Andersen JN, Myers MP, Tonks NK, Barford D. Molecular basis for the dephosphorylation of the activation segment of the insulin receptor by protein tyrosine phosphatase 1B. Mol Cell 2000;6(6):1401–12.
[74] Eckstein JW, Beer-Romero P, Berdo I. Identification of an essential acidic residue in Cdc25 protein phosphatase and a general three-dimensional model for a core region in protein phosphatases. Protein Sci 1996;5(1):5–12.

[75] Fauman EB, Cogswell JP, Lovejoy B, Rocque WJ, Holmes W, Montana VG, et al. Crystal structure of the catalytic domain of the human cell cycle control phosphatase, Cdc25A. Cell 1998; 93(4):617–25.
[76] Reynolds RA, Yem AW, Wolfe CL, Deibel MR, Jr., Chidester CG, Watenpaugh KD. Crystal structure of the catalytic subunit of Cdc25B required for G2/M phase transition of the cell cycle. J Mol Biol 1999;293(3):559–68.
[77] Arantes GM. The catalytic acid in the dephosphorylation of the Cdk2-pTpY/CycA protein complex by Cdc25B phosphatase. J Phys Chem B 2008;112(47):15244–7.
[78] Chen W, Wilborn M, Rudolph J. Dual-specific Cdc25B phosphatase: in search of the catalytic acid. Biochemistry 2000;39(35):10781–9.
[79] Rudolph J. Catalytic mechanism of Cdc25. Biochemistry 2002;41(49):14613–23.
[80] Barr AJ, Ugochukwu E, Lee WH, King ON, Filippakopoulos P, Alfano I, et al. Large-scale structural analysis of the classical human protein tyrosine phosphatome. Cell 2009;136(2):352–63.
[81] Critton DA, Tautz L, Page R. Visualizing active-site dynamics in single crystals of HePTP: opening of the WPD loop involves coordinated movement of the E loop. J Mol Biol 2011; 405(3):619–29.
[82] Wang S, Tabernero L, Zhang M, Harms E, Van Etten RL, Stauffacher CV. Crystal structures of a low-molecular weight protein tyrosine phosphatase from Saccharomyces cerevisiae and its complex with the substrate p-nitrophenyl phosphate. Biochemistry 2000;39(8):1903–14.
[83] Sarmiento M, Puius YA, Vetter SW, Keng YF, Wu L, Zhao Y, et al. Structural basis of plasticity in protein tyrosine phosphatase 1B substrate recognition. Biochemistry 2000;39(28):8171–9.
[84] Song H, Hanlon N, Brown NR, Noble MEM, Johnson LN, Barford D. Phosphoprotein–protein interactions revealed by the crystal structure of kinase-associated phosphatase in complex with phosphoCDK2. Mol Cell 2001;7(3):615–26.
[85] Garton AJ, Flint AJ, Tonks NK. Identification of p130(cas) as a substrate for the cytosolic protein tyrosine phosphatase PTP-PEST. Mol Cell Biol 1996;16(11):6408–18.
[86] Xie L, Zhang YL, Zhang ZY. Design and characterization of an improved protein tyrosine phosphatase substrate-trapping mutant. Biochemistry 2002;41(12):4032–9.
[87] Heo YS, Ryu JM, Park SM, Park JH, Lee HC, Hwang KY, et al. Structural basis for inhibition of protein tyrosine phosphatases by Keggin compounds phosphomolybdate and phosphotungstate. Exp Mol Med 2002;34(3):211–23.
[88] Madan LL, Goutam K, Gopal B. Inter-domain interactions influence the stability and catalytic activity of the bi-domain protein tyrosine phosphatase PTP99A. Biochim Biophys Acta (BBA) – Proteins Proteomics 2012;1824(8):983–90.
[89] Mustelin T, Tautz L, Page R. Structure of the hematopoietic tyrosine phosphatase (HePTP) catalytic domain: structure of a KIM phosphatase with phosphate bound at the active site. J Mol Biol 2005;354(1):150–63.
[90] Sun JP, Wu L, Fedorov AA, Almo SC, Zhang ZY. Crystal structure of the Yersinia protein-tyrosine phosphatase YopH complexed with a specific small molecule inhibitor. J Biol Chem 2003; 278(35):33392–9.
[91] Wiesmann C, Barr KJ, Kung J, Zhu J, Erlanson DA, Shen W, et al. Allosteric inhibition of protein tyrosine phosphatase 1B. Nat Struct Mol Biol 2004;11(8):730–7.
[92] Begley MJ, Taylor GS, Kim SA, Veine DM, Dixon JE, Stuckey JA. Crystal structure of a phosphoinositide phosphatase, MTMR2: insights into myotubular myopathy and Charcot-Marie-Tooth syndrome. Mol Cell 2003;12(6):1391–402.
[93] Sarmiento M, Zhao Y, Gordon SJ, Zhang ZY. Molecular basis for substrate specificity of protein-tyrosine phosphatase 1B. J Biol Chem 1998;273(41):26368–74.
[94] Huang Z, Zhou B, Zhang ZY. Molecular determinants of substrate recognition in hematopoietic protein-tyrosine phosphatase. J Biol Chem 2004;279(50):52150–9.

[95] Zhao Y, Zhang ZY. The mechanism of dephosphorylation of extracellular signal-regulated kinase 2 by mitogen-activated protein kinase phosphatase 3. J Biol Chem 2001;276(34):32382–91.
[96] Peters GH, Iversen LF, Branner S, Andersen HS, Mortensen SB, Olsen OH, et al. Residue 259 is a key determinant of substrate specificity of protein-tyrosine phosphatases 1B and alpha. J Biol Chem 2000;275(24):18201–9.
[97] Xin Z, Oost TK, Abad-Zapatero C, Hajduk PJ, Pei Z, Szczepankiewicz BG, et al. Potent, selective inhibitors of protein tyrosine phosphatase 1B. Bioorg Med Chem Lett 2003;13(11):1887–90.
[98] Desmarais S, Friesen RW, Zamboni R, Ramachandran C. [Difluro(phosphono)methyl]phenylalanine-containing peptide inhibitors of protein tyrosine phosphatases. Biochem J 1999; 337 (Pt 2):219–23.
[99] Kuznetsova E, Nocek B, Brown G, Makarova KS, Flick R, Wolf YI, et al. Functional diversity of haloacid dehalogenase superfamily phosphatases from saccharomyces cerevisiae: biochemical, structural, and evolutionary insights. J Biol Chem 2015;290(30):18678–98.
[100] Wang W, Kim R, Jancarik J, Yokota H, Kim SH. Crystal structure of phosphoserine phosphatase from Methanococcus jannaschii, a hyperthermophile, at 1.8 A resolution. Structure 2001; 9(1):65–71.
[101] Allen KN, Dunaway-Mariano D. Phosphoryl group transfer: evolution of a catalytic scaffold. Trends Biochem Sci 2004;29(9):495–503.
[102] Burroughs AM, Allen KN, Dunaway-Mariano D, Aravind L. Evolutionary genomics of the HAD superfamily: understanding the structural adaptations and catalytic diversity in a superfamily of phosphoesterases and allied enzymes. J Mol Biol 2006;361(5):1003–34.
[103] Jung SK, Jeong DG, Chung SJ, Kim JH, Park BC, Tonks NK, et al. Crystal structure of ED-Eya2: insight into dual roles as a protein tyrosine phosphatase and a transcription factor. FASEB J 2010;24(2):560–9.
[104] Aravind L, Galperin MY, Koonin EV. The catalytic domain of the P-type ATPase has the haloacid dehalogenase fold. Trends Biochem Sci 1998;23(4):127–9.
[105] Ridder IS, Dijkstra BW. Identification of the Mg2+-binding site in the P-type ATPase and phosphatase members of the HAD (haloacid dehalogenase) superfamily by structural similarity to the response regulator protein CheY. Biochem J 1999;339 (Pt 2):223–6.
[106] Collet JF, Stroobant V, Van Schaftingen E. Mechanistic studies of phosphoserine phosphatase, an enzyme related to P-type ATPases. J Biol Chem 1999;274(48):33985–90.
[107] Allen KN, Dunaway-Mariano D. Markers of fitness in a successful enzyme superfamily. Curr Opin Struct Biol 2009;19(6):658–65.

4 The receptor protein tyrosine phosphatases: Structure and function

4.1 Classification of protein tyrosine phosphatases

The class I classical protein tyrosine phosphatase (PTP) family has been thoroughly analyzed for the sequence evolution of their catalytic domains using phylogenetic tree analysis [1]. The various branches of the phylogenetic tree have allowed for the classification of PTPs into 17 distinct groups (Figure 4.1) [1, 2]. Essentially there are two main types of PTPs: the membrane-bound receptor PTPs and the cytosolic non-receptor PTPs. The sequence ontology-based approach allows for their further classification into eight types of receptor PTPs (Figure 4.2) and nine types of non-receptor PTPs. A previous classification of the receptor PTPs grouped them into nine types based on their extracellular domains [3]. However, based on their functional PTP catalytic domains, these receptor PTPs were reclassified using sequence-based ontology. This has allowed for the reclassification of various proteins like the chicken PTPλ, which was earlier a stand-alone member of the R6 subtype, but its sequence closeness with CD45 has allowed for the formation of a composite R1/R6 category [1, 2]. Interestingly, some receptor PTP types also contain non-receptor PTPs due to the high sequence identity between their catalytic PTP domains. For example, the R7 subtype includes the cytosolic striatal-enriched PTP (STEP) and HePTP. Similarly, the R3 subtype includes both the transmembrane and cytosolic isoform of GLEPP1 (mouse PTPϕ) [4] and the R4 subtype includes both the transmembrane and cytosolic isoform of PTPε [5]. Also, under this classification, the receptor PTPs that contained two tandem PTP catalytic domains clustered separately from the ones having only one catalytic PTP domain. This has provided for a PTP "supertype" that includes the classes R1/R6, R2A, R2B, R4 and R5. Interestingly, the D1 domains of this supertype share a much higher sequence identity (60–80%) when compared to the sequence identity between the PTP domains of the R3 type (45–60%). Also, the D2 domains of the supertype cluster separately into a distinct branch of the phylogenetic tree [2]. This sequence analysis hence confirms prior speculations [6] that intragenic duplication leading to formation of bi-domain proteins has preceded gene duplication that gave rise to various subtypes of receptor PTPs. A thorough tabulation of the 22 human receptor PTPs along with their mouse, rat or chicken homologues is provided in Tables 4.1 and 4.2 (adapted from [1]). Table 4.1 also includes their synonymous names used in literature.

4.2 The R1/R6 subtype

The only human gene that is a part of the R1/R6 subtype of receptor PTPs is *PTPrc* that encodes for CD45 (cluster of differentiation 45). CD45 was the first receptor PTP to

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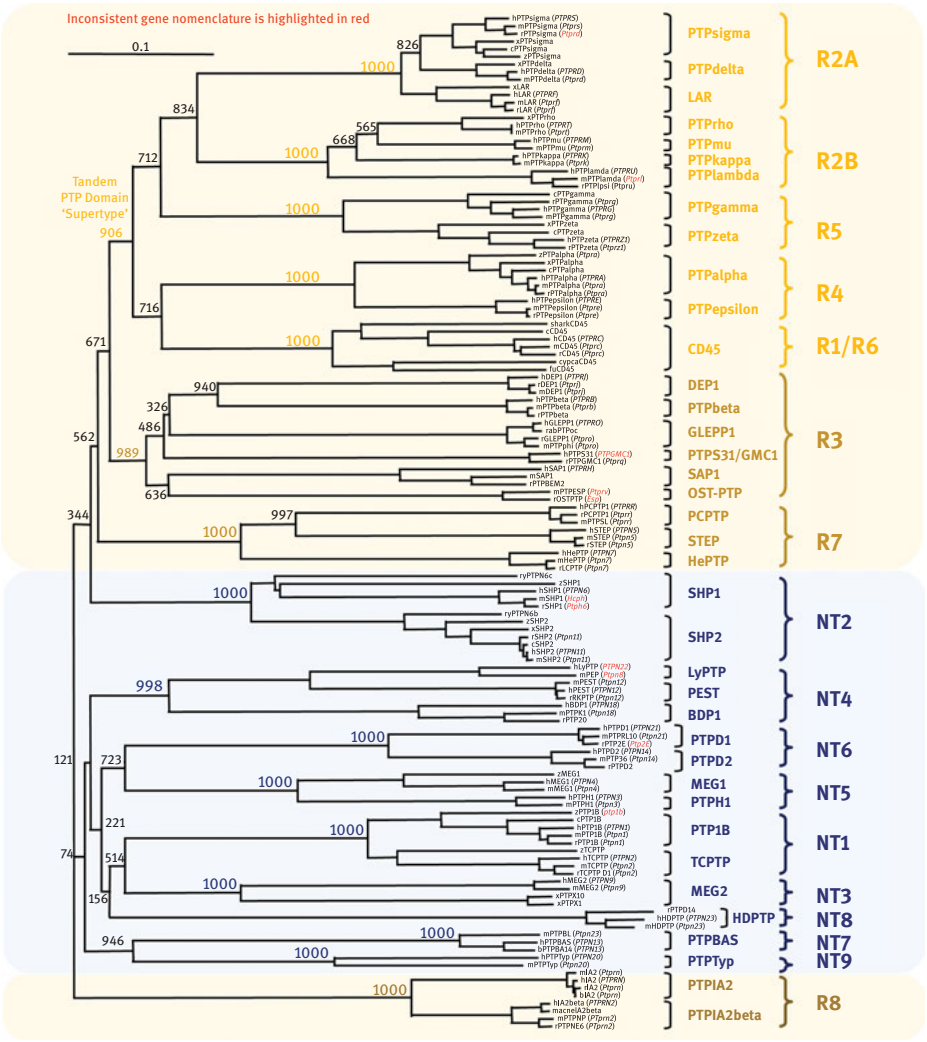


Figure 4.1: Evolutionary classification of the catalytic domains of classic protein tyrosine phosphatases. Adapted from Refs [1, 2].

be discovered in 1988 [7, 8], based on its high sequence homology with PTP1B. Understandably, it served as the prototype for understanding the receptor PTPs for sometime. CD45 was earlier known as leukocyte common antigen (LCA) as it is specifically expressed in hematopoietic cells and regulates B-cell and T-cell antigen receptor (TCR) signaling. The gene is located on chromosome 1q and is encoded by 34 exons. Three exons on the primary transcript can be alternatively spliced to generate the RA, RB and RC isoforms [9]. Using all splicing combinations, about eight different CD45 isoforms are generated: CD45RA, CD45RB, CD45RC, CD45RAB, CD45RAC,

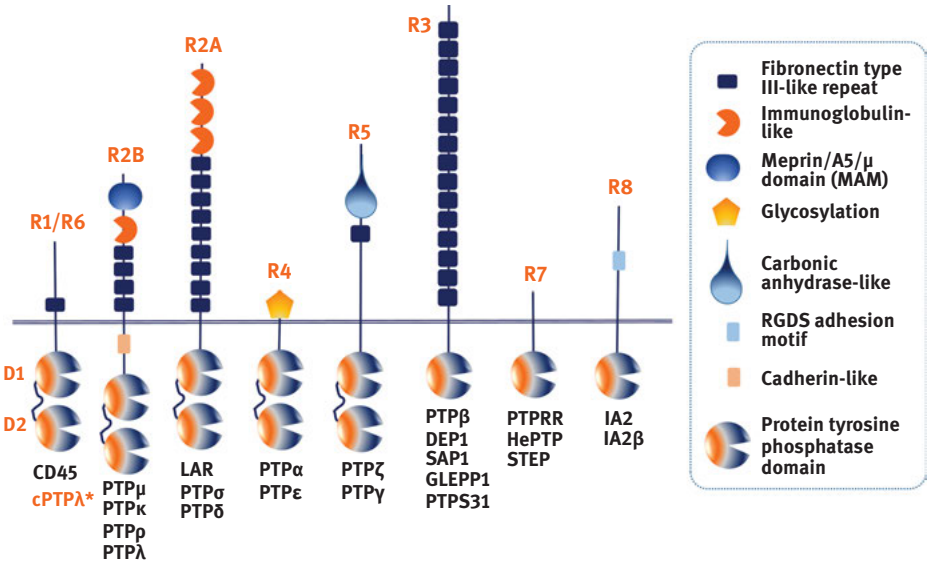


Figure 4.2: The receptor protein tyrosine phosphatases and their various subtypes.

Table 4.1: Tabulation of the 22 human receptor PTPs along with their mouse, rat or chicken homologues.

Subtype	Name	Full name	Other names in literature
R1/R6	hCD45	Cluster of differentiation 45	Leukocyte common antigen (LCA), T200, PTPRC
	mCD45	Cluster of differentiation 45	LCA, T200, Ly5
	rCD45	Cluster of differentiation 45	Leukocyte common antigen
	cPTPlambda	PTPlambda	
R2B	hPTPlambda	RPTPlambda	PCP2, PTPomicron, PTPfmi,PTPpi, PTPi, PTPRO
	mPTPlambda	RPTPlambda	PTPftp1, PTPpsi
	rPTPpsi	RPTPpsi	
	hPTPkappa	RPTPkappa	
	mPTPkappa	RPTPkappa	
	hPTPmu	RPTPmu	
	mPTPmu	RPTPmu	
	hPTPrho	RPTPrho	
	mPTPrho	RPTPrho	
R2A	hLAR	LCA-related PTP	PTP-LAR
	mLAR	LCA-related PTP	PTP-LAR
	rLAR	LCA-related PTP	PTP-LAR
	hPTPdelta	RPTPdelta	

(*continued*)

Table 4.1: (*continued*)

Subtype	Name	Full name	Other names in literature
	mPTPdelta	RPTPdelta	
	cLAR	LCA-related PTP	CRYPalpha
	hPTPsigma	RPTPsigma	
	rPTPsigma	RPTPsigma	LAR-PTP2, PTP-PS, PTP-P1
	mPTPNU3		PTPsigma, PTPT9a, PTPT9b
R3	hPTPS31		
	rPTPGMC	Glomerular mesangial cell receptor	PTPRQ, PTPGMC1
	hGLEPP1	Glomerular epithelial protein 1	PTPU2, PTProt
	mPTPphi		PTP-BK, PTP-ro, mGLEPP1
	rPTPBEM1	Brain-enriched membrane-associated PTP1	PTPD30, BSM-1
	rabPTPoc	Osteoclastic PTP	
	cPTPcryp2		CRYP-2
	hPTPbeta	RPTP	
	mPTPbeta		Vascular endothelial PTP(VE-PTP)
	hDEP1	Density-enhanced PTP	PTPeta, CD148, F-36-12
	mPTPBYP	RPTPbeta-like PTP	PTPeta
	rDEP1	Density enhanced PTP	Vascular PTP-1
	hSAP1	Stomach cancer-associated PTP	hPTPH
	rPTPBEM2	Brain-enriched membrane-associated PTP2	
	mPTPesp	Embryonic sterm cell PTP	OST-PTP
	rOSTPTP	Osteotesticular PTP	
R4	hPTPalpha	RPTPalpha	
	mPTPalpha	RPTPalpha	LCA-related PTP
	rPTPalpha	RPTPalpha	
	cPTPalpha	RPTPalpha	
	hPTPepsilon	RPTPepsilon	
	mPTPepsilon	RPTPepsilon	
	rPTPepsilon	RPTPepsilon	
R5	hPTPgamma	RPTPgamma	
	mPTPgamma	RPTPgamma	
	cPTPgamma	RPTPgamma	
	cPTPzeta	RPTPzeta	
	hPTPzeta	RPTPzeta	
	rPTPzeta	RPTPzeta	
R7	hPCPTP1	PC12-derived PTP	PTPch1g, PTPCOM1,hCh1PTP, PTPEC
	rPCPTP1	PC12-derived PTP	PC12-PTP1, CBPTP

Table 4.1: (*continued*)

Subtype	Name	Full name	Other names in literature
	mPTPSL		PTPBR7, PTP-SL, PC12-PTP1
	hSTEP	Striatum-enriched phosphatase	
	mSTEP61	Striatum-enriched phosphatase	
	rSTEP	Striatum-enriched phosphatase	
	hHePTP	Hematopoietic PTP	Leukocyte PTP
	rLCPTP	Leukocyte PTP	Hematopoietic PTP
R8	hPTPIA2	Islet cell antigen	Islet cell antigen, ICA- 512
	mPTPIA2	Islet cell antigen	PTP35
	rPTPIA2	Islet cell antigen	BEM-3, PTPN, ICA105,PTPLP
	bPTPIA2	Islet cell antigen	ICA512
	hPTPIA2beta	PTP-IA-2beta	IAR, RPTPX
	mPTPNP	Nervous system andpancreatic PTP	IA2beta, RPTPX,PTPNP-2
	macPTPIA2beta		IA2beta
	rPTPNE6		IA2beta, phogrin

Table 4.2: Physiological substrates and disease associations of various receptor PTPs.

Protein *(Gene)*	Chromosome location	Subtype	Major substrate	Related diseases
CD45*(Ptprc)*	1q	R1/R6	Janus kinase, Src-family kinases	Autoimmune disorders
PTPκ*(Ptprk)*	1p	R2A	EGFR, β-catenin	Cancer
LAR*(Ptprf)*	6q	R2B	β-Catenin, p130, Src-family of kinases, EGFR, RET	Cancer
DEP-1*(Ptprj)*	11p	R3	PDGFR, HGFR, Src-family of kinases, VEGFR2, Erk	Cancer
PTPα*(Ptpra)*	20p	R4	Src-family kinases	Cancer, gastric ulcers
PTPζ*(Ptprz)*	7q	R5	β-Catenin, Git1, Magi1, RhoGAP, β-adducin, ALK, TrkA, Src-family kinases	Parkinson's disease, Gastric ulcers, demyelinating disease
PTPRR*(Ptprr)*	12p	R7	Erk	Cancer, psychological disorders, endometriosis
IA2*(Ptprn)*	2q	R8	N/A (inactive phosphatase)	Diabetes

EGFR, epidermal growth factor receptor; HGFR, hepatocyte growth factor receptor; DEP-1, density-enhanced phosphatase; PDGFR, platelet-derived growth factor receptor; VEGFR2, vascular endothelial growth factor receptor 2.

CD45RBC, CD45RO and CD45R (ABC) [10]. CD45RO lacks the RA, RB and RC exons, and is the smallest isoform. CD45RO is expressed by memory or activated T cells, while the naïve T cells express CD45RA.

The largest isoform is CD45R that migrates on the gel at a molecular weight of 200 kDa. B cells express a heavily glycosylated CD45R that runs slower on a gel with a molecular weight of 220 kDa. When isolated in the glycosylated form, CD45R is also called B220. Although there is limited evidence for the functional variation in the CD45 isoforms, it is clear that expression levels of these specific isoforms are critical at different stages of leukocyte development [11]. Isoforms CD45R, CD45RB and CD45RO when expressed alone are unable to sustain normal B-cell maturation but are competent to support peripheral T-cell function and development [12]. Expression of a CD45 minigene has been reported to restore B-cell and T-cell function, but the optimal expression requirements and isoform combinations for this result are still unclear [13].

Two polymorphisms in the CD45 gene have been reported to play important roles in human physiology. A high-frequency CD45 polymorphism is the A138G allele that mutates its Thr47 to an alanine. This substitution removes a glycosylation site from its N-terminal region [14, 15]. This polymorphism is advantageous in preventing Graves' thyroiditis and also provides immunity against hepatitis B. The other CD45 polymorphism is the C77G mutation in exon 4 that prevents its excision [16]. Affected populations with this polymorphism are unable to express the isoform CD45RO in their memory/effector T cells. These individuals hence have compromised immunity. The frequency of this mutation is seen to be elevated in patients with multiple sclerosis, autoimmune hepatitis, Langerhans cell histiocytosis and human immunodeficiency virus infections [17, 18].

The extracellular region of CD45 consists of distinct structural regions. The N-terminal region has an extended conformation and contains multiple sites for O-linked glycosylations. This region provides the CD45 isoforms of all their variation. Of course, the smallest isoform CD45RO is least glycosylated and the largest isoform CD45R is the most glycosylated. The variable glycosylation of CD45 isoforms allows for specification in shape, and charge influences its ligand-binding properties and intracellular signaling [19]. The variable N-terminal region is followed by a cysteine-rich domain rich in β-sheets but lacking any α-helices. Mammalian CD45 contains five conserved cysteines in this region that engage in two intra-domain disulfide bonds and a fifth disulfide bond with a cysteine from the adjacent fibronectin type III repeat [20]. The extracellular region of CD45 also has two to three fibronectin type III domains that contain sites for N-linked glycosylations. These N-linked glycosylations are crucial for the proper biological function of CD45 including its proper transport and presentation on the cell surface [21]. These glycosylations also serve as ligand-binding sites for extracellular ligands such as galectins, CD22 and macrophage mannose receptors [22, 23]. Galectins are β-galactoside-binding proteins that contain carbohydrate recognition sites [24]. Galectin-1 and galectin-3 have been reported to be extracellular binding partners for CD45 and also PTPκ. Galectin-1 is the ligand for CD45 expressed on T cells

[25, 26]. Galectin-1 interaction with CD45 is crucial for regulation of T-cell death at various stages of their maturation and development [27]. Galectin-1 binding induces clustering of CD45 leading to the inhibition of its intracellular PTP activity [28]. Several binding sites on CD45 assist in its interaction with galectin-1, all which are dependent on the glycosylation state of CD45 [22]. Hence, isoform diversity, glycosylation levels and galectin-1 binding, all contribute to various stages of T-cell development.

A single transmembrane helix connects the extracellular region to the cytosolic region that has a juxtamembrane region with a wedge-like structure that is followed by two tandem PTP domains and short ≅80 amino acid C-tail (Figure 4.3) [29]. Of the two tandem PTP domains, the membrane proximal domain is known as the D1 domain and the membrane distal domain is termed as the D2 domain (Figure 4.2). Almost all of CD45 protein's phosphatase activity is limited to its D1 domain [30]. The D2 domain serves as a cognate activator of the D1 domain [31] and is reported to be catalytically active in certain deletion constructs of the D1–D2 bidomain protein [32]. The D2 domain of CD45 has critical mutations in its WPD-loop and P-loop, although it does possess the conserved active site cysteine [33]. Major mutations that contribute to inactivity of the D2 domain are the conserved acid/base aspartate (WPD loop) mutation to valine (Val1102), and the conserved HCS-motif serine (P-loop) mutation to arginine (Arg1145). The electrostatics of the active site is disturbed by the presence of aspartate (Asp1146). Also, a glutamine residue (Q1149) projects into the active site, while the conserved tyrosine/phenylalanine of the phosphotyrosine recognition loop is mutated to asparagine (Asn949).

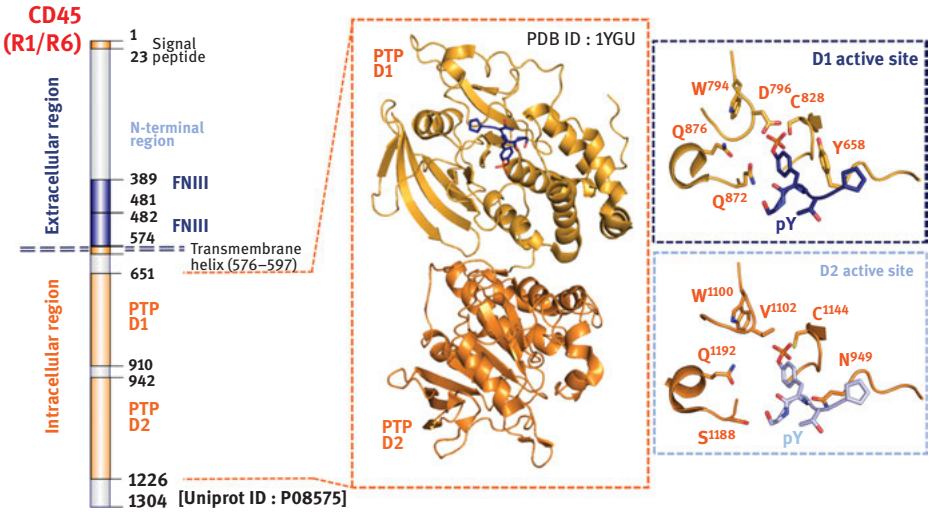


Figure 4.3: The R1/R6 subtype of receptor protein tyrosine phosphatases and the crystal structure of CD45. Active site arrangement of the membrane proximal domain (D1) and membrane distal domain (D2) show their differences.

CD45 is an important player in the immune system development. CD45 is required for antigen-specific lymphocyte stimulation, maintaining lymphocyte survival and modulating cytokine response. CD45 negatively regulates cytokine receptor activation by dephosphorylating Janus kinases [34]. In B cells and T cells, CD45 regulates the phosphorylation levels of Src family of protein tyrosine kinases including Fyn and Lck [35]. CD45 knockout mouse have been used to understand thymocyte development dependent on Fyn and Lck signaling [36, 37]. Fyn and Lck protein tyrosine kinases phosphorylate the signal-transducing subunits CD3 and TCRζ of the TCR. Phosphorylation of these subunits creates docking sites for the Src homology 2 (SH2) domains of ZAP-70 that is also phosphorylated by Fyn and Lck leading to its activation. Activated ZAP-70 now functions as a protein kinase to phosphorylate LAT and SLP-76 proteins that regulate T-cell development and maturation [38, 39] . In CD45-deficient thymocytes, Fyn and Lck remain inactivated; hence, the TCR also remains unphosphorylated. Owing to its critical role in immune development and regulation, CD45 deficiency or suboptimal function gives rise to various diseases [17]. The severe combined immunodeficiency phenotype results from CD45 deficiency [40, 41].

4.3 The R2A subtype

The R2A family of receptor PTPs is composed of proteins having immunoglobulin-like (C2-set) domains and fibronectin type III (FNIII) repeats in their extracellular portions. A single transmembrane helix connects the extracellular region to two tandem PTP domains in the intracellular space (Figure 4.4) [33, 42]. Members of this subtype include PTPσ, PTPδ and the Leukocyte common Antigen-Related (LAR) [43]. The extracellular domains of this subtype can undergo proteolytic cleavage to form two fragments that continue to associate noncovalently [44]. Proteolytic cleavage occurs by subtilisin-like endoprotease and is predicted to occur intracellularly. The cleavage site is a paired basic amino acid sequence, which can block proteolysis via a single point mutation. In LAR, the two cleaved fragments are called E-subunit and P-subunit, respectively [45]. The E-subunit contains all the extracellular domains required for cell adhesion. The P-domain contains the transmembrane region and the two tandem PTP domains. The E-subunit of LAR is discarded during cell growth in a process called "ectodomain shedding." This is an extremely specific and well-regulated process wherein only about 2% of cell receptors shed their ectodomains in the extracellular milieu [46]. Also, LAR undergoes tightly regulated alternate splicing to generate isoforms that have variations in their extracellular domains [47]. Another soluble form of the ectodomain region called LARFN5C is formed by the N-terminal signaling sequence and has the fifth FNIII repeat at the C-terminal. This segment makes homophilic interactions to bind the FNIII repeats of full-length LAR in neurons and this interaction is required for mediating neurite outgrowth [48, 49].

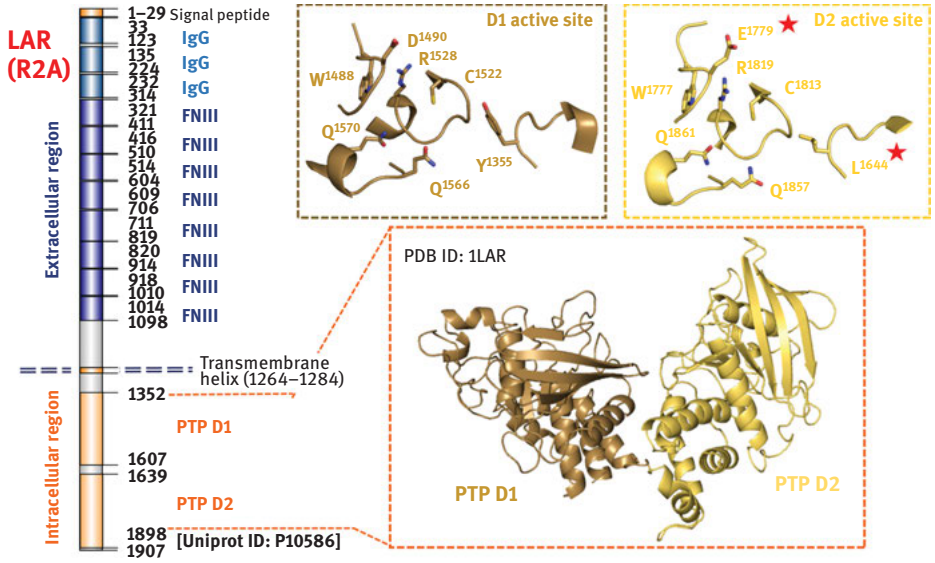


Figure 4.4: Domain organization and crystal structure of the R2A subtype LAR protein tyrosine phosphatase.

Members of the R2A subtype are the main modulators of signaling pathways for a variety of protein tyrosine kinases including those of epidermal growth factor receptor (EGFR), insulin receptor (IR), hepatocyte growth factor receptor (HGFR)/MET, Lck and Fyn [50–52]. LAR, PTPσ and PTPδ show overlapping expression in a variety of tissues [53]. LAR is reported to coimmunoprecipitate alongside IR [54], and its overexpression in mice models have suggested its role in insulin resistance and diabetes [55]. LAR knockout mice are unable to mediate normal differentiation of alveoli during pregnancy and have abnormally developed mammary glands [56]. These mice also have abnormal forebrain size and hippocampal organization [57]. Similarly, mice deficient in PTPδ also show retardation in growth, deficiency in hippocampal long-term potentiation and also abnormal behavior [58, 59]. The role of PTPδ has been assessed in glioblastoma multiforme, the most aggressive form of brain cancer [60]. PTPδ homozygous deletions have been reported in melanomas [61], and re-expression of PTPδ is seen to curb cell growth and promote their apoptosis [60]. Overexpression of LAR is associated with thyroid cancers and also breast cancer, small-cell lung cancer and colon cancer [62–65].

LAR protein is seen to be localized on focal adhesions, and its phosphatase activity is crucial for maintenance of focal adhesion turnover [66]. Focal adhesion molecules, β-catenin and p130cas, have been reported to be substrates of LAR [67]. The multifunctional signal assembly protein p130cas co-localizes on focal adhesions and stress fibers with LAR. Dephosphorylation of p130cas by LAR induces apoptosis through the caspase-dependent pathway [68]. Association between LAR

and the catherin–catenin complex is required for abscising tumor formation in nude mice [69]. *Drosophila* LAR (DLAR), along with Lirpin-α, is required for normal morphology of neuromuscular synapsis [70]. DLAR also associates with two heparin sulfate proteoglycans named syndecan (sdc) and Dallylike (Dlp) [71]. Sdc binds the Ig repeats of DLAR and decreases its phosphatase activity. This allows for promotion of neuromuscular junction formation in *Drosophila*. Suppression of synaptic morphogenesis is mediated by the binding of Dlp to DLAR. Hence, sdc and Dlp compete for DLAR to balance the biological process of synaptic development [72]. From the extracellular matrix, laminins and nidogen are reported to be ligands for LAR [73].

The two tandem domains of the R2A family are closely related in sequence, wherein the sequence similarity between the D2 domains of LAR, PTPσ and PTPδ is higher than the similarity of these D2 domains with their cognate D1 domains [74]. Also, the sequence similarity between the D1 and D2 domains of this family is more than the similarity between their D1 domains [74]. This observation alongside the clear clustering of D2 domains in a separate phylogenetic branch has provided evidence that intragenic gene duplication to yield tandem PTP domains occurred early in evolution [75]. These D2 domains have acquired only two crucial point mutations around their active site that makes them inactive phosphatases [30, 31]. The first point mutation is in the WPD-loop that has a mutated general acid/base aspartate to glutamate. An additional methylene group in the longer side chain of glutamate doesn't allow for oxyanion formation and proper functioning of the WPD loop acid/base during catalysis [76]. Also, interaction of the carboxylate group of this glutamate with the main chain of nearby residues compromises the opening–closing dynamics of the WPD loop. The second mutation occurs in the phosphotyrosine recognition loop, wherein the substrate-guiding tyrosine/phenylalanine is mutated to a smaller aliphatic residue like leucine [42]. Interestingly, reverse double mutations of these residues (L1644Y of the pY-loop and E1779D of the WPD loop) are enough to activate the D2 domain of LAR [42].

While the D2 domain of this subfamily is inactive, its presence is seen to modulate the activity of its cognate D1 domain [31, 77]. The removal of the D2 domain of human Lar results in a twofold increase in the activity of its D1 domain [30]. The active site cysteine to serine mutant of the D2 domain (C2823S in human LAR) increases the activity of the D1 domain for Raytide, Maltose Binding Protein (MBP) and IR substrates [30, 52]. Additional experiments in COS-7 cells have revealed the requirement of an intact D2 domain of LAR for proper association with the IR as a substrate. Mutation in the D2 active site P-loop (C2823S) or the double mutant of both D1 and D2 (C1522S/C2823S) were unable to immunoprecipitate the LAR–IR complex [52]. These studies explain how the D2 domain in these bi-domain receptor PTPs functions in substrate recruitment. A more convincing result was obtained in *Drosophila* embryos where the DLAR cytoplasmic PTP domains were required to rescue lethality [78]. In this system, the D1 and D2 domains of DLAR were expressed

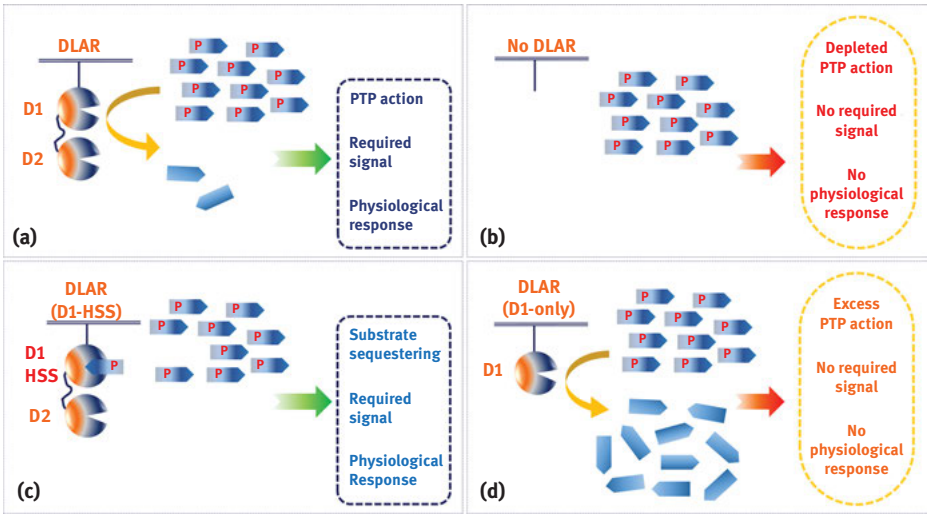


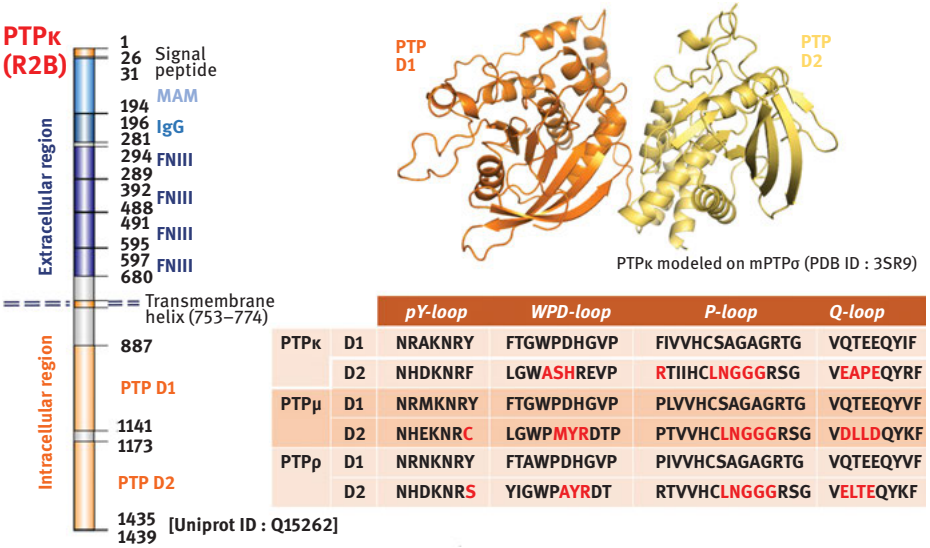
Figure 4.5: Biological experiments using lethality rescue of *Drosophila* embryos allow for examining the role of the inactive D2 domain of DLAR protein. Experiments using wild-type DLAR (a), absence of D1 DLAR (b), serine mutant of the D1 domain of DLAR (c) and D1 domain alone of DLAR (d) explain the sequestering effect of the D2 domain in contributing toward physiological function.

in various combinations of their P-loop mutants under the control of a galactose operon in a DLAR $^{-}/^{-}$ background. Embryo lethality would be rescued in cases where the D1 and/or D2 domains would be able to signal their downstream substrate proteins (Figure 4.5). Intriguingly, while the D1 domain has a higher catalytic activity than the D1D2 bi-domain protein [77], it can only rescue about 50% of the embryos. These studies explain how the D2 domain allows for the modulation of DLAR activity in "space" and "time." The D2 domain binds substrates to either sequester them away from D1, or only make the appropriate substrate available to its D1 domain (modulation in space). The D2 domain also slows down the catalytic turnover rates of the D1 domain so that only a well-quantitated required signal is allowed to pass through (modulation in time). Thus while the active site serine mutant of the D1 domain (D1 HSS/D2 construct) is able to rescue embryo lethality as it has a functional D2 domain, the lack of D2 domain completely results in embryo death (Figure 4.5) [78]. Biochemistry experiments with the recombinant forms of the DLAR D1 and D2 domains have confirmed the modulatory properties of the D2 domain [77]. These studies demonstrate how the active site of the D2 domain is equally accessible, yet independent from its D1 domain. The D1 and D2 domains individually expressed recombinant constructs bind the small molecule *p*-Nitrocatechol Sulfate with comparable affinities (K_D D1 = 234.8 μM; K_D D2 = 214.4 μM) [77]. The D1D2 bidomain recombinant protein shows two site binding of the small molecule with comparable binding affinities for the two sites (K_{D1} = 159.6 μM; K_{D2} = 172.7 μM). Surface plasmon

resonance studies on tyrosine phosphorylated peptides showed efficient binding of substrate peptide by the D2 domain, with a distinct preference for flanking regions of the phosphotyrosine [77].

4.4 The R2B subtype

Members of the R2B subtype are recognized by the unique meprin/A5(neuropilin)/μ (MAM) domain in their extracellular regions. Subtype includes four members: PTPκ, PTPμ, PTPρ and PTPλ. They also have Ig and FNIII repeats in their extracellular regions. Their intracellular regions contain a juxtamembrane region with very high sequence similarity to classical cadherins. Just like their related R2A subtype members, these receptor PTPs also have two tandem PTP catalytic domains in their cytosolic portions. The overall sequence identity between the members is about 55% but the catalytic domains are about 68% identical in member sequences [79]. All of the catalytic activity is localized to the D1 domains where the active site cysteine is apt at acting as a nucleophile and has a low p*K*a of about 5.5. The crystal structure of the D1 domain of PTPκ has been characterized, but the structure of the D1D2 bidomain protein remains unavailable [80]. The D2 domains lack activity and show heterogeneous mutations around the active site (Figure 4.6). These domains hence cannot be reverse activated by simple point mutations [81]. Mutations are seen



		pY-loop	WPD-loop	P-loop	Q-loop
PTPκ	D1	NRAKNRY	FTGWPDHGVP	FIVVHCSAGAGRTG	VQTEEQYIF
	D2	NHDKNRF	LGWASHREVP	RTIIHCLNGGGRSG	VEAPEQYRF
PTPμ	D1	NRMKNRY	FTGWPDHGVP	PLVVHCSAGAGRTG	VQTEEQYVF
	D2	NHEKNRC	LGWPMYRDTP	PTVVHCLNGGGRSG	VDLLDQYKF
PTPρ	D1	NRNKNRY	FTAWPDHGVP	PIVVHCSAGAGRTG	VQTEEQYVF
	D2	NHDKNRS	YIGWPAYRDT	RTVVHCLNGGGRSG	VELTEQYKF

Figure 4.6: The R2B subtype of receptor protein tyrosine phosphatases. Mutations in key motifs of their D2 domains explain their inactivity.

to disrupt especially the P-loop and the Q-loops of the catalytic domain, which allow for complete breakdown of the D2 domain's catalytic machinery.

Members of the R2B subtype have been reported to sustain homophilic interactions through their ectodomains that mediate cell–cell aggregation [79, 82–84]. Homophilic interaction-mediated cell aggregation is reported for PTPμ, PTPρ and PTPκ [85–87]. PTPλ seems to be the exception in this case [79]. Initially, the Ig domain of PTPμ was reported to be the mediator of these homophilic interactions [88, 89], but now the MAM domain and the first three FNIII repeats are also known to participate in the interaction [79]. Crystal structures of the N-terminal region of PTPμ showed that the MAM and Ig domains form a seamless interface. These are collectively called the MIg domain [90]. The structure of the full ectodomain was later analyzed by the same research group [91] that showed two PTPμ molecules to make a homophilic antiparallel dimer in an extended conformation (Figure 4.7). At the dimer interface, the MAM and Ig domains of one monomer clamp onto the FN1 and FN2 FNIII repeats of the other monomer. Formation of aggregates as a measure of ectodomain adhesion was tested in Sf9 cells that were transfected by baculoviral expression constructs of PTPμ. Immuno-electron microscopy studies were used to visualize the intermembrane spacing in cells expressing different lengths of PTPμ, keeping the MAM-Ig-FN1-FN2 unit untouched (Figure 4.7). The intermembrane distance in cells expressing full-length PTPμ is about 23.7 nm, which is a signature of cadherin-mediated cell junctions [92]. Upon deletion of the ectodomain C-terminal region that includes the linker of the FN4 domain, the intermembrane spacing shrinks to about 17.2 nm. Further deletion of the FN4 domain decreases the

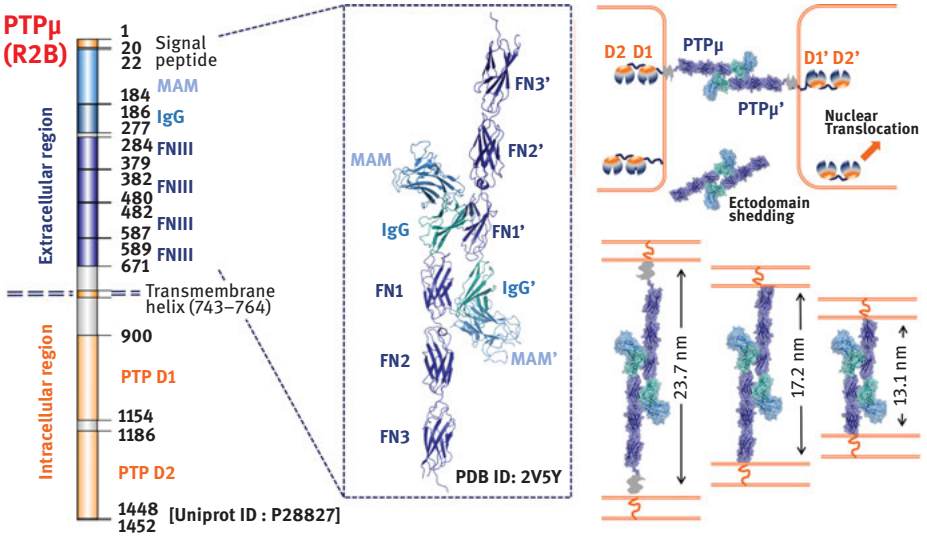


Figure 4.7: Homophilic interactions mediated by the MAM and Ig containing ectodomains of PTPμ.

intermembrane spacing by another 4 nm to become 13.1 nm [91]. This ectodomain-regulated localization of PTPμ is seen to play an important role in its function. Ectodomain size and rigidity are key to modulate intercellular spacing and also intracellular assembly of multimolecular substrates. Several components of the cadherin–catenin complexes are reported to the substrates of R2B receptor PTPs [93, 94]. Also, a highly similar mode of molecular interactions allows these receptor PTPs to directly associate with cadherins and modulate their adhesive properties [95]. Surface presentation (but not gene expression) of PTPμ is seen to increase by almost threefold as cells reach confluence upon growing. Almost all the PTPμ is localized to the cell–cell junctions [96, 97]. Deletion mutants of PTPμ lacking the Ig domain show a diffused expression pattern in confluent cells indicating that homophilic binding is key to their localization at the membrane [88]. The present model of adhesion-regulated PTPμ signaling focuses on the *trans*-interaction of its ectodomains that matches cadherin-mediated cell contacts. This allows the ectodomains of PTPμ of two interacting cells to lock each other at cell junctions so that phosphatase activity of PTPμ is also localized to the cell contacts.

The R2B-type receptor PTPs use their extracellular regions as a "spacer clamp" to hold their positions on cell–cell contacts. The polar nature of the homophilic ectodomain interface ensures that homodimers are not formed in the secretory vesicles that have an acidic pH [98]. The receptor PTPs keep re-circulating from the membrane, until another molecule contacts it from the neighboring cell. The slightly basic pH of the extracellular space (pH 7.4) allows for a strong dimer formation with high buried molecular surface of about 1,630 $Å^2$ per molecule of the PTP ectodomain [91]. Modularity in cell–cell contacts is achieved by the proteolytic processing of these R2B subtype members. Protein convertases can cleave the FN4 domain from the extracellular regions while the receptor PTP re-circulates through the trans-Golgi network of the cell [99]. ADAM 10 protease cleaves the ectodomains at the C-terminal region close to the transmembrane helix. This contributes to "ectodomain shedding" and allows for the circulation of homodimeric ectodomains in the extracellular milieu [100]. This may be followed by cleavage of the tandem PTP domains from the transmembrane helix by a γ-secretase-mediated intramembrane proteolytic cleavage. In the case of PTPκ, this process allows for the release of its phosphatase intracellular catalytic (PIC) fragment from the membrane and its subsequent translocation to the nucleus. In the nucleus, the PIC of PTPκ dephosphorylates β-catenin and enhances its transcriptional activity [100].

PTPκ uses both β-catenin and γ-catenin as its substrates [94] and also regulates EGFR signaling by dephosphorylating its C-terminal phosphotyrosine residues [101]. As cells reach confluence, the expression of PTPκ is enhanced in order to negatively regulate EGFR activity [102]. Transforming growth factor-β induces the expression of PTPκ; hence serving as an inhibitor of EGFR activity. As EGFR is a mitogen, its negative regulator PTPκ functions as a tumor suppressor. Genetic data of cancer patients shows that 18 of 29 primary nervous system lymphomas map to a 140 kb

deletion in the PTPκ gene [103]. Also, PTPκ lies on chromosome 6 on a region reported to be frequently associated with deletions that cause ovary carcinomas, melanomas and hematological neoplasia. All these cancers are associated with increase in EGFR activity and an abnegated PTPκ function. In fact PTPκ is reduced and almost undetectable in almost 20% of melanoma biopsies [104].

4.5 The R3 subtype

The R3 subtype includes the members, density-enhanced phosphatase (DEP-1) (also called as CD148 and PTPη), SAP1, GLEPP1 and PTPS31. The well-studied invertebrate R3 subtype members include PTP10D, PTP52F and PTP4E from *Drosophila melanogaster* and DEP-1/F44G4.8 from *Caenorhabditis elegans* [105]. All these members are active PTPs, except PTPS31 that dephosphorylates phosphatidylinositol moieties [106, 107]. The R3 members have varying numbers of FNIII repeats in their extracellular regions, a single transmembrane helix and an active single PTP catalytic domain. The FNIII repeats of the R2 members allow for the control of their biological function by regulation of their cellular localization [108]. Genetic studies elucidate that R3 subtype members are crucial for tubular organ development in both vertebrates and invertebrates. They also serve important functions in nervous system development and serve as tumor suppressors in various cancers [53]. Disruption of DEP-1 gene in transgenic mice leads to embryonic lethality and mutations in the gene are associated with vasculature deformities and growth retardation [109]. The phosphotyrosine phosphatase activity of the R3 members is key to their role in endothelial vessel formation and their promise as therapeutic targets for angiogenesis-related diseases [110].

DEP-1 was the first receptor PTP to be implicated as a tumor suppressor when its role as a negative regulator of platelet-derived growth factor receptor (PDGFR) was studied in detail [111]. Inhibition of PDGFR signaling was reported to depend on the phosphatase activity of DEP-1. DEP-1 catalytic domain active site cysteine mutant (C1239S) enhanced cell migration and was a dominant negative over the wild-type endogenous DEP-1 gene. In contrast, the C1239S mutant delayed cell–substrate interaction that was promoted by the wild-type DEP-1, hence showing a positive role of DEP-1 in adhesion-stimulated downstream Src kinase signaling. Similarly, the antiproliferative effects of DEP-1 have been reported in various systems where DEP-1 negatively regulates the activity of various tyrosine kinases, including PDGFR, VEGFR2 (vascular endothelial growth factor receptor 2), HGFR, RET and Erk [112–116]. DEP-1 is reported to control various stages of EGFR signaling including its endocytosis and recycling at the membrane [117]. DEP-1 also regulates the internalization of VEGFR complex and negatively controls VEGFR signaling to block cell proliferation upon reaching confluence [113, 118]. In various forms of cancers, loss of heterozygosity has been mapped to the DEP-1 gene locus [119]. Various polymorphisms have been mapped to the DEP-1 gene

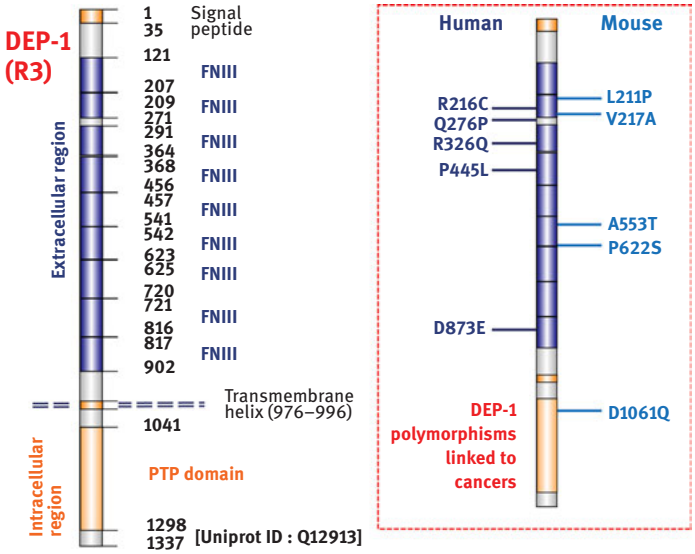


Figure 4.8: Domain organization of the R3 subtype of receptor protein tyrosine phosphatase DEP-1. Polymorphism linked to cancers map to various regions on the protein.

and are associated with various forms of cancer in mouse models and also human breast, thyroid, colon and lung cancers (Figure 4.8) [110, 120, 121].

4.6 The R4 subtype

The R4 subtype members are unique as they contain no cell adhesion molecules (CAM)-like domains in the extracellular regions. Their short extracellular region is a highly glycosylated segment that is connected to two tandem PTP domains via a transmembrane helix [122]. Two receptor PTPs of the human phosphatome, PTPα and PTPε, belong to this subtype. No extracellular ligands have been reported for PTPα and PTPε; however, PTPα is reported to interact with cell surface receptors of contactin (CNTN) and N-CAM [123]. PTPα is reported to cocluster with N-CAM in lipid rafts in antibody-mediated clustering assays [124]. This coclustering is thought to promote localization-dependent access of PTPα to Fyn and other Src family kinases that are PTPα substrates.

The two tandem PTP domains of this subtype share a high sequence similarity and also molecular structure [125]. Interestingly, unlike the other bi-domain containing receptor PTPs, PTPα is reported to exhibit phosphatase activity in both its tandem PTP domains [126, 127]. This is despite Y536V and D671E mutations that occur in its phosphotyrosine recognition loop and the WPD-loop. In this way the R4 subtype tandem PTP domains resemble those of the R2A family members [31]. The D2 domain

of PTPα shows a distinct substrate preference form its cognate D1 domain and is also reported to bind the SH2 domain of Src kinase [128]. Reverse mutations in the D2 domain in its phosphotyrosine recognition loop and WPD-loop (V536Y/E671D double mutant) allow for a 90-fold increase in catalytic efficiency [129]. In contrast, introducing these mutations in the D1 domain (Y243V/D382V double mutant) decreases the D1 domain's catalytic efficiency by 150,000-fold [130].

PTPα is reported to exist predominantly as a dimer when expressed exogenously which also reduces its catalytic activity [131, 132]. Molecular analysis of the crystal structure of the PTPα D1 domain showed that an N-terminal wedge of the PTP domain made of a helix-turn-helix motif that allowed for its dimerization [133]. In the symmetrical PTPα D1–D1 dimer, the wedge from one monomer swaps over to bind the active site of the other monomer and occludes any substrate access (Figure 4.9). This study allowed for the scientific community to suggest a dimerization-based inhibition of receptor PTPs. Supporting evidence came from the studies on R2A subtype member LAR, where the peptides containing the wedge region of LAR were successful in inhibiting LAR activity by homophilic binding to the LAR catalytic domains [134]. Similarly, PTPμ wedge peptides showed inhibitory effects on the interaction of PTPμ with its substrates. However, as the structure of PTPμ bidomain protein was published, this model of inhibition became less relevant. Subsequent analysis of the bi-domain structures of PTPμ, LAR and CD45 indicated that the placement of the D2

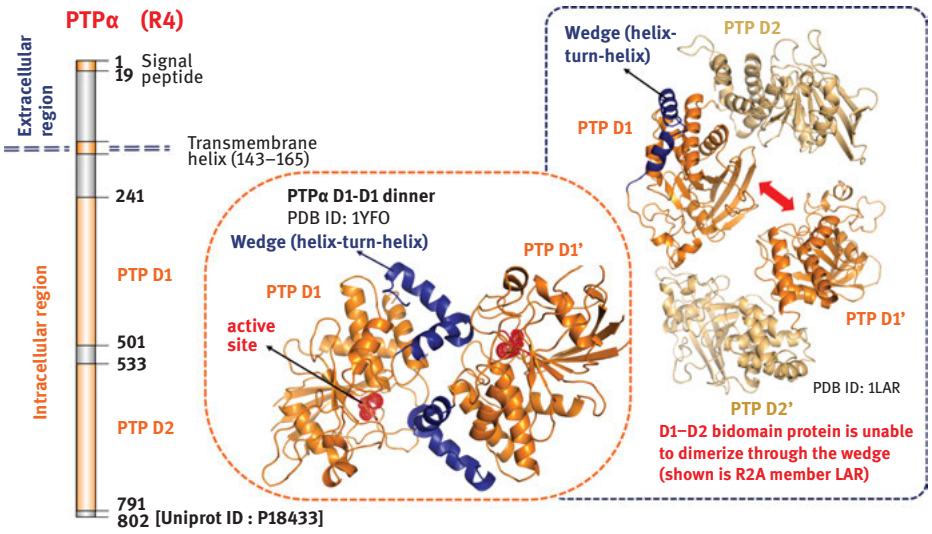


Figure 4.9: Domain organization and crystal structure of the R4 subtype PTPα. Crystal structure of the D1 domain of PTPα shows the N-terminal α-helices to form an inhibitory wedge that occludes the active site of its cognate partner. This wedge-mediated dimerization is contra-indicated in the D1–D2 structures of double-domain receptor protein tyrosine phosphatases.

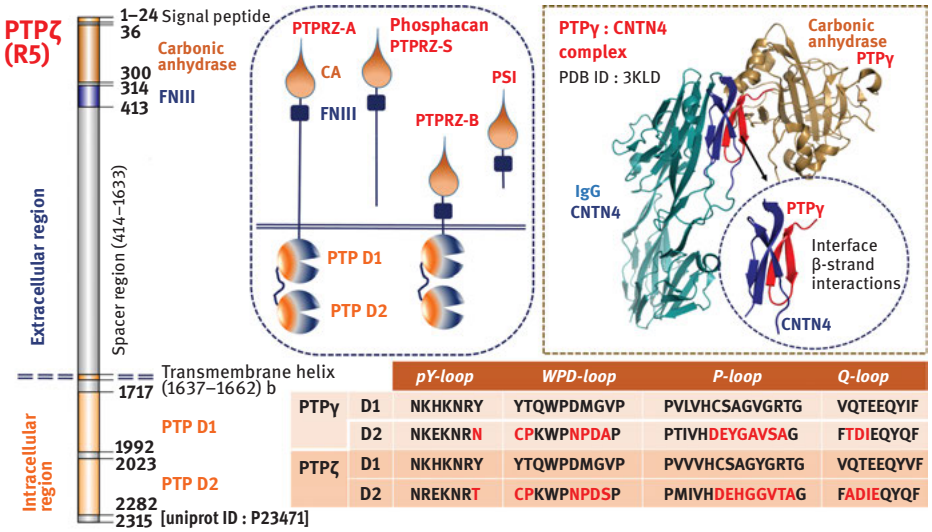
domain does not allow for wedge region-based dimerization (Figure 4.9) [33, 42]. Consequently, a head-to-tail dimer was seen and analyzed for the bi-domain PTPγ [135]. The exact physiological relevance of this dimerization is a subject for further studies.

The catalytic activity of PTPα is regulated by its phosphorylation at serine and tyrosine residues by protein kinases. The helix-turn-helix wedge domain of PTPα is phosphorylated at Ser180 and Ser240 by protein kinase C [136]. This is speculated to alter PTPα activity by interfering with its dimerization as described earlier. PTPα is also reported to be constitutively phosphorylated at its C-terminal Tyr789 [137]. This allows PTPα to interact with Grb-2 in a phosphorylation-dependent manner [138]. Phosphorylation of Tyr789 is crucial for the interaction of PTPα and its substrate Src [139]. pTyr789 is speculated to bind the Src SH2 domain and allow for the release of the locked Src conformation that would release the Src C-terminal pY527 to be dephosphorylated by PTPα. An interplay between Grb-2 binding and Src SH2 domain binding allows for the maneuvering of Src kinase signaling pathways. Consistent with this model, Y789F mutation of PTPα abrogates PTPα–Src interaction [139]. As the C-terminal inhibitory pY527 of Src is a substrate for PTPα, PTPα functions to release Src inhibition to allow for its activation. Understandably, the PTPα knockout mice show reduced Src and Fyn kinase activity [140], while overexpressed PTPα allows for enhanced Src activity in cells [141]. Activation of Fyn by PTPα is crucial for α_v/β_3-integrin-based cell matrix connection formation [142]. PTPα is reported to colocalize with α_v/β_3-integrins and also immunoprecipitate with α_v-integrins during cell spreading on a fibronectin matrix.

PTPα is reported to be a potential suppressor of breast cancer wherein high levels of PTPα correlated with low tumor grades. Also, expression of PTPα in carcinoma cells leads to delayed tumor growth [143]. Mutations in PTPα at F245 (extracellular region), G445 (P-loop of D1 domain) and L652 (in the D2 domain) are linked to neoplasia and reported in about 30% of breast, liver and colon cancers [144]. PTPα is also linked to gastric ulcer pathogenesis caused by *Helicobacter pylori*. PTPα is reported to associate with *Helicobacter* secreted vacuolating cytotoxin VacA allowing for its association with epithelial cells [145]. Entry of the cytotoxin causes progressive vacuolation, mitochondrial damage and subsequent apoptosis of gastric epithelial cells.

4.7 The R5 subtype

The R5 subtype of receptor PTPs includes PTPζ (also known as PTPβ in literature) and PTPγ. These proteins are characterized by a carbonic anhydrase domain and a single FNIII domain in their extracellular regions [146]. The carbonic anhydrase domain lacks its active site histidine residue and is an inactive anhydrase that cannot catalyze the hydration of carbon dioxide. However, this atypical carbonic



		pY-loop	WPD-loop	P-loop	Q-loop
PTPγ	D1	NKHKNRY	YTQWPDMGVP	PVLVHCSAGVGRTG	VQTEEQYIF
	D2	NKEKNRN	CPKWPNPDAP	PTIVHDEYGAVSAG	FTDIEQYQF
PTPζ	D1	NKHKNRY	YTQWPDMGVP	PVVVHCSAGYGRTG	VQTEEQYVF
	D2	NREKNRT	CPKWPNPDSP	PMIVHDEHGGVTAG	FADIEQYQF

Figure 4.10: The R5 subtype of receptor protein tyrosine phosphatases. Mutations of the *P*-loop, *WPD* loop and *Q*-loop explain the lack of activity in their D2 domains. PTPζ uses its extracellular carbonic anhydrase domain to bind contactins. A secretory version of the protein contains only the extracellular regions.

anhydrase domain can use its non-catalytic active site as a hydrophobic pocket for binding extracellular ligands [147]. The intracellular region of the R5 members includes two tandem PTP catalytic domains. Similar to the other bi-domain receptor PTPs, all the catalytic PTP activity is limited to the D1 domain. The D2 domain has several mutations in the P-loop, WPD-loop and Q-loop, wherein the active site cysteine, the water activating glutamine and the conserved acid/base aspartate are all modified (Figure 4.10) [31].

The R5 subtype is especially characterized for its role in axon guidance and neurite outgrowth in the vertebrate nervous system [53]. PTPζ and PTPγ are both expressed in the developing and also the adult vertebrate brain. PTPζ is predominantly found oligodendrocytes and astrocytes whereas PTPγ is expressed in the neurons [148]. Both PTPζ and PTPγ bind cell adhesion molecules (CAMs) called contactins (CNTNs) that are expressed in various stages of neural development and in the adult vertebrate brain. Binding assays report that PTPγ binds CNTN3, CNTN4, CNTN5 and CNTN6, whereas PTPζ is more specific for CNTN1 [148]. These CNTNs are extracellular proteins that contain six immunoglobulin-like domains, four FNIII domains and a C-terminal glycophosphatidylinositol group that anchors them to the outside of the cell membrane. CNTNs use their Ig domain to bind to the atypical carbonic anhydrase domains of PTPζ and PTPγ. Structure of PTPγ:CNTN4 complex and the PTPζ:CNTN1 complex is very similar [148, 149]. The complexes show the PTPγ/PTPζ using their carbonic anhydrase domain's β-hairpin loop to hold the Ig

domains 2 and 3 of the CNTN in a horseshoe conformation (Figure 4.10). Two β-strands of the carbonic anhydrase domain combine with three antiparallel β-strands of the binding CNTN molecule. The CNTN4 binding interface residues are conserved in other CNTN isoforms including CNTN3, CNTN5 and CNTN6 indicating a similar mode of binding to PTPγ. The specific binding interface in the PTPζ:CNTN1 complex is contributed by key interface residues unique to PTPζ and CNTN1.

PTPζ has three alternatively spliced isoforms, two receptor forms and also a secreted isoform. The alternatively spliced isoforms include the full-length 2,315 amino acid protein, a shorter receptor form that has a deletion in the extracellular region (755–1,614) and another isoform with a short seven amino acid deletion in the intracellular region [150]. The two receptor forms of PTPζ are known as PTPRZ-A and PTPRZ-B [151]. The secretory variant of the full-length protein is PTPRZ-S (also called phosphacan) that contains only the extracellular region [152]. Metalloproteinases allow for the cleavage of the extracellular region to release the secreted isoform. The leftover membrane-tethered fragment is then acted about by γ-secretases to release the tandem PTP domains into the cytoplasm [151]. The released PTP domains are reported to exist both in the cytoplasm and in the nucleus. Another isoform of PTPζ called the phosphacan short isoform (PSI) is expressed specifically in the central nervous system [153]. This small secreted isoform ends 196 residues after the FNIII domain (Figure 4.10). The various isoforms of PTPζ show punctuated spatiotemporal expression in various developmental processes of the central nervous system. These developmental processes include cell synaptogenesis, myelination, cell migration, wounding and regeneration [154–156]. The present model of PTPζ-mediated development of neuronal cells is based on the isoform-specific interaction of PTPζ (expressed on glial cells) with CNTN1 expressed on oligodendrocyte precursor cells (OPCs) that develop into mature neurons. In this model, the PTPRZ-B form is released into the extracellular matrix to interact with the CNTN1 expressed on the OPC and inhibit their proliferation. Formation of the PTPRZ-B:CNTN1 complex allows for the OPCs to differentiate into immature oligodendrocytes that express lower levels of CNTN1. Now the glial cells switch the expression of PTPRZ-B to the receptor isoform PTPRZ-A. Subsequently, the PTPRZ-B:CNTN1 complex is substituted by the PTPRZ-A:CNTN1 complex that is smaller in number but membrane bound. This signal allows for the differentiation of immature oligodendrocytes to mature oligodendrocytes and the myelination of their axons [149]. Cell culture assays show that the carbonic anhydrase domain of PTPζ is sufficient to inhibit OPC proliferation. However, morphological maturation of oligodendrocytes requires PTPζ with its carbonic anhydrase, FNIII domain and spacer region. It is speculated that PTPζ uses its spacer region to recruit differentiation-inducing molecules like tenascins [157].

PTPζ is reported to use multiple substrates including β-catenin, G-protein-coupled receptor interactor (Git1), Magi1, Fyn kinase, β-adducin, ALK kinase, TrkA kinase and p190 Rho-GAP [158–162]. Phosphatase activity of PTPζ is seen to be

modulated by binding extracellular ligands pleiotrophin (also known as heparin-binding brain mitogen or neurite growth-promoting factor) and midkine [152]. Ligand-binding-induced dimerization of PTPζ is speculated to inhibit its enzyme activity by blocking of substrate access to its PTP domains inside the cell [158, 163]. Pleiotrophin-induced inhibition of PTPζ activity increases the cellular levels of phosphorylated β-catenin, Fyn and β-adducin. The increased levels of these phosphorylated proteins allow for proliferation of dopaminergic progenitors and their survival and differentiation. In this way, pleiotrophin contributes to differentiation of dopaminergic neurons and also contributes to nervous system repair by binding and inhibiting PTPζ [164]. Reported upregulation of pleiotrophin during nervous system repair and its function in mice models of Parkinson's disease make PTPζ a lucrative target for inhibitor discovery required for therapeutic intervention of neurological disorders [165].

4.8 The R7 subtype

The R7 subtype includes receptor PCPTP1 (also called as PTPRR and PTPB7) and the non-receptor STEP and HePTP proteins. The receptor PTPRR has a single PTP catalytic domain that is attached to a short extracellular region that lacks any known homology domains. These receptor PTPs are also expressed mainly in neuronal cells. Four isoforms of PTPRR allow for its spatiotemporal function in the needs of the developing neuronal cells [166]. Isoforms are created in a unique way by differential utilization of three distinct transcription start sites and inclusion/exclusion of a unique 5′-untranslated region in the shorted transcript [167, 168]. The two receptor isoforms are called PTPBR7 and PTP-SL. Both the isoforms contain two hydrophobic regions, one of which forms the transmembrane region. As the PTP-SL was reported to lack the signal peptide sequence, there were speculations about it being a membrane-associated protein [169]. Cell permeabilization assays and use of specific anti-sera against the PTPRR domains reported the PTP-SL isoform to be a type III transmembrane protein with the C-terminal facing in the cytosol. Both the isoforms are detected in trans-Golgi network and the endocytic vesicles but only the PTPBR7 isoform is expressed on the membrane [166]. The two cytosolic isoforms are PTPPBSγ-42 and PTPPBSγ-37 (Figure 4.11). All the four isoforms contain the PTP catalytic domain and a kinase-interacting motif (KIM). The receptor isoforms of PTPRR are reported to oligomerize that allows for attenuation of their catalytic activity [170] . The cytosolic isoforms occur as monomers and their activity is independent of the effects of ligand binding [171]. Expression of the four isoforms is precisely timed and is unique to specific regions of the brain as studied in mouse models. PTPBR7 is expressed at early embryogenesis in the Purkinje cells and the spinal ganglia. PTP-SL is expressed only postnatally and is speculated to gradually replace the decreasing PTPBR7 levels during embryo maturation.

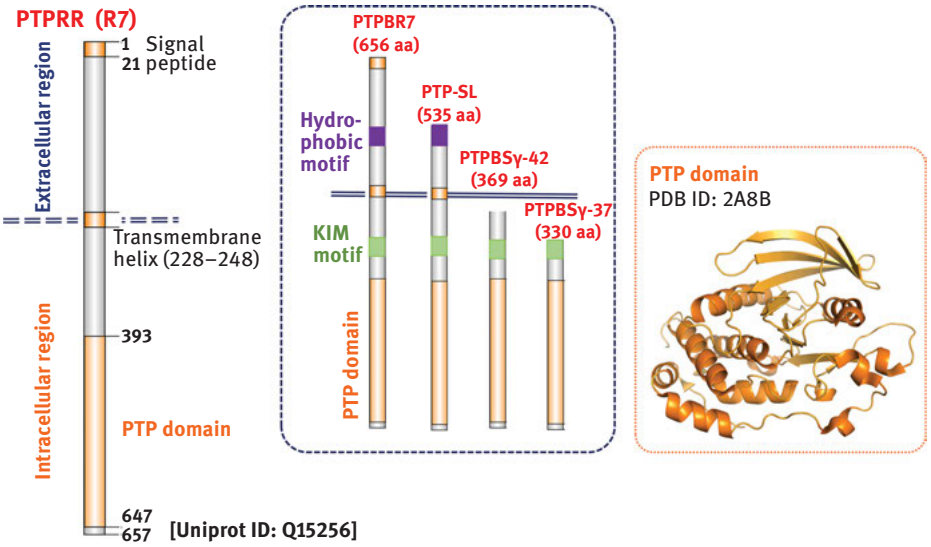


Figure 4.11: The kinase interaction motif (KIM) containing R7 subtype of receptor protein tyrosine phosphatases.

Eventually, PTP-SL is exclusively expressed in the adult cerebellar Purkinje cells and only in trace amounts in the midbrain, cortex or the brain stem. PTPBR7 is present in all other areas of the adult mouse and rat brain [169, 172]. The cytosolic PTPPBSγ isoforms are expressed in low levels in the brain and also seen in trace amounts in developing cartilage and the gastrointestinal tract [168].

All four PTPRR isoforms have a KIM motif that allows them to interact with the mitogen-activated protein kinases (MAPKs) Erk1 and Erk2 [171]. These MAPKs are dually phosphorylated on their activation loops on the pT-E-pY motif, and their signaling can be terminated by the dephosphorylation of their the phosphotyrosine or the phosphothreonine. The phosphotyrosine of the Erk1/2/5 are substrates for PTPRR [173, 174]. Increased activity and expression of PTPRR is associated with decreased expression of functional Erk1/2 as seen in the prefrontal cortex and hippocampal tissue of suicide victims when compared to the normal human patients [175]. In females, PTPRR expression levels are seen to increase in the transition phase from proliferation to secretion during the menstrual cycle. PTPRR is hence reported to be a predisposing factor for endometriosis where the endometrium starts to grow and expand outside the uterus [176]. Methylation of the PTPRR gene and its loss of expression and activity is associated with invasive cervical cancer [177]. In acute myelogenous leukemia, the PTPRR is reported to form a fusion protein with the TEL gene due to the fusion of their exons. This TEL–PTPRR fusion protein is an inactive PTP that compromises the appropriate maintenance of STAT3-mediated signaling pathway contributing to leukemogenesis [178].

4.9 The R8 subtype

The R8 subfamily of the receptor PTPs includes two members that share about 74% sequence identity [179]. Human islet antigen-2 (IA2 or ICA512) and IAβ (phogrin) are both expressed in β-cells of the pancreases and are located in insulin secretory granule membrane [180]. Antibodies against these autoimmune proteins are used as a marker for detection of onset of clinical type I diabetes [181]. *Prprn* gene encodes IA2 that contains an N-terminal signal peptide followed by a long cysteine-rich extracellular domain. A single transmembrane helix connects the extracellular domain to a single PTP domain that lacks any enzymatic activity. The lack of phosphatase activity in the domain is due to two critical point mutations. The first mutation converts the conserved acid/base aspartate of the WPD-loop into alanine (Ala877 in IA2). The second mutation occurs in the P-loop, where a small hydrophobic alanine next to the HCS sequence is replaced by a polar aspartate (Asp911 in IA2). This substitution of a short-chain hydrophobic residue with a negatively charged aspartate severely compromises the chemical integrity of the active site. Reverse mutations of these residues to abide by the consensus motifs is seen to be successful in converting these pseudophosphatase domains into active enzymes (ascertained using generic phosphatase substrates).

Both IA2 and IA2β are granule-associated proteins that are expressed in β-cells and participate in insulin granule turnover in the pancreas [182]. IA2 and IA2β are expressed as pro-proteins that mature alongside insulin in the secretory granules. The mature IA2 and IA2β have their pseudophosphatase domain facing the cytoplasm and their extracellular region faces the inside of the secretory granule. Upon exocytosis and membrane integration of the granules, these proteins are present on the membrane like canonical receptor PTPs. The pseudophosphatase domain binds an F-actin-associated protein called β2-syntrophin. The presence of glucose serves as a stimulus for the exocytosis of granules containing insulin. As the stimulus is received, the IA2 subpopulation presented on the cell membrane (also called ICA512-TMF) undergoes calpain-1-mediated cleavage to release its pseudophosphatase domain into the cytoplasm (Figure 4.12). This fragment called as ICA512-CCF is now free to either translocate to the nucleus where it enhances the transcription of granule genes using STAT signaling or to bind and displace the IA2- β2-syntrophin complex. ICA512-CCF can effectively bind to either component of the IA2- β2-syntrophin complex and allow for its disruption. This displaces the secretory granules from actin fibers, thus increasing their mobility as required for active exocytosis. As more and more granules are exocytosed and more ICA512-CCF is generated in the cytosol, the population that reaches the nucleus gradually increases to enhance transcription of granule genes. This positive feedback allows for concurrent cycling of insulin granules in the β-cells in need of glucose absorption and signaling.

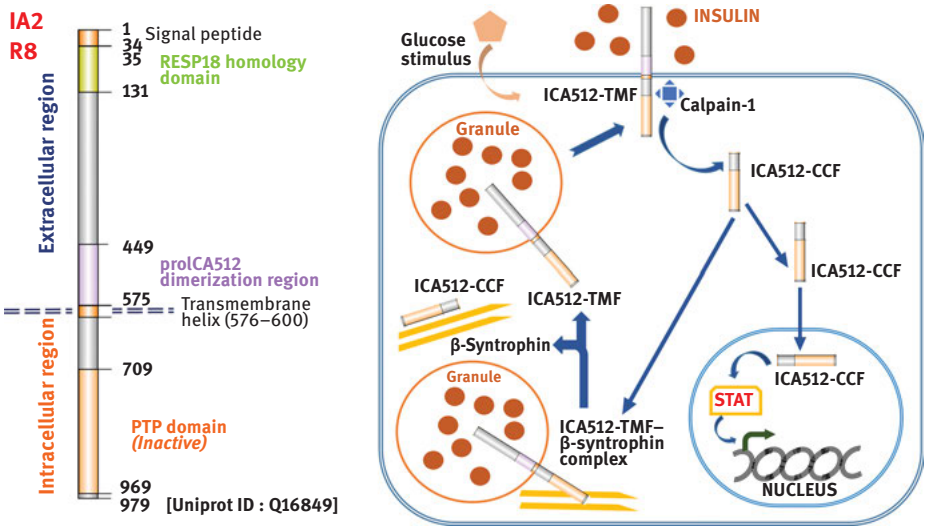


Figure 4.12: The R8 subtype of protein tyrosine phosphatase has a single inactive catalytic domain. This protein participates in insulin granule turnover in the human pancreas.

Alongside their role in regulating insulin secretory granule cycling, the subtype R8 members have also been reported for β-cell growth [183]. Knockout mice lacking IA2 and IA2β have as much as 50% reduced insulin secretion and are glucose intolerant [184, 185]. Conversely, overexpression of IA2 in mouse β-cells allows for better glucose uptake and efficiently increased insulin secretion [186]. Autoimmune antibodies secreted against these R8 subtype members are a major cause of type I diabetes in humans [187]. IA2 antibody screening is used as a clinical diagnostic test for type I diabetes. As many as 70% patients with type I diabetes show elevated antibodies for IA2 [181], antibodies are reported to appear years before the clear onset of diabetes. Patients showing the presence of serum antibodies against IA2, IA2β and another protein GAD65 (a glutamic acid decarboxylase) are reported to develop type I diabetes within just five years [187].

References

[1] Andersen JN, Mortensen OH, Peters GH, Drake PG, Iversen LF, Olsen OH, et al. Structural and evolutionary relationships among protein tyrosine phosphatase domains. Mol Cell Biol 2001;21(21):7117–36.

[2] Andersen JN, Jansen PG, Echwald SM, Mortensen OH, Fukada T, Del Vecchio R, et al. A genomic perspective on protein tyrosine phosphatases: gene structure, pseudogenes, and genetic disease linkage. FASEB J 2004;18(1):8–30.

[3] Brady-Kalnay SM, Tonks NK. Protein tyrosine phosphatases as adhesion receptors. Current Opinion in Cell Biol 1995;7(5):650–7.

[4] Pixley FJ, Lee PS, Dominguez MG, Einstein DB, Stanley ER. A heteromorphic protein-tyrosine phosphatase, PTP phi, is regulated by CSF-1 in macrophages. J Biol Chem 1995;270(45): 27339–47.
[5] Elson A, Leder P. Identification of a cytoplasmic, phorbol ester-inducible isoform of protein tyrosine phosphatase epsilon. Proc Natl Acad Sci 1995;92(26):12235–9.
[6] Hooft van Huijsduijnen R. Protein tyrosine phosphatases: counting the trees in the forest. Gene 1998;225(1–2):1–8.
[7] Tonks NK, Charbonneau H, Diltz CD, Fischer EH, Walsh KA. Demonstration that the leukocyte common antigen CD45 is a protein tyrosine phosphatase. Biochemistry 1988;27(24): 8695–701.
[8] Charbonneau H, Tonks NK, Walsh KA, Fischer EH. The leukocyte common antigen (CD45): a putative receptor-linked protein tyrosine phosphatase. Proc Natl Acad Sci USA 1988;85 (19):7182–6.
[9] Trowbridge IS, Thomas ML. CD45: an emerging role as a protein tyrosine phosphatase required for lymphocyte activation and development. Ann Rev Immunol 1994;12:85–116.
[10] Hermiston ML, Zikherman J, Zhu JW. CD45, CD148, and Lyp/Pep: critical phosphatases regulating Src family kinase signaling networks in immune cells. Immunol Rev 2009;228(1): 288–311.
[11] Holmes N. CD45: all is not yet crystal clear. Immunology 2006;117(2):145–55.
[12] Ogilvy S, Louis-Dit-Sully C, Cooper J, Cassady RL, Alexander DR, Holmes N. Either of the CD45RB and CD45RO isoforms are effective in restoring T cell, but not B cell, development and function in CD45-null mice. J Immunol 2003;171(4):1792–800.
[13] Virts EL, Diago O, Raschke WC. A CD45 minigene restores regulated isoform expression and immune function in CD45-deficient mice: therapeutic implications for human CD45-null severe combined immunodeficiency. Blood 2003;101(3):849–55.
[14] Stanton T, Boxall S, Hirai K, Dawes R, Tonks S, Yasui T, et al. A high-frequency polymorphism in exon 6 of the CD45 tyrosine phosphatase gene (PTPRC) resulting in altered isoform expression. Proc Natl Acad Sci USA 2003;100(10):5997–6002.
[15] Boxall S, Stanton T, Hirai K, Ward V, Yasui T, Tahara H, et al. Disease associations and altered immune function in CD45 138G variant carriers. Hum Mol Genet 2004;13(20):2377–84.
[16] Lynch KW, Weiss A. A CD45 polymorphism associated with multiple sclerosis disrupts an exonic splicing silencer. J Biol Chem 2001;276(26):24341–7.
[17] Tchilian EZ, Beverley PCL. Altered CD45 expression and disease. Trends Immunol 2006; 27(3):146–53.
[18] Do HT, Baars W, Borns K, Windhagen A, Schwinzer R. The 77C->G mutation in the human CD45 (PTPRC) gene leads to increased intensity of TCR signaling in T cell lines from healthy individuals and patients with multiple sclerosis. J Immunol 2006;176(2):931–8.
[19] Novak TJ, Farber D, Leitenberg D, Hong SC, Johnson P, Bottomly K. Isoforms of the transmembrane tyrosine phosphatase CD45 differentially affect T cell recognition. Immunity 1994;1 (2):109–19.
[20] Symons A, Willis AC, Barclay AN. Domain organization of the extracellular region of CD45. Protein Eng 1999;12(10):885–92.
[21] Pulido R, Sanchez-Madrid F. Glycosylation of CD45: carbohydrate processing through Golgi apparatus is required for cell surface expression and protein stability. Eur J Immunol 1992; 22(2):463–8.
[22] Earl LA, Bi S, Baum LG. N- and O-glycans modulate galectin-1 binding, CD45 signaling, and T cell death. J Biol Chem 2010;285(4):2232–44.
[23] Earl LA, Baum LG. CD45 glycosylation controls T-cell life and death. Immunol Cell Biol 2008; 86(7):608–15.

[24] Barondes SH, Castronovo V, Cooper DN, Cummings RD, Drickamer K, Feizi T, et al. Galectins: a family of animal beta-galactoside-binding lectins. Cell 1994;76(4):597–8.
[25] Perillo NL, Pace KE, Seilhamer JJ, Baum LG. Apoptosis of T cells mediated by galectin-1. Nature 1995;378(6558):736–9.
[26] Walzel H, Schulz U, Neels P, Brock J. Galectin-1, a natural ligand for the receptor-type protein tyrosine phosphatase CD45. Immunol Lett 1999;67(3):193–202.
[27] Nguyen JT, Evans DP, Galvan M, Pace KE, Leitenberg D, Bui TN, et al. CD45 modulates galectin-1-induced T cell death: regulation by expression of core 2 O-glycans. J Immunol 2001; 167(10):5697–707.
[28] Pace KE, Lee C, Stewart PL, Baum LG. Restricted receptor segregation into membrane microdomains occurs on human T cells during apoptosis induced by galectin-1. J Immunol 1999; 163(7):3801–11.
[29] Fischer EH, Charbonneau H, Tonks NK. Protein tyrosine phosphatases: a diverse family of intracellular and transmembrane enzymes. Science 1991;253(5018):401–6.
[30] Streuli M, Krueger NX, Thai T, Tang M, Saito H. Distinct functional roles of the two intracellular phosphatase like domains of the receptor-linked protein tyrosine phosphatases LCA and LAR. EMBO J 1990;9(8):2399–407.
[31] Ahuja LG, Gopal B. Bi-domain protein tyrosine phosphatases reveal an evolutionary adaptation to optimize signal transduction. Antioxid Redox Signal 2014;20(14):2141–59.
[32] Tan X, Stover DR, Walsh KA. Demonstration of protein tyrosine phosphatase activity in the second of two homologous domains of CD45. J Biol Chem 1993;268(10):6835–8.
[33] Nam HJ, Poy F, Saito H, Frederick CA. Structural basis for the function and regulation of the receptor protein tyrosine phosphatase CD45. J Exp Med 2005;201(3):441–52.
[34] Irie-Sasaki J, Sasaki T, Matsumoto W, Opavsky A, Cheng M, Welstead G, et al. CD45 is a JAK phosphatase and negatively regulates cytokine receptor signalling. Nature 2001;409 (6818):349–54.
[35] Hurley TR, Hyman R, Sefton BM. Differential effects of expression of the CD45 tyrosine protein phosphatase on the tyrosine phosphorylation of the lck, fyn, and c-src tyrosine protein kinases. Mol Cell Biol 1993;13(3):1651–6.
[36] Kong YY, Kishihara K, Yoshida H, Mak TW, Nomoto K. Generation of T cells with differential responses to alloantigens in CD45 exon 6-deficient mice. J Immunol 1995;154(11): 5725–35.
[37] Byth KF, Conroy LA, Howlett S, Smith AJ, May J, Alexander DR, et al. 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 1996;183(4):1707–18.
[38] Finco TS, Kadlecek T, Zhang W, Samelson LE, Weiss A. LAT is required for TCR-mediated activation of PLCgamma1 and the Ras pathway. Immunity 1998;9(5):617–26.
[39] Yablonski D, Kuhne MR, Kadlecek T, Weiss A. Uncoupling of nonreceptor tyrosine kinases from PLC-gamma1 in an SLP-76-deficient T cell. Science 1998;281(5375):413–6.
[40] Kung C, Pingel JT, Heikinheimo M, Klemola T, Varkila K, Yoo LI, et al. Mutations in the tyrosine phosphatase CD45 gene in a child with severe combined immunodeficiency disease. Nat Med 2000;6(3):343–5.
[41] Tchilian EZ, Wallace DL, Wells RS, Flower DR, Morgan G, Beverley PC. A deletion in the gene encoding the CD45 antigen in a patient with SCID. J Immunol 2001;166(2):1308–13.
[42] Nam HJ, Poy F, Krueger NX, Saito H, Frederick CA. Crystal structure of the tandem phosphatase domains of RPTP LAR. Cell 1999;97(4):449–57.
[43] Streuli M, Krueger NX, Hall LR, Schlossman SF, Saito H. A new member of the immunoglobulin superfamily that has a cytoplasmic region homologous to the leukocyte common antigen. J Exp Med 1988;168(5):1523–30.

[44] Streuli M, Krueger NX, Ariniello PD, Tang M, Munro JM, Blattler WA, et al. Expression of the receptor-linked protein tyrosine phosphatase LAR: proteolytic cleavage and shedding of the CAM-like extracellular region. EMBO J 1992;11(3):897–907.
[45] Serra-Pages C, Saito H, Streuli M. Mutational analysis of proprotein processing, subunit association, and shedding of the LAR transmembrane protein tyrosine phosphatase. J Biol Chem 1994;269(38):23632–41.
[46] Hayashida K, Bartlett AH, Chen Y, Park PW. Molecular and cellular mechanisms of ectodomain shedding. Anat Rec: Adv Integr Anat Evol Biol 2010;293(6):925–37.
[47] Chagnon MJ, Uetani N, Tremblay ML. Functional significance of the LAR receptor protein tyrosine phosphatase family in development and diseases. Biochem Cell Biol 2004;82(6): 664–75.
[48] Yang T, Bernabeu R, Xie Y, Zhang JS, Massa SM, Rempel HC, et al. Leukocyte antigen-related protein tyrosine phosphatase receptor: a small ectodomain isoform functions as a homophilic ligand and promotes neurite outgrowth. J Neurosci 2003;23(8):3353–63.
[49] Yang T, Yin W, Derevyanny VD, Moore LA, Longo FM. Identification of an ectodomain within the LAR protein tyrosine phosphatase receptor that binds homophilically and activates signalling pathways promoting neurite outgrowth. Eur J Neurosci 2005;22(9):2159–70.
[50] Suarez Pestana E, Tenev T, Gross S, Stoyanov B, Ogata M, Bohmer FD. The transmembrane protein tyrosine phosphatase RPTPsigma modulates signaling of the epidermal growth factor receptor in A431 cells. Oncogene 1999;18(28):4069–79.
[51] Kulas DT, Goldstein BJ, Mooney RA. The transmembrane protein-tyrosine phosphatase LAR modulates signaling by multiple receptor tyrosine kinases. J Biol Chem 1996;271(2):748–54.
[52] Tsujikawa K, Kawakami N, Uchino Y, Ichijo T, Furukawa T, Saito H, et al. Distinct functions of the two protein tyrosine phosphatase domains of LAR (leukocyte common antigen-related) on tyrosine dephosphorylation of insulin receptor. Mol Endocrinol 2001;15(2):271–80.
[53] Johnson KG, Van Vactor D. Receptor protein tyrosine phosphatases in nervous system development. Physiol Rev 2003;83(1):1–24.
[54] Mooney RA, LeVea CM. The leukocyte common antigen-related protein LAR: candidate PTP for inhibitory targeting. Curr Top Med Chem 2003;3(7):809–19.
[55] Zabolotny JM, Kim YB, Peroni OD, Kim JK, Pani MA, Boss O, et al. Overexpression of the LAR (leukocyte antigen-related) protein-tyrosine phosphatase in muscle causes insulin resistance. Proc Natl Acad Sci USA 2001;98(9):5187–92.
[56] Schaapveld RQ, Schepens JT, Robinson GW, Attema J, Oerlemans FT, Fransen JA, et al. Impaired mammary gland development and function in mice lacking LAR receptor-like tyrosine phosphatase activity. Dev Biol 1997;188(1):134–46.
[57] Yeo TT, Yang T, Massa SM, Zhang JS, Honkaniemi J, Butcher LL, et al. Deficient LAR expression decreases basal forebrain cholinergic neuronal size and hippocampal cholinergic innervation. J Neurosci Res 1997;47(3):348–60.
[58] Uetani N, Kato K, Ogura H, Mizuno K, Kawano K, Mikoshiba K, et al. Impaired learning with enhanced hippocampal long-term potentiation in PTPdelta-deficient mice. EMBO J 2000; 19(12):2775–85.
[59] Navis AC, van den Eijnden M, Schepens JT, Hooft van Huijsduijnen R, Wesseling P, Hendriks WJ. Protein tyrosine phosphatases in glioma biology. Acta Neuropathol 2010;119(2):157–75.
[60] Solomon DA, Kim JS, Cronin JC, Sibenaller Z, Ryken T, Rosenberg SA, et al. Mutational inactivation of PTPRD in glioblastoma multiforme and malignant melanoma. Cancer Res 2008;68(24):10300–6.
[61] Solomon DA, Kim JS, Yang XR, Tucker MA, Goldstein AM, Samuels Y, et al. Lack of inherited mutations of PTPRD in familial melanoma and melanoma-astrocytoma syndrome. Pigment Cell Melanoma Res 2009;22(4):489–91.

[62] Konishi N, Tsujikawa K, Yamamoto H, Ishida E, Nakamura M, Shimada K, et al. Overexpression of leucocyte common antigen (LAR) P-subunit in thyroid carcinomas. Br J Cancer 2003; 88(8):1223–8.
[63] Harder KW, Saw J, Miki N, Jirik F. Coexisting amplifications of the chromosome 1p32 genes (PTPRF and MYCL1) encoding protein tyrosine phosphatase LAR and L-myc in a small cell lung cancer line. Genomics 1995;27(3):552–3.
[64] Wang Z, Shen D, Parsons DW, Bardelli A, Sager J, Szabo S, et al. Mutational analysis of the tyrosine phosphatome in colorectal cancers. Science 2004;304(5674):1164–6.
[65] Yang T, Zhang JS, Massa SM, Han X, Longo FM. Leukocyte common antigen-related tyrosine phosphatase receptor: increased expression and neuronal-type splicing in breast cancer cells and tissue. Mol Carcinog 1999;25(2):139–49.
[66] Serra-Pages C, Kedersha NL, Fazikas L, Medley Q, Debant A, Streuli M. The LAR transmembrane protein tyrosine phosphatase and a coiled-coil LAR-interacting protein co-localize at focal adhesions. EMBO J 1995;14(12):2827–38.
[67] Kypta RM, Su H, Reichardt LF. Association between a transmembrane protein tyrosine phosphatase and the cadherin-catenin complex. J Cell Biol 1996;134(6):1519–29.
[68] Weng LP, Wang X, Yu Q. Transmembrane tyrosine phosphatase LAR induces apoptosis by dephosphorylating and destabilizing p130Cas. Genes Cells 1999;4(3):185–96.
[69] Muller T, Choidas A, Reichmann E, Ullrich A. Phosphorylation and free pool of beta-catenin are regulated by tyrosine kinases and tyrosine phosphatases during epithelial cell migration. J Biol Chem 1999;274(15):10173–83.
[70] Kaufmann N, DeProto J, Ranjan R, Wan H, Van Vactor D. Drosophila Liprin-α and the Receptor Phosphatase Dlar Control Synapse Morphogenesis. Neuron 2002;34(1):27–38.
[71] Fox AN, Zinn K. The heparan sulfate proteoglycan syndecan is an in vivo ligand for the drosophila LAR receptor tyrosine phosphatase. Curr Biol 2005;15(19):1701–11.
[72] Johnson KG, Tenney AP, Ghose A, Duckworth AM, Higashi ME, Parfitt K, et al. The HSPGs Syndecan and Dallylike bind the receptor phosphatase LAR and exert distinct effects on synaptic development. Neuron 2006;49(4):517–31.
[73] O'Grady P, Thai TC, Saito H. The laminin-nidogen complex is a ligand for a specific splice isoform of the transmembrane protein tyrosine phosphatase LAR. J Cell Biol 1998;141(7): 1675–84.
[74] Krueger NX, Streuli M, Saito H. Structural diversity and evolution of human receptor-like protein tyrosine phosphatases. EMBO J 1990;9(10):3241–52.
[75] Ono K, Suga H, Iwabe N, Kuma K-i, Miyata T. Multiple protein tyrosine phosphatases in sponges and explosive gene duplication in the early evolution of animals before the parazoan–eumetazoan split. J Mol Evol 1999;48(6):654–62.
[76] Jia Z, Barford D, Flint AJ, Tonks NK. Structural basis for phosphotyrosine peptide recognition by protein tyrosine phosphatase 1B. Science 1995;268(5218):1754–8.
[77] Madan LL, Veeranna S, Shameer K, Reddy CCS, Sowdhamini R, Gopal B. Modulation of catalytic activity in multi-domain protein tyrosine phosphatases. PLOS ONE 2011;6(9):e24766.
[78] Krueger NX, Reddy RS, Johnson K, Bateman J, Kaufmann N, Scalice D, et al. Functions of the ectodomain and cytoplasmic tyrosine phosphatase domains of receptor protein tyrosine phosphatase Dlar in vivo. Mol Cell Biol 2003;23(19):6909–21.
[79] Becka S, Zhang P, Craig SE, Lodowski DT, Wang Z, Brady-Kalnay SM. Characterization of the adhesive properties of the type IIb subfamily receptor protein tyrosine phosphatases. Cell Commun Adhes 2010;17(2):34–47.
[80] Eswaran J, Debreczeni JE, Longman E, Barr AJ, Knapp S. The crystal structure of human receptor protein tyrosine phosphatase kappa phosphatase domain 1. Protein Sci 2006; 15(6):1500–5.

[81] Pils B, Schultz Jr. Evolution of the multifunctional protein tyrosine phosphatase family. Mol Biol Evol 2004;21(4):625–31.
[82] Zondag GC, Koningstein GM, Jiang Y-P, Sap J, Moolenaar WH, Gebbink MF. Homophilic interactions mediated by receptor tyrosine phosphatases μ and κ. A critical role for the novel extracellular MAM domain. J Biol Chem 1995;270(24):14247–50.
[83] Cheng J, Wu K, Armanini M, O'Rourke N, Dowbenko D, Lasky LA. A novel protein-tyrosine phosphatase related to the homotypically adhering κ and μ receptors. J Biol Chem 1997; 272(11):7264-77.
[84] Sap J, Jiang Y-P, Friedlander D, Grumet M, Schlessinger J. Receptor tyrosine phosphatase R-PTP-kappa mediates homophilic binding. Mol Cell Biol 1994;14(1):1–9.
[85] Sap J, Jiang YP, Friedlander D, Grumet M, Schlessinger J. Receptor tyrosine phosphatase R-PTP-kappa mediates homophilic binding. Mol Cell Biol 1994;14(1):1–9.
[86] Yu J, Becka S, Zhang P, Zhang X, Brady-Kalnay SM, Wang Z. Tumor-derived extracellular mutations of PTPRT/PTPrho are defective in cell adhesion. Mol Cancer Res 2008;6(7): 1106–13.
[87] Zhang P, Becka S, Craig SE, Lodowski DT, Brady-Kalnay SM, Wang Z. Cancer-derived mutations in the fibronectin III repeats of PTPRT/PTPrho inhibit cell-cell aggregation. Cell Commun Adhes 2009;16(5–6):146–53.
[88] Del Vecchio RL, Tonks NK. The conserved immunoglobulin domain controls the subcellular localization of the homophilic adhesion receptor protein-tyrosine phosphatase μ. J Biol Chem 2005;280(2):1603–12.
[89] Brady-Kalnay SM, Tonks NK. Identification of the homophilic binding site of the receptor protein tyrosine phosphatase PTP mu. J Biol Chem 1994;269(45):28472–7.
[90] Aricescu AR, Hon WC, Siebold C, Lu W, van der Merwe PA, Jones EY. Molecular analysis of receptor protein tyrosine phosphatase mu-mediated cell adhesion. EMBO J 2006;25(4): 701–12.
[91] Aricescu AR, Siebold C, Choudhuri K, Chang VT, Lu W, Davis SJ, et al. Structure of a tyrosine phosphatase adhesive interaction reveals a spacer-clamp mechanism. Science 2007; 317(5842):1217–20.
[92] Staehelin LA. Structure and function of intercellular junctions. Int Rev Cytol 1974;39:191–283.
[93] Besco JA, Hooft van Huijsduijnen R, Frostholm A, Rotter A. Intracellular substrates of brain-enriched receptor protein tyrosine phosphatase rho (RPTPrho/PTPRT). Brain Res 2006; 1116(1):50–7.
[94] Fuchs M, Muller T, Lerch MM, Ullrich A. Association of human protein-tyrosine phosphatase kappa with members of the armadillo family. J Biol Chem 1996;271(28):16712–9.
[95] Verin AD. Tyrosine phosphorylation and endothelial cell barrier regulation. Am J Pathol 2005;166(4):955–7.
[96] Del Vecchio RL, Tonks NK. The conserved immunoglobulin domain controls the subcellular localization of the homophilic adhesion receptor protein-tyrosine phosphatase mu. J Biol Chem 2005;280(2):1603–12.
[97] Sörby M, östman A. Protein-tyrosine phosphatase-mediated decrease of epidermal growth factor and platelet-derived growth factor receptor tyrosine phosphorylation in high cell density cultures. J Biol Chem 1996;271(18):10963–6.
[98] Cismasiu VB, Denes SA, Reilander H, Michel H, Szedlacsek SE. The MAM (meprin/A5-protein/PTPmu) domain is a homophilic binding site promoting the lateral dimerization of receptor-like protein-tyrosine phosphatase mu. J Biol Chem 2004;279(26):26922–31.
[99] Coles CH, Jones EY, Aricescu AR. Extracellular regulation of type IIa receptor protein tyrosine phosphatases: mechanistic insights from structural analyses. Semin Cell Dev Biol 2015;37: 98–107.

[100] Anders L, Mertins P, Lammich S, Murgia M, Hartmann D, Saftig P, et al. Furin-, ADAM 10-, and gamma-secretase-mediated cleavage of a receptor tyrosine phosphatase and regulation of beta-catenin's transcriptional activity. Mol Cell Biol 2006;26(10):3917–34.
[101] Wang SE, Wu FY, Shin I, Qu S, Arteaga CL. Transforming growth factor {beta} (TGF-{beta})-Smad target gene protein tyrosine phosphatase receptor type kappa is required for TGF-{beta} function. Mol Cell Biol 2005;25(11):4703–15.
[102] Xu Y, Tan LJ, Grachtchouk V, Voorhees JJ, Fisher GJ. Receptor-type protein-tyrosine phosphatase-kappa regulates epidermal growth factor receptor function. J Biol Chem 2005; 280(52):42694–700.
[103] Nakamura M, Kishi M, Sakaki T, Hashimoto H, Nakase H, Shimada K, et al. Novel tumor suppressor loci on 6q22-23 in primary central nervous system lymphomas. Cancer Res 2003;63(4):737–41.
[104] Novellino L, Renkvist N, Rini F, Mazzocchi A, Rivoltini L, Greco A, et al. Identification of a mutated receptor-like protein tyrosine phosphatase kappa as a novel, class II HLA-restricted melanoma antigen. J Immunol 2003;170(12):6363–70.
[105] Jeon M, Zinn K. R3 receptor tyrosine phosphatases: conserved regulators of receptor tyrosine kinase signaling and tubular organ development. Semin Cell Dev Biol 2015;37:119–26.
[106] Seifert RA, Coats SA, Oganesian A, Wright MB, Dishmon M, Booth CJ, et al. PTPRQ is a novel phosphatidylinositol phosphatase that can be expressed as a cytoplasmic protein or as a subcellularly localized receptor-like protein. Exp Cell Res 2003;287(2):374–86.
[107] Yu KR, Kim YJ, Jung SK, Ku B, Park H, Cho SY, et al. Structural basis for the dephosphorylating activity of PTPRQ towards phosphatidylinositide substrates. Acta Crystallogr D Biol Crystallogr 2013;69(Pt 8):1522–9.
[108] Iuliano R, Raso C, Quintiero A, Pera IL, Pichiorri F, Palumbo T, et al. The eighth fibronectin type III domain of protein tyrosine phosphatase receptor J influences the formation of protein complexes and cell localization. J Biochem 2009;145(3):377–85.
[109] Takahashi T, Takahashi K, St John PL, Fleming PA, Tomemori T, Watanabe T, et al. A mutant receptor tyrosine phosphatase, CD148, causes defects in vascular development. Mol Cell Biol 2003;23(5):1817–31.
[110] Takahashi T, Takahashi K, Mernaugh RL, Tsuboi N, Liu H, Daniel TO. A monoclonal antibody against CD148, a receptor-like tyrosine phosphatase, inhibits endothelial-cell growth and angiogenesis. Blood 2006;108(4):1234–42.
[111] Jandt E, Denner K, Kovalenko M, Ostman A, Bohmer FD. The protein-tyrosine phosphatase DEP-1 modulates growth factor-stimulated cell migration and cell-matrix adhesion. Oncogene 2003;22(27):4175–85.
[112] Kovalenko M, Denner K, Sandstrom J, Persson C, Gross S, Jandt E, et al. Site-selective dephosphorylation of the platelet-derived growth factor beta-receptor by the receptor-like protein-tyrosine phosphatase DEP-1. J Biol Chem 2000;275(21):16219–26.
[113] Lampugnani MG, Orsenigo F, Gagliani MC, Tacchetti C, Dejana E. Vascular endothelial cadherin controls VEGFR-2 internalization and signaling from intracellular compartments. J Cell Biol 2006;174(4):593–604.
[114] Iervolino A, Iuliano R, Trapasso F, Viglietto G, Melillo RM, Carlomagno F, et al. The receptor-type protein tyrosine phosphatase J antagonizes the biochemical and biological effects of RET-derived oncoproteins. Cancer Res 2006;66(12):6280–7.
[115] Palka HL, Park M, Tonks NK. Hepatocyte growth factor receptor tyrosine kinase met is a substrate of the receptor protein-tyrosine phosphatase DEP-1. J Biol Chem 2003;278(8):5728–35.
[116] Sacco F, Tinti M, Palma A, Ferrari E, Nardozza AP, van Huijsduijnen RH, et al. Tumor suppressor density-enhanced phosphatase-1 (DEP-1) inhibits the RAS pathway by direct dephosphorylation of ERK1/2 kinases. J Biol Chem 2009;284(33):22048–58.

[117] Tarcic G, Boguslavsky SK, Wakim J, Kiuchi T, Liu A, Reinitz F, et al. An unbiased screen identifies DEP-1 tumor suppressor as a phosphatase controlling EGFR endocytosis. Curr Biol 2009; 19(21):1788–98.
[118] Grazia Lampugnani M, Zanetti A, Corada M, Takahashi T, Balconi G, Breviario F, et al. Contact inhibition of VEGF-induced proliferation requires vascular endothelial cadherin, beta-catenin, and the phosphatase DEP-1/CD148. J Cell Biol 2003;161(4):793–804.
[119] Ruivenkamp CA, van Wezel T, Zanon C, Stassen AP, Vlcek C, Csikos T, et al. Ptprj is a candidate for the mouse colon-cancer susceptibility locus Scc1 and is frequently deleted in human cancers. Nat Genet 2002;31(3):295–300.
[120] Mita Y, Yasuda Y, Sakai A, Yamamoto H, Toyooka S, Gunduz M, et al. Missense polymorphisms of PTPRJ and PTPN13 genes affect susceptibility to a variety of human cancers. J Cancer Res Clin Oncol 2010;136(2):249–59.
[121] Iuliano R, Le Pera I, Cristofaro C, Baudi F, Arturi F, Pallante P, et al. The tyrosine phosphatase PTPRJ/DEP-1 genotype affects thyroid carcinogenesis. Oncogene 2004; 23(52):8432–8.
[122] Kaplan R, Morse B, Huebner K, Croce C, Howk R, Ravera M, et al. Cloning of three human tyrosine phosphatases reveals a multigene family of receptor-linked protein-tyrosine-phosphatases expressed in brain. Proc Natl Acad Sci USA 1990;87(18):7000–4.
[123] Bodrikov V, Leshchyns'ka I, Sytnyk V, Overvoorde J, den Hertog J, Schachner M. RPTPalpha is essential for NCAM-mediated p59fyn activation and neurite elongation. J Cell Biol 2005; 168(1):127–39.
[124] Zeng L, D'Alessandri L, Kalousek MB, Vaughan L, Pallen CJ. Protein tyrosine phosphatase alpha (PTPalpha) and contactin form a novel neuronal receptor complex linked to the intracellular tyrosine kinase fyn. J Cell Biol 1999;147(4):707–14.
[125] den Hertog J. Protein-tyrosine phosphatases in development. Mech Dev 1999;85(1–2):3–14.
[126] Wang Y, Pallen CJ. The receptor-like protein tyrosine phosphatase HPTP alpha has two active catalytic domains with distinct substrate specificities. EMBO J 1991;10(11):3231–7.
[127] Wu L, Buist A, den Hertog J, Zhang ZY. Comparative kinetic analysis and substrate specificity of the tandem catalytic domains of the receptor-like protein-tyrosine phosphatase alpha. J Biol Chem 1997;272(11):6994–7002.
[128] Sonnenburg ED, Bilwes A, Hunter T, Noel JP. The structure of the membrane distal phosphatase domain of RPTPalpha reveals interdomain flexibility and an SH2 domain interaction region. Biochemistry 2003;42(26):7904–14.
[129] Buist A, Zhang YL, Keng YF, Wu L, Zhang ZY, den Hertog J. Restoration of potent protein-tyrosine phosphatase activity into the membrane-distal domain of receptor protein-tyrosine phosphatase alpha. Biochemistry 1999;38(3):914–22.
[130] Lim KL, Kolatkar PR, Ng KP, Ng CH, Pallen CJ. Interconversion of the kinetic identities of the tandem catalytic domains of receptor-like protein-tyrosine phosphatase PTPalpha by two point mutations is synergistic and substrate-dependent. J Biol Chem 1998;273(44): 28986–93.
[131] Jiang G, den Hertog J, Hunter T. Receptor-like protein tyrosine phosphatase alpha homodimerizes on the cell surface. Mol Cell Biol 2000;20(16):5917–29.
[132] Jiang G, den Hertog J, Su J, Noel J, Sap J, Hunter T. Dimerization inhibits the activity of receptor-like protein-tyrosine phosphatase-alpha. Nature 1999;401(6753):606–10.
[133] Bilwes AM, den Hertog J, Hunter T, Noel JP. Structural basis for inhibition of receptor protein-tyrosine phosphatase-alpha by dimerization. Nature 1996;382(6591):555–9.
[134] Xie Y, Massa SM, Ensslen-Craig SE, Major DL, Yang T, Tisi MA, et al. Protein-tyrosine phosphatase (PTP) wedge domain peptides: a novel approach for inhibition of PTP function and augmentation of protein-tyrosine kinase function. J Biol Chem 2006;281(24):16482–92.

[135] Barr AJ, Ugochukwu E, Lee WH, King ON, Filippakopoulos P, Alfano I, et al. Large-scale structural analysis of the classical human protein tyrosine phosphatome. Cell 2009; 136(2):352–63.
[136] Tracy S, van der Geer P, Hunter T. The receptor-like protein-tyrosine phosphatase, RPTP alpha, is phosphorylated by protein kinase C on two serines close to the inner face of the plasma membrane. J Biol Chem 1995;270(18):10587–94.
[137] Lammers R, Lerch MM, Ullrich A. The carboxyl-terminal tyrosine residue of protein-tyrosine phosphatase alpha mediates association with focal adhesion plaques. J Biol Chem 2000; 275(5):3391–6.
[138] Su J, Yang LT, Sap J. Association between receptor protein-tyrosine phosphatase RPTPalpha and the Grb2 adaptor. Dual Src homology (SH) 2/SH3 domain requirement and functional consequences. J Biol Chem 1996;271(45):28086–96.
[139] Zheng XM, Resnick RJ, Shalloway D. A phosphotyrosine displacement mechanism for activation of Src by PTPalpha. EMBO J 2000;19(5):964–78.
[140] Ponniah S, Wang DZ, Lim KL, Pallen CJ. Targeted disruption of the tyrosine phosphatase PTPalpha leads to constitutive downregulation of the kinases Src and Fyn. Curr Biol 1999; 9(10):535–8.
[141] Zheng XM, Wang Y, Pallen CJ. Cell transformation and activation of pp60c-src by overexpression of a protein tyrosine phosphatase. Nature 1992;359(6393):336–9.
[142] von Wichert G, Jiang G, Kostic A, De Vos K, Sap J, Sheetz MP. RPTP-alpha acts as a transducer of mechanical force on alphav/beta3-integrin-cytoskeleton linkages. J Cell Biol 2003;161(1): 143–53.
[143] Ardini E, Agresti R, Tagliabue E, Greco M, Aiello P, Yang LT, et al. Expression of protein tyrosine phosphatase alpha (RPTPalpha) in human breast cancer correlates with low tumor grade, and inhibits tumor cell growth in vitro and in vivo. Oncogene 2000;19(43):4979–87.
[144] Huang J, Yao L, Xu R, Wu H, Wang M, White BS, et al. Activation of Src and transformation by an RPTPalpha splice mutant found in human tumours. EMBO J 2011;30(15):3200–11.
[145] Yahiro K, Wada A, Nakayama M, Kimura T, Ogushi K, Niidome T, et al. Protein-tyrosine phosphatase alpha, RPTP alpha, is a Helicobacter pylori VacA receptor. J Biol Chem 2003; 278(21):19183–9.
[146] Barnea G, Silvennoinen O, Shaanan B, Honegger AM, Canoll PD, D'Eustachio P, et al. Identification of a carbonic anhydrase-like domain in the extracellular region of RPTP gamma defines a new subfamily of receptor tyrosine phosphatases. Mol Cell Biol 1993;13(3): 1497–506.
[147] Peles E, Nativ M, Campbell PL, Sakurai T, Martinez R, Lev S, et al. The carbonic anhydrase domain of receptor tyrosine phosphatase beta is a functional ligand for the axonal cell recognition molecule contactin. Cell 1995;82(2):251–60.
[148] Bouyain S, Watkins DJ. The protein tyrosine phosphatases PTPRZ and PTPRG bind to distinct members of the contactin family of neural recognition molecules. Proc Natl Acad Sci USA 2010;107(6):2443–8.
[149] Lamprianou S, Chatzopoulou E, Thomas JL, Bouyain S, Harroch S. A complex between contactin-1 and the protein tyrosine phosphatase PTPRZ controls the development of oligodendrocyte precursor cells. Proc Natl Acad Sci USA 2011;108(42):17498–503.
[150] Levy JB, Canoll PD, Silvennoinen O, Barnea G, Morse B, Honegger AM, et al. The cloning of a receptor-type protein tyrosine phosphatase expressed in the central nervous system. J Biol Chem 1993;268(14):10573–81.
[151] Chow JP, Fujikawa A, Shimizu H, Suzuki R, Noda M. Metalloproteinase- and gamma-secretase-mediated cleavage of protein-tyrosine phosphatase receptor type Z. J Biol Chem 2008; 283(45):30879–89.

[152] Maeda N, Noda M. Involvement of receptor-like protein tyrosine phosphatase zeta/RPTPbeta and its ligand pleiotrophin/heparin-binding growth-associated molecule (HB-GAM) in neuronal migration. J Cell Biol 1998;142(1):203–16.
[153] Heck N, Klausmeyer A, Faissner A, Garwood J. Cortical neurons express PSI, a novel isoform of phosphacan/RPTPbeta. Cell Tissue Res 2005;321(3):323–33.
[154] Haunsø A, Celio MR, Margolis RK, Menoud P-A. Phosphacan immunoreactivity is associated with perineuronal nets around parvalbumin-expressing neurones. Brain Res 1999;834(1): 219–22.
[155] Meyer-Puttlitz B, Junker E, Margolis RU, Margolis RK. Chondroitin sulfate proteoglycans in the developing central nervous system. II. Immunocytochemical localization of neurocan and phosphacan. J Comp Neurol 1996;366(1):44–54.
[156] Garwood J, Rigato F, Heck N, Faissner A. Tenascin glycoproteins and the complementary ligand DSD-1-PG/phosphacan – structuring the neural extracellular matrix during development and repair. Restor Neurol Neurosci 2001;19(1,2): 51–64.
[157] Czopka T, von Holst A, ffrench-Constant C, Faissner A. Regulatory mechanisms that mediate tenascin C-dependent inhibition of oligodendrocyte precursor differentiation. J Neurosci 2010;30(37):12310–22.
[158] Meng K, Rodriguez-Pena A, Dimitrov T, Chen W, Yamin M, Noda M, et al. Pleiotrophin signals increased tyrosine phosphorylation of beta beta-catenin through inactivation of the intrinsic catalytic activity of the receptor-type protein tyrosine phosphatase beta/zeta. Proc Natl Acad Sci USA 2000;97(6):2603–8.
[159] Kawachi H, Fujikawa A, Maeda N, Noda M. Identification of GIT1/Cat-1 as a substrate molecule of protein tyrosine phosphatase zeta/beta by the yeast substrate-trapping system. Proc Natl Acad Sci USA 2001;98(12):6593–8.
[160] Pariser H, Ezquerra L, Herradon G, Perez-Pinera P, Deuel TF. Fyn is a downstream target of the pleiotrophin/receptor protein tyrosine phosphatase beta/zeta-signaling pathway: regulation of tyrosine phosphorylation of Fyn by pleiotrophin. Biochem Biophys Res Commun 2005; 332(3):664–9.
[161] Perez-Pinera P, Zhang W, Chang Y, Vega JA, Deuel TF. Anaplastic lymphoma kinase is activated through the pleiotrophin/receptor protein-tyrosine phosphatase beta/zeta signaling pathway: an alternative mechanism of receptor tyrosine kinase activation. J Biol Chem 2007; 282(39):28683–90.
[162] Fukada M, Kawachi H, Fujikawa A, Noda M. Yeast substrate-trapping system for isolating substrates of protein tyrosine phosphatases: Isolation of substrates for protein tyrosine phosphatase receptor type z. Methods 2005;35(1):54–63.
[163] Fukada M, Fujikawa A, Chow JP, Ikematsu S, Sakuma S, Noda M. Protein tyrosine phosphatase receptor type Z is inactivated by ligand-induced oligomerization. FEBS Lett 2006; 580(17):4051–6.
[164] Hida H, Jung CG, Wu CZ, Kim HJ, Kodama Y, Masuda T, et al. Pleiotrophin exhibits a trophic effect on survival of dopaminergic neurons in vitro. Eur J Neurosci 2003;17(10):2127–34.
[165] Herradon G, Ezquerra L. Blocking receptor protein tyrosine phosphatase beta/zeta: a potential therapeutic strategy for Parkinson's disease. Curr Med Chem 2009;16(25): 3322–9.
[166] Hendriks WJ, Dilaver G, Noordman YE, Kremer B, Fransen JA. PTPRR protein tyrosine phosphatase isoforms and locomotion of vesicles and mice. Cerebellum 2009;8(2): 80–8.
[167] Chirivi RG, Dilaver G, van de Vorstenbosch R, Wanschers B, Schepens J, Croes H, et al. Characterization of multiple transcripts and isoforms derived from the mouse protein tyrosine phosphatase gene Ptprr. Genes Cells 2004;9(10):919–33.

[168] Augustine KA, Silbiger SM, Bucay N, Ulias L, Boynton A, Trebasky LD, et al. Protein tyrosine phosphatase (PC12, Br7,S1) family: expression characterization in the adult human and mouse. Anat Rec 2000;258(3):221–34.
[169] Van Den Maagdenberg AM, Bachner D, Schepens JT, Peters W, Fransen JA, Wieringa B, et al. The mouse Ptprr gene encodes two protein tyrosine phosphatases, PTP-SL and PTPBR7, that display distinct patterns of expression during neural development. Eur J Neurosci 1999; 11(11):3832–44.
[170] Noordman YE, Augustus ED, Schepens JT, Chirivi RG, Rios P, Pulido R, et al. Multimerisation of receptor-type protein tyrosine phosphatases PTPBR7 and PTP-SL attenuates enzymatic activity. Biochim Biophys Acta 2008;1783(2):275–86.
[171] Chirivi RG, Noordman YE, Van der Zee CE, Hendriks WJ. Altered MAP kinase phosphorylation and impaired motor coordination in PTPRR deficient mice. J Neurochem 2007;101(3):829–40.
[172] Watanabe Y, Shiozuka K, Ikeda T, Hoshi N, Hiraki H, Suzuki T, et al. Cloning of PCPTP1-Ce encoding protein tyrosine phosphatase from the rat cerebellum and its restricted expression in Purkinje cells. Brain Res Mol Brain Res 1998;58(1–2):83–94.
[173] Buschbeck M, Eickhoff J, Sommer MN, Ullrich A. Phosphotyrosine-specific phosphatase PTP-SL regulates the ERK5 signaling pathway. J Biol Chem 2002;277(33):29503–9.
[174] Munoz JJ, Tarrega C, Blanco-Aparicio C, Pulido R. Differential interaction of the tyrosine phosphatases PTP-SL, STEP and HePTP with the mitogen-activated protein kinases ERK1/2 and p38alpha is determined by a kinase specificity sequence and influenced by reducing agents. Biochem J 2003;372(Pt 1):193–201.
[175] Thalmeier A, Dickmann M, Giegling I, Schneider B, A MH, Maurer K, et al. Gene expression profiling of post-mortem orbitofrontal cortex in violent suicide victims. Int J Neuropsychopharmacol 2008;11(2):217–28.
[176] Sherwin JR, Sharkey AM, Mihalyi A, Simsa P, Catalano RD, D'Hooghe TM. Global gene analysis of late secretory phase, eutopic endometrium does not provide the basis for a minimally invasive test of endometriosis. Hum Reprod 2008;23(5):1063–8.
[177] Su PH, Lin YW, Huang RL, Liao YP, Lee HY, Wang HC, et al. Epigenetic silencing of PTPRR activates MAPK signaling, promotes metastasis and serves as a biomarker of invasive cervical cancer. Oncogene 2013;32(1):15–26.
[178] Nakamura F, Nakamura Y, Maki K, Sato Y, Mitani K. Cloning and characterization of the Novel Chimeric Gene TEL/PTPRR in acute myelogenous leukemia with inv(12)(p13q13). Cancer Res 2005;65(15):6612–21.
[179] Lan MS, Lu J, Goto Y, Notkins AL. Molecular cloning and identification of a receptor-type protein tyrosine phosphatase, IA-2, from human insulinoma. DNA Cell Biol 1994;13(5):505–14.
[180] Solimena M, Dirkx R, Jr., Hermel JM, Pleasic-Williams S, Shapiro JA, Caron L, et al. ICA 512, an autoantigen of type I diabetes, is an intrinsic membrane protein of neurosecretory granules. EMBO J 1996;15(9):2102–14.
[181] Lan MS, Wasserfall C, Maclaren NK, Notkins AL. IA-2, a transmembrane protein of the protein tyrosine phosphatase family, is a major autoantigen in insulin-dependent diabetes mellitus. Proc Natl Acad Sci USA 1996;93(13):6367–70.
[182] Trajkovski M, Mziaut H, Schubert S, Kalaidzidis Y, Altkruger A, Solimena M. Regulation of insulin granule turnover in pancreatic beta-cells by cleaved ICA512. J Biol Chem 2008; 283(48):33719–29.
[183] Torii S. Expression and function of IA-2 family proteins, unique neuroendocrine-specific protein-tyrosine phosphatases. Endocr J 2009;56(5):639–48.
[184] Saeki K, Zhu M, Kubosaki A, Xie J, Lan MS, Notkins AL. Targeted disruption of the protein tyrosine phosphatase-like molecule IA-2 results in alterations in glucose tolerance tests and insulin secretion. Diabetes 2002;51(6):1842–50.

[185] Kubosaki A, Nakamura S, Notkins AL. Dense core vesicle proteins IA-2 and IA-2beta: metabolic alterations in double knockout mice. Diabetes 2005;54 Suppl 2:S46–51.
[186] Harashima S, Clark A, Christie MR, Notkins AL. The dense core transmembrane vesicle protein IA-2 is a regulator of vesicle number and insulin secretion. Proc Natl Acad Sci USA 2005; 102(24):8704–9.
[187] Notkins AL. Immunologic and genetic factors in type 1 diabetes. J Biol Chem 2002; 277(46):43545–8.

5 The double-domain receptor protein tyrosine phosphatases

5.1 Tandem PTP domains in receptor PTPs

A unique characteristic of certain subtypes of receptor protein tyrosine phosphatases (PTPs) is the presence of two tandem tyrosine phosphatase domains in their intracellular regions. These subtypes include R1/R6 subtype (CD45, cPTPλ), R2A subtype (LAR, PTPσ, PTPδ), R2B subtype (PTPμ, PTPλ, PTPρ, PTPκ), R4 subtype (PTPα, PTPε) and R5 subtype (PTPζ, PTPγ; Figure 5.1) [1, 2]. The fruit fly genome of *Drosophila melanogaster* and the closely related mosquito genome of *Aedes aegypti* contain R3 subtype members that are have two tyrosine phosphatase domains [3, 4]. In literature, the two tandem domains are distinctly referred to as the membrane proximal D1 domain and the membrane distal D2 domain (Figure 5.2). In almost all reported double-domain receptor PTPs, the D1 domain carries the entire catalytic activity, while the D2 domain is a pseudophosphatase [5]. As an exception, the D2 domain of PTPα has been reported to display catalytic activity toward discrete substrates [6, 7]. CD45 was the first double-domain receptor PTP that was reported as early as in 1988 [8]. Structural exploration of these double-domain PTPs began with LAR in 1999 [9]. Since then, structures of CD45, PTPσ, PTPγ and PTPε have been reported [10].

The domain architecture of the D1 and D2 domain is extremely well conserved. Despite sequence differences in the two cognate D1 and D2 domains, their Cα traces superimpose with very low root mean square deviations. For example, in the case of LAR, the two domains only differ by a rotation of about 3.5 Å and an average difference in the Cα traces of about 1.6 Å [9]. If the differentiating loops of the two domains are not included in the superimposition, the remaining trace of about 205aa of the two domains superimposes with only 0.67 Å root mean square deviation. Similarly, the two domains of CD45 superimpose with an overall root mean square deviation of about 2.9 Å of their backbone positions [11]. Regions of structural uniqueness of the domains are localized to the surface loops that account for their surface properties (Figure 5.2). In general, the loops of the D2 domain are more surface exposed and extended when compared to the D1 domain. There is also a characteristic longer loop between the α1′ and α2′ helices in the D2 domains. Loops of the D2 domain of CD45 are associated with its molecular function in T-lymphocytes that uses CD3-mediated activation [12]. The D2 domain of CD45 also contains an acidic and a basic loop that are located on the side of the D2 domain facing the D1 domain. The acidic loop contains a phosphorylation site for casein kinase 2 [13]. Deletions in these loop regions of CD45 compromise its activity and stability [14].

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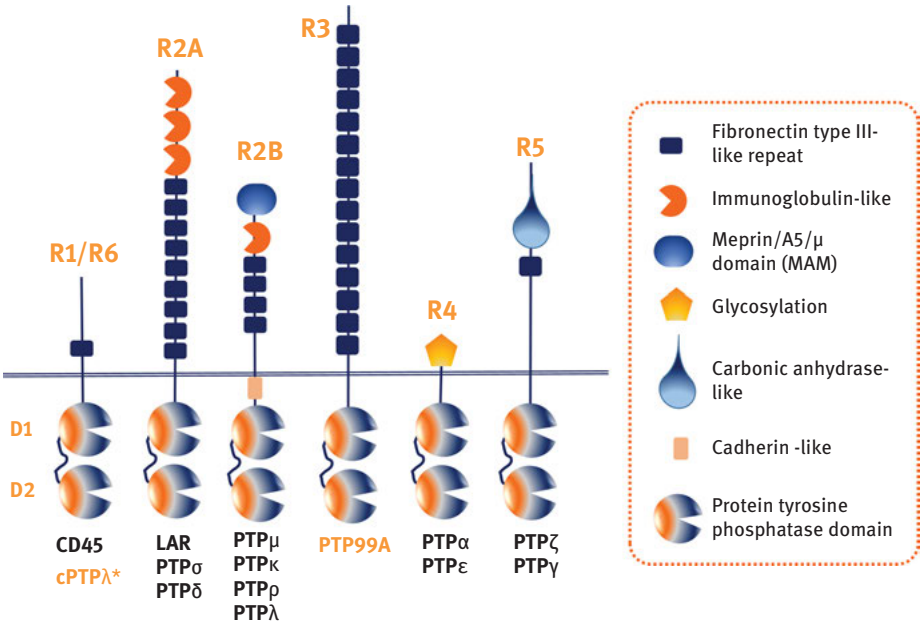


Figure 5.1: The double-domain containing receptor PTP subtypes.

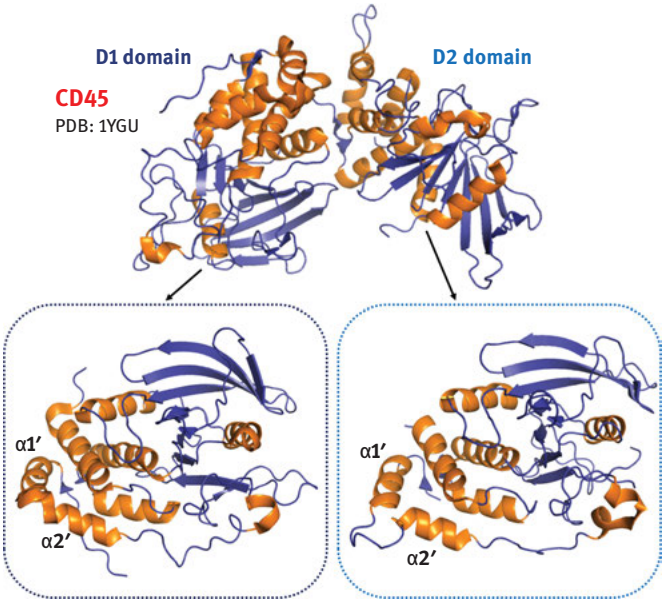


Figure 5.2: Differences in the secondary structural elements in the two tandem PTP domain of double-domain CD45.

5.2 Interdomain interactions holding the tandem PTP domains

The overall orientation of the D1 and D2 domains with respect to each other is well conserved. The two domains are organized such that their active sites are face away from each other at about 90° (Figure 5.3). The molecular separation between their active sites is about 40 Å. A short linker of ~12 amino acids connects the two domains that also share an extensive interdomain interface that buries about 2,000 Å^2 of surface area [10]. The buried surface includes interface residues that use multiple physical forces including *van der Waals* interactions, electrostatic salt bridges and hydrophobic interactions to hold the two domains together. A detailed hydrogen bonding network can be seen in the structure of LAR and CD45. This network is formed by residues from the β9, β10 strands and α3 helix from the D1 domain and α4 helix and the β2–β3 loop of their D2 domains [9, 11]. Surface residues from the α3 and α6 helices of the D1 domains complement residues from the α4 and α5 helices of the D2 domain. Conserved hydrophobic interactions have been mapped to the aromatic rings of Phe890 (α6 helix in the D1 domain) and Phe1173 (α5 helix in the D2 domain) in CD45.

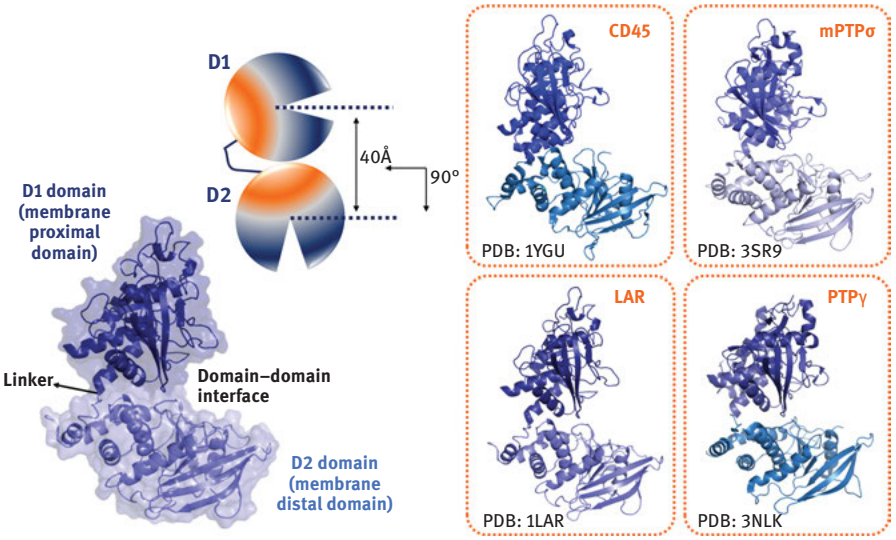


Figure 5.3: Relative orientation and organization of the two tandem D1 and D2 domains of double-domain receptor PTPs.

Conserved salt bridges include interactions between the positively charged Arg811 and Arg812 from the D1 domain of CD45 with the negatively charged Glu1167 and Asp1171 of the D2 domain of CD45. This interdomain buried surface occupies a unique pocket at the backside of the active sites of the D1 domain. Intriguingly, this pocket is an

allosteric site in the non-receptor PTP catalytic domains like PTP1B [2]. The unique placement of the D2 domains at the allosteric pocket of the D1 domains explains the modulatory role of the D2 domains and their evolutionary presence. A single-point mutation of Glu1180 to glycine in CD45 or Lys990 to alanine in PTP99A is enough to destabilize the tandem domain construct and alter their activity [4, 14]. Biochemistry experiments report that LAR mutants lacking the N-terminal residues of the D2 domain that are a part of the interdomain interface show altered catalytic kinetics for raytide and maltose-binding protein (MBP)-based substrates [15]. Insertion of a 19 amino acid peptide between the β1–β2 strands of the D2 domain of CD45 alters the interdomain interface and changes its catalytic properties [15].

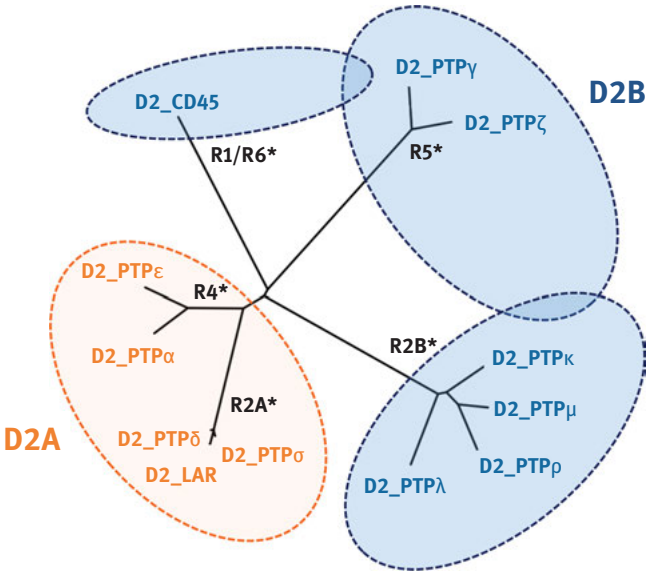
5.3 Linker connecting the two domains

The linker connecting the D1 and D2 domain is varied in both sequence and length, but the average length is about 12 amino acids. A conserved motif G[D/E]TE is located in the D1 domain at the start of the linker sequence [2]. The glycine residue in the linker functions to allow the linker to maintain an extended conformation and also makes hydrogen-bonding interactions with the α3 helix of the D1 domain. Structural analysis of the linker region elucidates its limited flexibility [9, 11] which is further confirmed by molecular dynamics simulations [16]. The extensive interdomain surface and the short length of the linker essentially lock the linker in a particular conformation. Functional significance of the linker has been elucidated in the case of CD45 where the two domains are unable to interact with each other if the linker region is absent [17]. The presence of the linker attached to the D2 domain of CD45 is crucial for its interaction with the D1 domain. Attaching the linker to the D1 domain does not compensate for the lack of linker region in the D2 domain, indicating that the linker–D2 construct is evolutionarily significant. Biochemical assays have been used to demonstrate direct interaction between the linker region and the D2 domain [17]. Site directed mutagenesis experiments that alter the linker region limit the interaction between the D1 and D2 domain. In the case of LAR, DLAR and CD45 proteins, the presence of the linker region is reported to be essential to obtain recombinant protein in soluble and stable form [14, 16, 18]. The linker of PTPα is reported to be proteolytically labile [19], suggesting that specific physiological conditions may allow for the separation of the D1 and D2 domains for the modulation of their activity and function.

5.4 Evolution of the D2 domain

Sequence alignment alongside phylogenetic analysis of the tandem tyrosine phosphatase domains of the double-domain receptor PTPs suggests that the D1

and D2 domains have evolved from a common ancestral domain and are probably products of gene duplication [2]. Sequence similarity between the D1 and D2 domains of the R2A family is more (~45%) when compared to the similarity between the D1 domains of closely related R2A and R2B subfamilies [20]. In the more carefully plotted phylogenetic trees, the D2 domain is seen to evolve independent of the D1 domain after its initial gene duplication event. This evolutionary process has given rise to two types of D2 domains that have accumulated mutations in distinct ways around the active site [21]. The first group shows more conservation with respect to its cognate D1 domains and is also capable of binding to phosphotyrosine substrates. Limited but critical mutations in the active site of these domains make them catalytically inactive. The second group has accumulated fast evolving mutations around the front as well as the back of the PTP domain-active site. These mutations result in a complete loss of catalytic or phosphotyrosine substrate-binding abilities of the D2 domain. These D2 domains are the most diverse in sequence and share poor homology among themselves when compared across the receptor PTP subfamilies. An unrooted neighbor joining phylogenetic tree of the D2 domains shows how members of the same receptor PTP subfamily cluster together on a single branch (Figure 5.4). However, they show less homology to other subfamily of D2 domains.



*Receptor protein tyrosine phosphatase subfamily

Figure 5.4: Phylogenetic tree of the D2 domains of various double-domain receptor PTPs.

5.4.1 The D2A subclass of D2 domains

The D2 domains of the R2A and R4 families of receptor PTP families including LAR, PTPσ, PTPδ, PTPα and PTPε belong to the D2A subtype. *Drosophila melanogaster* receptor PTPs DLAR and PTP69D also contain the D2A subtype of membrane distal domains. Their conserved active sites are well formed and they also have the active-site cysteine present in the conserved His–Cys–Ser triad of their P-loop. However, these D2 domains have critical point mutations in their phosphotyrosine recognition loop (pY-loop; Motif 1) wherein the phosphotyrosine recognition aromatic tyrosine or phenylalanine residue in the KNRY motif sequence is substituted for a smaller aliphatic chain amino acid like valine or leucine (Figure 5.5). This point mutation increases the $t_{1/2}$ of the tyrosine phosphatase reaction by threefold and reduces its catalytic efficiency by about 60-fold [5]. They also contain a point mutation in their WPD-loop (Motif 8) wherein the conserved acid–base aspartate is replaced by a longer glutamate residue. The longer side chain of glutamic acid obstructs its proper oxyanion formation at the tyrosine phosphatase-active site and makes the D2 domain inactive [7, 22]. The structure of LAR protein shows that the substituted glutamate of the WPD-loop (Glu 1779) interacts with the main chain of a methionine of a neighboring loop (Met 1718), making the WPD-loop rigid and less responsive to closing in the presence of a phosphotyrosine substrate [9].

D2A

	pY-loop	WPD-loop	P-loop	Q-loop
LAR	NKFKNRL	FTDWPEQGVP	PITVHCSAGVGRTG	VQTEDQYQL
PTPδ	NKFKNRL	FTDWPEQGVP	PISVHCSAGVGRTG	VQTEDQYQF
PTPσ	NKFKNRL	FTDWPEQGVP	PISVHCSAGVGRTG	VQTEDEYQF
PTPα	NMKKNRV	FHGWPEVGIP	PISVHCSAGVGRTG	VQTLDQYQF
PTPε	NMKKARV	FHGWPEIGIP	PITVHCSAGVGRTG	VQTLDQYQF

D2B

	pY-loop	WPD-loop	P-loop	Q-loop
PTPκ	NHDKNRF	YLGWASHREVP	RTIIHCLNGGGRSG	VEAPEQYRF
PTPμ	NHEKNRC	FLGWPMYRDTP	PTVVHCLNGGGRSG	VDLLDQYKF
PTPρ	NHDKNRS	YIGWPAYRDT	RTVVHCLNGGGRSG	VETLDQYKF
PTPγ	NHEKNRN	CPKWPNPDAP	PTIVHDEYGAVSAG	FTDIEQYQF
PTPζ	NREKNRT	CPKWPNPDSP	PMIVHDEHGGVTAG	FADIEQYQF
PTPλ	NRDKNRS	FLRWSAYRDTP	RTIVHCLNGGGRSG	VETMDQYHF
CD45	NKSKNRN	YTNWSVEQLP	PLLIHCRDGSQQTG	VSTFEQYQF

Figure 5.5: The two evolutionary subtypes of D2 domains. The classification is based on the sequence variation seen in the active site motifs of the pY-loop, WPD-loop, P-loop and Q-loop of these domains.

While the D2A subtype of D2 domains cannot catalyze phosphotransfer, they are reported to bind phosphotyrosine substrates with comparable affinities as their cognate D1 domains [16]. In this way, the D2A subtype functions as a "substrate trap" and participates in substrate recruitment for their D1 domains [22, 23]. Reverse engineering has been reported for these D2 domains where mutations are used to reconstitute the consensus KNRY and WPD-loop. In the case of the D2 domain of LAR, just two point mutations, Leu 1644 to Tyr in the pY-loop and Glu 1779 to Asp in the WPD-loop, are sufficient to activate its phosphatase reaction [9] Interestingly, the wild-type D2 domain of PTPα has been reported to show phosphatase activity comparable to that of its D1 domain when small molecules are used as substrates [6]. The unique D2 domain is also reported to interact with the SH2 domain of Src protein tyrosine kinase [19] Corresponding mutations to reconstitute the consensus KNRY and WPD-loops in the D2 domain of PTPα enhance its catalytic activity by 100-fold [24, 25].

5.4.2 The D2B subclass of D2 domains

The D2B subclass of the membrane distal domains have accumulated diverse mutations in their active sites including mutations in the critical P-loop. The D2B subclass includes the D2 domains of R1/R6, R2B and R5 subfamilies of receptor PTPs. These include CD45, PTPκ, PTPλ, PTPμ, PTPρ, PTPζ and PTPγ. The accumulated mutations are variable and do not show any sequence patterns that can allow for their reactivation using a simple set of reverse amino acid substitutions. This peculiarity distinguishes the D2B subtype domains from D2A even when both may carry an active site cysteine in their P-loops [26]. The D2 domain of CD45 has been studied for its sequence and structural properties [26]. Critical mutations in the D2 domain of CD45 occur around the active site cysteine (IHCRDGSQQTG instead of the consensus (V/I)HCSAGxGR(T/S)G) such that His–Cys–Ser triad of the P-loop is severely disturbed. In addition, mutation of the critical arginine of the P-loop to a shorter glutamine compromises the ability of the active to accept a negatively charged phosphotyrosine residue as a substrate. Also, the general acid–base of the WPD-loop is substituted for an aliphatic valine (Val1102). Moreover, two acidic side chains of Asp1146 and Glu1149 occupy the active site and make the active site electrostatics repulsive for the incoming phosphotyrosine substrate. Although the proteolytically cleaved D2 domain of CD45 has been shown to harbor some phosphatase activity in specialized research experiments [27] these studies are not re—substantiated using elaborate biochemical or structural studies.

The D2 domains of the R5 subtype of receptor PTPs are characterized by the mutation of their active site cysteine to a negatively charged aspartate residue (Figure 5.5) [2]. The negative charge of the aspartate makes the D2 domain of the R5 subtype most unresponsive to binding a phosphotyrosine. Similarly, in the *Drosophila melanogaster* PTP99A,

the P-loop consensus sequence is altered drastically to substitute the consensus (V/I) HCSAGxR(T/S)G for an incompatible ICDRIGGAQA sequence [4, 28]. Biochemical experiments to study the binding of phosphotyrosine memetic *para*-Nitrocatechol Sulfate (PNC) to the D2 domain of PTP99A reveal a poor binding affinity of ~800 μM as opposed to the ~95 μM dissociation constant for the cognate D1 domain [4, 5].

5.5 D2 domains affect substrate selection of the cognate D1 domains

The double-domain receptor PTPs have been extensively characterized as both double-domain and single PTP domain constructs using biochemical experiments [4, 6, 7, 15, 20, 28–32]. Some the earliest experiments done in the 1990s demonstrated that the two tyrosine phosphatase domains of PTPα showed distinct substrate specificities. Three distinct substrates were used. These included the phosphorylated MBP, the peptide RR-Src and the small molecule *para*-Nitrophenyl Phosphate (*p*NPP). The D2 domain of PTPα shows a substantially higher substrate preference for the small-molecule *p*NPP over the peptide or protein substrate (*p*NPP ⋙ RR-Src > MBP) as compared to its cognate D1 domain that showed a preference order as MBP~RR-Src > *p*NPP. These observations also showed that PTPα was unique in having a D2 domain that was not inactive. However, the D2 domain of PTPα only showed a substantial and comparable phosphatase activity for the small-molecule *p*NPP as compared to peptide and protein-based substrates. This conundrum is probably due to the conformational restraints of the phosphotyrosine in accessing the active site of the D2 domain of PTPα. Studies to understand the hypothesis of conformational dependence of peptide binding and catalysis at the PTPα D2 domain-active site show contrasting results [25]. Mutations in the pY-loop Val536 to tyrosine and the WPD-loop Glu671 to aspartate allowed for only 90-fold increase in its catalytic efficiency. This was in contrast to a 150,000-fold decrease in the catalytic activity of the cognate D1 domain of PTPα if corresponding mutations were introduced it its pY- and WPD-loop (Tyr243 to valine and Asp382 to glutamate). These studies indicated that while the microenvironment of the active site would be crucial for deciding if the domain has phosphatase activity, the synergetic association of the two domains and surface charges around their active sites would be critical factors in deciding their substrate specificity.

5.6 D2 modulates the activity of its cognate D1 domain

Biochemical studies to understand the role of the D2 domain have been reported for various receptor PTPs. Early studies on the individual domains of CD45 showed an absolute necessity for the presence of the D2 domain for CD45 to demonstrate tyrosine phosphatase activity [15, 18]. The D2 domain of CD45 is reported to directly participate

in substrate recruitment and binds the TCR-ζ chain in vivo [33] The catalytic efficiency of the D1 domain of CD45 is compromised by as much as 1,000-fold in the absence of its cognate D2 domain. Constructs with deletions in the N-terminal region of the D2 domain retain their efficiency for Raytide substrate but show a sixfold increase in using MBP as the substrate. Studies using small-molecule *p*NPP show that the D1 domain of CD45 is only half as catalytically efficient when compared to the double-domain construct [14]. The presence of D2 domain allows for increased efficiency of the D1 domain in utilizing CD3ζ, Fyn, Src, cdc2 and PDGR phosphopeptides as substrates. Thus, the D2 domain of CD45 activates its D1 domain and also modulates its biological substrate recruitment. This activation of the D1 domain is reported to be self-specific as domain-swapped constructs using the D1 domain of CD45 and the D2 domain of LAR fail to show comparable catalytic activities [15, 18, 33].

The D2 domains of PTP99A and CD45 have been reported to contribute to the structural stability of their cognate D1 domain [4, 14]. CD45 loses catalytic activity and also shows thermos-sensitivity in the absence of its D2 domain [14, 18] Reconstitution of the recombinant form of D1 of CD45 with the recombinant form of its D2 domain increases its catalytic activity by various folds and also relieves its thermos-sensitivity. This indicates toward an evolutionary role of the D2 domain of CD45 in cofolding and D1 domain stabilization. Another D2B subtype of D2 domain studied for its stabilizing effect on its D1 domain is that of the *Drosophila melanogaster* PTP99A. PTP99A belongs to the R3 subfamily of receptor PTP and is unique in harboring two tandem tyrosine phosphatase domains as an R3 subtype. PTP99A does not have any homologues in the human genome but finds many in the fly and mosquito phosphatome. The D2 domain of PTP99A belongs to the D2B subtype and lacks a well-formed active site P-loop and also the active site cysteine. Biochemical experiments have been reported for the recombinant expressed individual tyrosine phosphatase domains of PTP99A and also for the double-domain protein. The D2 domain of PTP99A is more stable than the cognate D1 domain as seen from its ~twofold higher change in heat capacity of unfolding (ΔC_p) and a 2 kcal/mol lower free energy of unfolding (ΔG) [4]. The thermal melting points (T_m) of the D1 and D2 domain of PTP99A are about 20° apart. D2 domain unfolds and aggregates at a much slower rate, with a lag time difference in the $t_{1/2}$ of aggregation at 38 sec when compared to the D1 domain. The double-domain PTP99A construct shows a biphasic unfolding curve that shows concerted unfolding of the two domains when present together. The double-domain construct is more stable than the D1 domain alone ($\Delta G_{unfolding}$ D1 domain = −6.8 kcal/mol; $\Delta G_{unfolding}$ D1D2 = −4.3 kcal/mol). External reconstitution of the D1 domain with the individually expressed D2 domain fails to provide it the same thermodynamic stability. These studies indicate how the D2B subtypes of D2 domains are crucial for stabilizing an otherwise weak tyrosine phosphatase domains in the double-domain proteins.

The D2 domain of PTP99A also serves as an activator of its cognate D1 domain [4]. The presence of the D2 domain enhances the catalytic activity of the D1 domain by as much as 250-fold for small molecules and more than 30-fold for

phosphopeptides-based substrates. The D2 domain of PTP99A shows low affinity binding to small molecules even in the absence of a functional P-loop. This D2 domain also effects the substrate preference of its D1 domain that changes from abelson > cuticle > nervous fingers > myospheroid > insulin receptor for the D2 domain to insulin receptor > myospheroid > abelson > cuticle > nervous fingers for the double-domain protein. The catalytic efficiency of the double-domain protein is substantially enhanced such that the difference in the most and least preferred substrate is about ~500-fold more than the D1 domain alone [30×10^2 sec^{-1} M^{-1} for the D1 domain; 152×10^4 sec^{-1} M^{-1} for the D1D2 double-domain protein). Titration of the D1 domain with increasing quantities of the D2 domain results in an increase in its catalytic turnover in accordance with the "nonessential partially mixed" model of enzyme activation (Figure 5.6). Here, the D2 domain serves as an activator that simultaneously increases the catalytic turnover rate of the D1 domain and also enhances its affinity for its substrate molecules. The domain–domain interface of the D1 and D2 domains of PTP99A is found to be crucial to their interaction, wherein a mutation in a semiconserved lysine (Lys990 to alanine) abrogates the nonessential partially mixed activation contributed by the D2 domain [4].

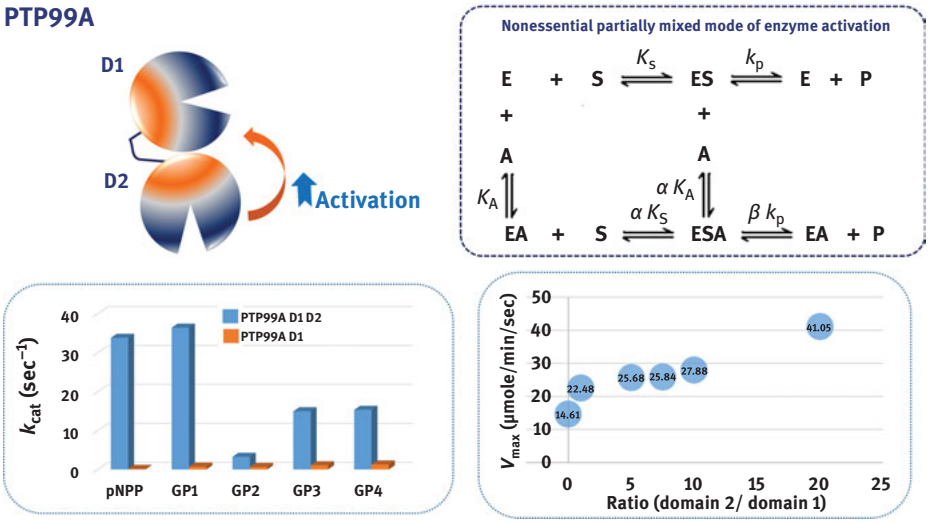


Figure 5.6: The D2 domain of PTP99A acts as a positive activator for catalytic activity of it cognate D1 domain.

While in the case of the D2B subtype of D2 domains of PTP99A and CD45 are reported to be activating modulators of their D1 domains, the D2A subtype of D2 domains of LAR and its *Drosophila melanogaster* homologue DLAR are reported to be inhibitory to their

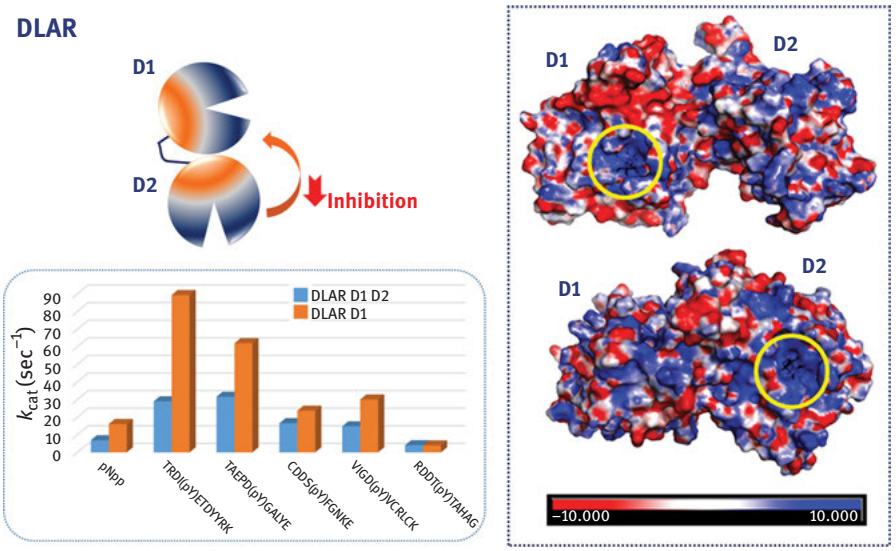


Figure 5.7: The D2 domain of DLAR shows a highly conserved active site, much like that of its D1 domain. The D2 domain of DLAR inhibits the activity of its cognate D1 domain.

D1 domains (Figure 5.7). Moreover these D2A subtype of D2 domains show an ability to bind substrates and creating a competition for their D1 domains in sequestering peptides. In the case of LAR, the catalytic activity of its D1 domain is about eightfold more in the absence of its D2 domain [15, 28]. Similarly the D1 domain of DLAR is much more active (specific activity$_{(pNPP)D1}$ = 20.6 µmol/min/sec) in the absence of the D2 domain (specific activity$_{(pNPP)D1D2}$ = 6.2 µmol/min/sec) [28]. The substrate preference of the two domains of DLAR has been characterized by enzyme kinetics and also surface plasmon resonance. The D2 domain of DLAR shows a preference pattern of insulin receptor > nervous fingers > cuticle > myospheroid > abelson phosphopeptides. This is distinct from that of the D1 domain as insulin receptor > cuticle > nervous fingers > myospheroid > abelson phosphopeptides. In contrast to PTP99A, the catalytic efficiency difference between the most and least preferred phosphopeptide for the DLAR double-domain protein is less [16×10^4 sec^{-1} M^{-1}) when compared to the D1 domain alone [130×10^4 sec^{-1} M^{-1}). Phosphopeptide binding to the double-domain protein shows that the D2 domain binds tighter to the insulin receptor and cuticle protein-based phosphopeptides and competes with the D1 domain for substrates. Mutation of the active site cysteine to a serine of the D2 domain in the double-domain construct leads to a 1,000-fold reduction in the association kinetics of the phosphopeptides [28]. Small-molecule binding experiments show that the D2 domain of DLAR can bind phosphotyrosine mimetics with marginally better affinity ($K_{D(PNC)}$ D1 domain = 235 µM; $K_{D(PNC)}$ D2 domain = 214 µM), indicating that the phosphotyrosine-binding environment of the two domains is comparable.

The physiological significance of the D2 domain and its modulation of the D1 domain have been studied for LAR and DLAR using Chinese hamster ovary (COS-7) lines and *Drosophila* embryos respectively. Like in all double-domain receptor PTPs, all the catalytic activity is limited to the D1 domain. However, the molecular mimicry seen in their D2 domain allows them to participate in substrate association. Studies using coimmunoprecipitation of the insulin receptor by various constructs of LAR overexpressed in COS-7 cells demonstrate this role of its D2 domain [29, 34]. Cells are stimulated with insulin that allows for phosphorylation of the receptor's activation loop in the protein tyrosine kinase domain. This segment of the insulin receptor serves as the substrate for LAR in COS-7 cells. COS-7 cells were transfected with wild-type LAR (D1/D2 LAR), a double-domain construct having the D1 domain-active site cysteine mutated to a serine (D1CS/D2; Cys1522 to serine), a double-domain construct having the D2 domain-active site cysteine mutated to a serine (D1/D2CS; Cys1813 to serine) or both the D1 and D2 domains mutated at their active sites (D1CS/D2CS). Cells lysates were prepared after 1 min of insulin stimulation to study the levels of phosphoinsulin receptor that coimmunoprecipitate with LAR. The phosphoinsulin receptor was efficiently coimmunoprecipitated by D1CS/D2 construct and moderately coimmunoprecipitated by D1/D2 LAR and D1CS/D2CS. The D1/D2CS construct showed negligible coimmunoprecipitation of the insulin receptor. In addition, the total tyrosine phosphorylation of the insulin receptor was least in the cell transfected with D1D2 LAR as compared to D1CS/D2, D1/D2CS or the D1CS/D2CS constructs. This indicated that both the D1 and D2 domains of LAR are important for insulin receptor binding, but the active site of the D2 domain is more crucial for its physiological association and activity. Correspondingly, experiments were done with various cysteine to serine mutants of DLAR in *Drosophila* embryos [23]. These constructs were expressed in embryos in under a GAL-4 promoter and would be able to rescue lethality depending on their ability to associate with their physiological substrates. Wild-type DLAR is able to rescue 100% lethality and serves as the control. The D1 domain when expressed alone only rescues about 50% of the embryos. This clearly shows that the presence of the D2 domain is crucial for substrate recognition and association.

5.7 Role of inter- and intradomain amino acid networks

The modulatory effect of the D2 domains on their partner D1 domains has also been explored using molecular dynamics simulations on the *Drosophila* receptor PTPs DLAR and PTP99A [28] Interatomic interaction matrices have been reported for short simulation of about 20 ns that allow for accessing the fast motions in the side chains of all amino acids in the catalytic domains. These matrices are then analyzed for functionally important residues (FIRs) that include various amino acids from the P-loop, pY-loop, WPD-loop and the Q-loop of both the D1 and D2 domains of DLAR and PTP99A. These networks are then compared to the network obtained for the

single domain cytosolic PTP1B that serves as the benchmark. These networks provide a molecular allostery-based explanation for the activating effect of the D2 domain in PTP99A and the contrasting inhibitory effect of the D2 domain of DLAR (Figure 5.8]. Optimal functioning of the catalytic center of the tyrosine phosphatase domain requires molecular integration of the FIRs. In the case of the D1 domain of PTP99A, the WPD-loop residues are seen to distinctly cluster away from the other FIRs when the D2 domain is absent. The presence of the D2 domains allows for the merging of the WPD-loop network with other elements of the active site and this activates the D1 domain. Correspondingly, the D2 domain of DLAR alters the interaction network of its D1 domain such that its Q-loop glutamine residues are clustered separately from the other FIRs. This D2 domain is hence inhibitory to its partner D1 domain.

5.8 Role of D2 domain as a redox protector

The reversible oxidation of the active site cysteine serves as an important regulatory mechanism for various receptor PTPs and is commonly seen in cells stimulated by EGF, PDGF and insulin [35–38]. The reactive oxygen species (ROS) include the bioactive form of peroxide (H_2O_2) that transfers its hydronium to the active site thiolate (S^-) of the tyrosine phosphatase catalytic domain. The sulfenic acid (S–OH) formed at the active site is inactive and no longer serves as a nucleophile to dephosphorylate a phosphotyrosine. This sulfenic acid is converted back to its thiolate form by glutathionylation and subsequent reduction by thioltransferases [39, 40]. This redox regulation of tyrosine phosphates is studied in most detail for the cytosolic PTP1B [40]. Redox regulation of double-domain receptor PTPs is reported for PTPα and PTPσ, wherein their D2 domain plays the most crucial role [41]. Comparison of the active sites of the D1 and D2 domains of PTPα shows that the D2 domain is more susceptible to reaction with ROS [42]. ROS oxidize the cysteine at the active site of the D2 domain of PTPα to the sulfenic acid form that causes a major protein conformational change. The linker region is strained such that a rotation between the two domains of one of the PTPα proteins allows it to dimerize with another PTPα via its D2 domain. This dimerization locks the D1 domains of the dimer in an inaccessible configuration, thereby shielding its active site from ROS. In this way, the D2 domain functions as a redox sensor that protects its cognate D1 domain under oxidative stress conditions in the cell. Another set of experiments elucidate that oxidative stress-induced dimerization of PTPα via its D2 domain is also relayed to the extracellular domains on the cell surface [43]. In this way, the D2 domain of PTPα participate in inside-out or reverse cell signaling that allows for modulation of PTPα in getting access to its extracellular ligands when the cells are facing oxidative stress internally.

The role of the D2 domain as a redox protector of the D1 domain has also been reported for PTPε [44]. The D2 domain of PTPε uses its N- and C-terminal tails as the redox sensor. Upon stimulation of cells with EGF and subsequent oxidative stress, the

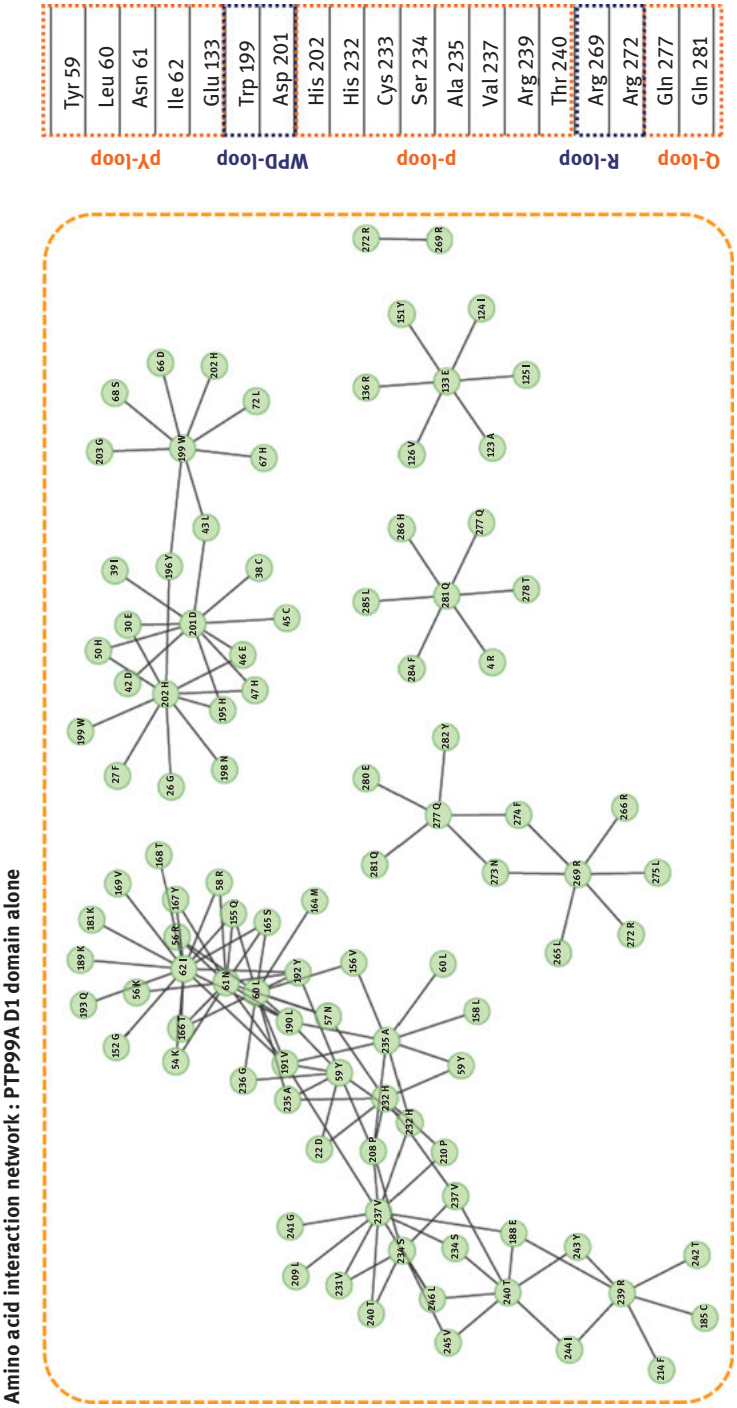


Figure 5.8: **(a)** Molecular dynamics simulations-based amino acid network map of the D1 domain (alone) of PTP99A. Adapted from the open resource at http://caps.ncbs.res.in/download/dlar_ptp99a/

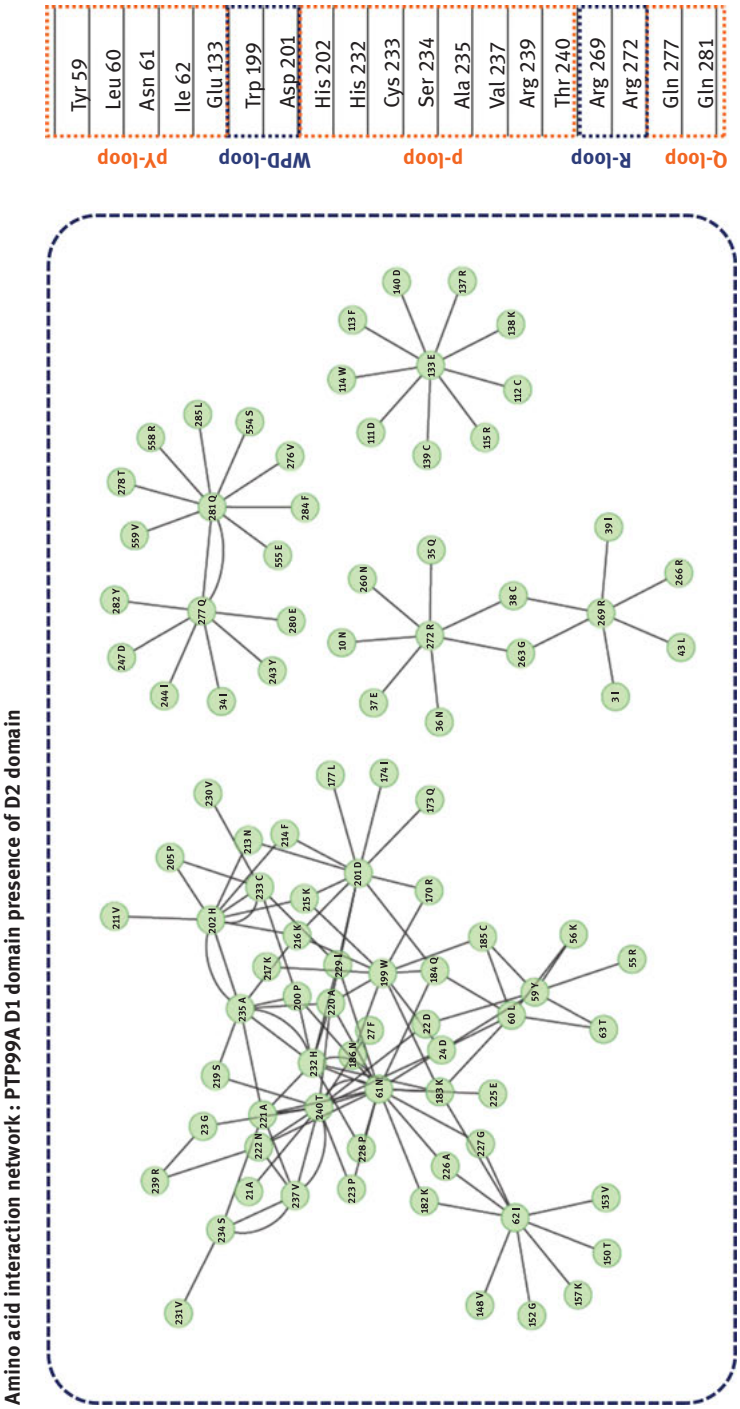


Figure 5.8: **(b)** Molecular dynamics simulations based amino acid network map of the D1 domain (with attached D2 domain) of PTP99A. Adapted from the open resource at http://caps.ncbs.res.in/download/dlar_ptp99a/

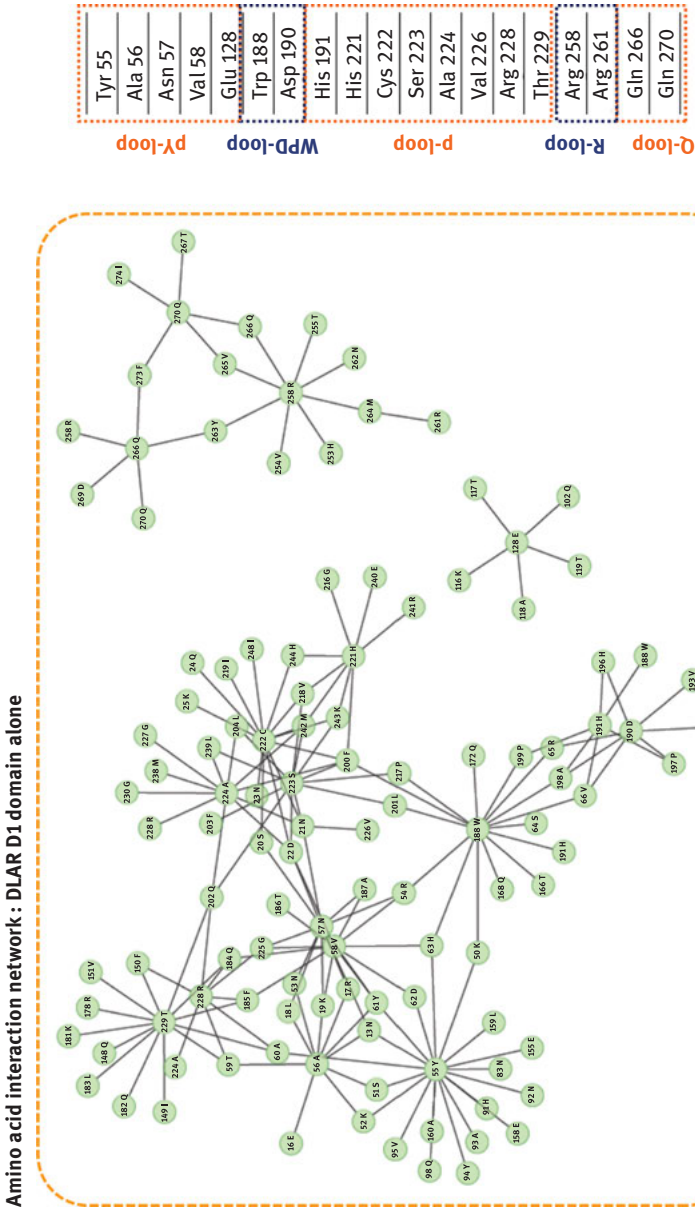


Figure 5.8: **(c)** Molecular dynamics simulations based amino acid network map of the D1 domain (alone) of DLAR. Adapted from the open resource at http://caps.ncbs.res.in/download/dlar_ptp99a/

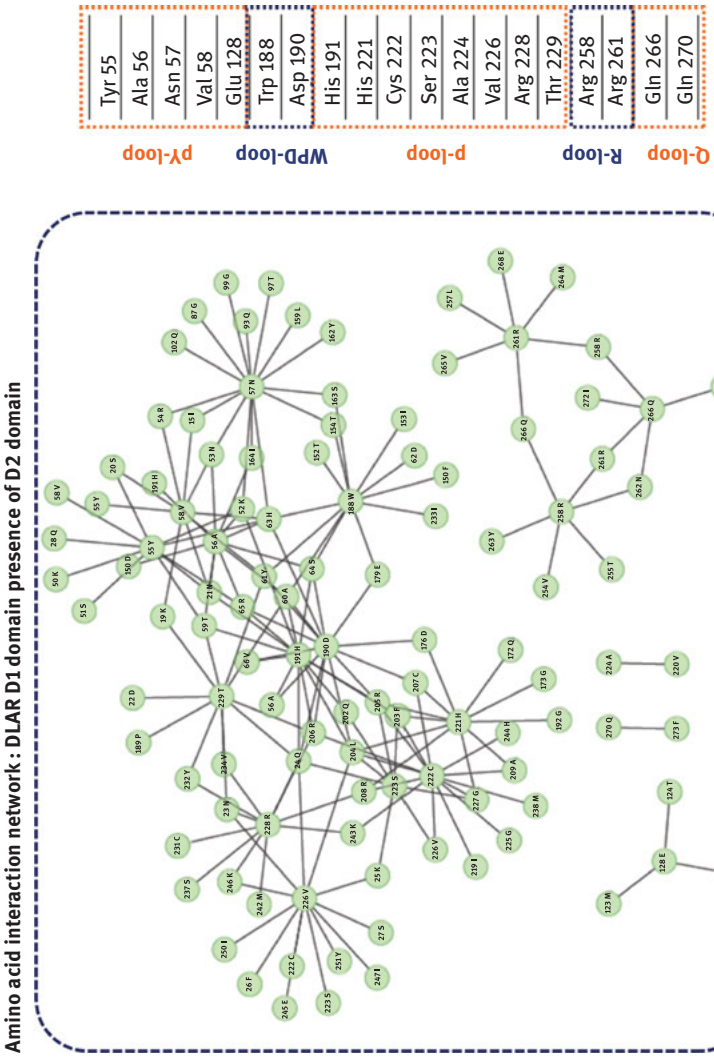


Figure 5.8: **(d)** Molecular dynamics simulations based amino acid network map of the D1 domain (with attached D2 domain) of DLAR. Adapted from the open resource at http://caps.ncbs.res.in/download/dlar_ptp99a/

monomeric PTPε dimerizes and is locked into an inactive state. This regulatory dimerization is dependent on residues 360–380 (N-terminal region of the D2 domain) and residues 621–643 (C-terminal region of the D2 domain). Deletion of these residues allows for a constitutive dimerization of PTPε that is inhibited as the active site of the D1 domains of the dimer is inaccessible. These studies indicate that the N- and C-terminal tails of the D2 domain allow for PTPε dimerization only in the need of the cell when it faces oxidative stress. In this way the D2 domain of PTPε serves as a redox shield for its D1 domain.

5.9 Dimerization in double-domain receptor PTPs

Receptor proteins are known to dimerize in the needs of the cell to propagate cellular signals across the cell surface [45]. The receptor protein tyrosine kinases use dimerization as an important mode of activation that allows the *trans*-phosphorylation of their protein kinase domains. However, just like the contrast in the functionality of protein kinases and protein phosphatases, the receptor PTPs are reported to be inhibited by dimerization [46, 47]. The first studies to understand the role of dimerization involved chimeras of CD45 with the receptor protein kinase EGFR [47]. The intracellular domains of EGFR were swapped for the intracellular double PTP domains of CD45. Upon stimulation with a ligand and the dimerization of the chimera via the extracellular region of EGFR, the CD45 double domains showed an inhibition in activity with a complete loss of TCR signaling. In subsequent cell-based experiments dimers of the intact wild-type CD45 were observed using chemical crosslinking and fluorescence resonance energy transfer techniques [48, 49]. Dimerization of CD45 is also reported to vary in its various isoforms [50].

Dimerization on the cell surface has also been reported for PTPα [51]. Conformational explanation of tyrosine phosphatase inhibition comes from the crystal structure of the PTPα dimer [52]. The crystal structure shows how the D1 domains of PTPα are inhibited by each other when an inhibitory wedge of one monomer occupies and blocks the active site of the other (Figure 5.9). This "wedge" is formed by the helices α1′ and α2′ that form the most diverse part of a tyrosine phosphatase domain [53]. The two helices make a helix-turn-helix turn motif in PTPα and these are present in the N-terminal region of the tyrosine phosphatase domain upstream of the pY-loop. Following these reports of the inhibitory wedge, another group reported that these wedge peptides could be used as inhibitors for other receptor PTPs [54]. In this study the wedge peptides of LAR were used to successfully inhibit its activity in biochemical experiments. However, the mechanism of domain swapping-based wedge-mediated inhibition has now been disapproved as a universal system of inhibition as the crystal structures of double-domain tyrosine phosphatases have become available. Prior studies were seen to be biased in having only the D1 domain in their experiments; or soluble short peptides corresponding to the wedge sequence were used. These did not

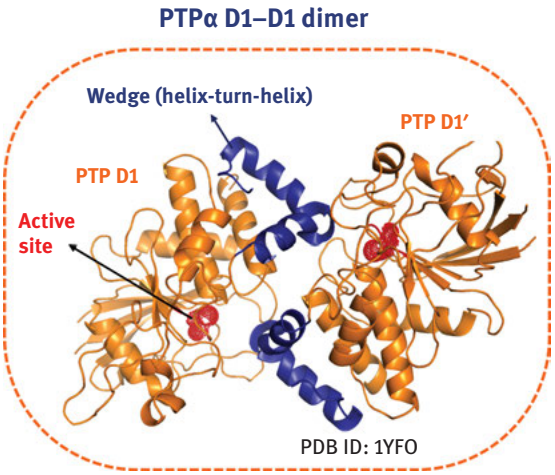


Figure 5.9: The inhibitory wedge domain as seen in the crystal structure of the dimer of the D1 domains of PTPα.

account for the conformational and steric clash that would be caused due to the presence of the D2 domain. Crystal structures of the double-domain constructs clearly demonstrate that wedge-mediated dimerization would be improbable in the presence of the D2 domain when two monomers try to dimerize head-to-head as seen for the D1 domains of PTPα [9–11]. However, crystal structures and biochemical experiments suggest a head-to-toe mode of dimerization of the double-domain proteins (Figure 5.10]. In this mode, the D1 domain of one monomer binds the D2 domain of the other. This model finds support in biochemical reports that show deletions in the

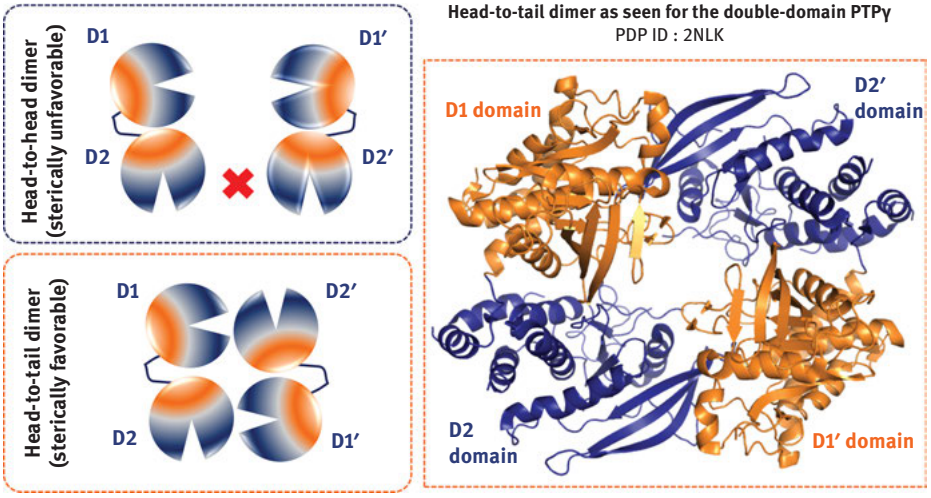


Figure 5.10: The head-to-tail dimerization mode of the double-domain PTPs.

wedge domains of the D1 domains of PTPα and LAR affect their ability to bind their D2 domains [55, 56]. Interestingly, deletion of the wedge region of the D1 domain of PTPα allows for its tighter association with its D2 domain. This reports hence suggest alternate and yet unexplored modes of tyrosine phosphatase dimerization in the double-domain proteins.

5.10 Role of D2 domain in receptor cross talk

Cross-talk between receptor proteins in signaling is known to be a common mode of interaction especially when multiple receptors are expressed at a common developmental stage and in related pathways [57, 58]. Cross-talk in receptor PTPs was first reported between the D1 domain of PTPσ with the D2 domain of PTPδ in yeast two hybrid screens [59] Binding of the D2 domain of PTPδ inhibited the catalytic activity of the D1 of PTPσ. The D1 domain of PTPσ was also reported to coimmunoprecipitate with the D2 domain of PTPδ when they were expressed in mammalian cells. This work also reported a weak binding interaction for the D1 domain of LAR and the D2 domain of PTPδ. Subsequently, cross-talk between various D1 and D2 domains of varied receptor PTPs has been reported in literature [55, 56]. The D2 domain of PTPσ is reported to bind the D1 domains of PTPα, PTPδ, PTPσ, PTPμ, LAR and CD45 with comparable affinities. The D2 domain of PTPε also binds the D1 domain of PTPα, PTPδ, PTPσ, PTPμ, LAR and CD45 and has a broad specificity. The D1 domain of PTPα interacts with the D2 domains of various double-domain receptor PTPs and shows a preference order of D2 PTPδ > D2 PTPσ > D2 LAR > D2 PTPα > D2 PTPμ. LAR and CD45 are reported to be the most stringent in receptor cross-talk. The D2 domain of LAR binds preferentially to the D1 domain of PTPα with high affinity. It binds with moderate affinity to the D1 domain of PTPε and shows poor affinity for the D1 domains of PTPσ, PTPδ and CD45. The D2 domain of CD45 is highly specific in its interaction. It only shows interactions with the D1 domains of PTPα and PTPε, that too with low affinity. The D2 domain of PTPμ binds only the D1 domain of LAR; however, the D2 domain of LAR does not cross-talk with the D1 domain of PTPμ.

Various regions in the double-domain protein constructs contribute to the interprotein cross-talk. The C-terminal tail of the D2 domains is an important determinant such that deletion in this segment of PTPδ abolishes its D2 domain's interaction with the D1 domain of PTPα. However, this deleted D2 domain of PTPδ now interacts with the D1 domains of PTPμ and LAR. These interactions are not seen for the undeleted D2 domain of PTPδ. Hence, the C-terminal tail functions as a sensor that decides the mode of cross-talk between the various double-domain proteins. A physiological significance of this cross-talk becomes more pertinent in the light of reports that suggest that proteolytic cleavage of the linker connecting the two domains to be proteolytically labile [19] This suggests an interesting scenario wherein the double-domain proteins could establish a critical interprotein cross-talk in the need of the

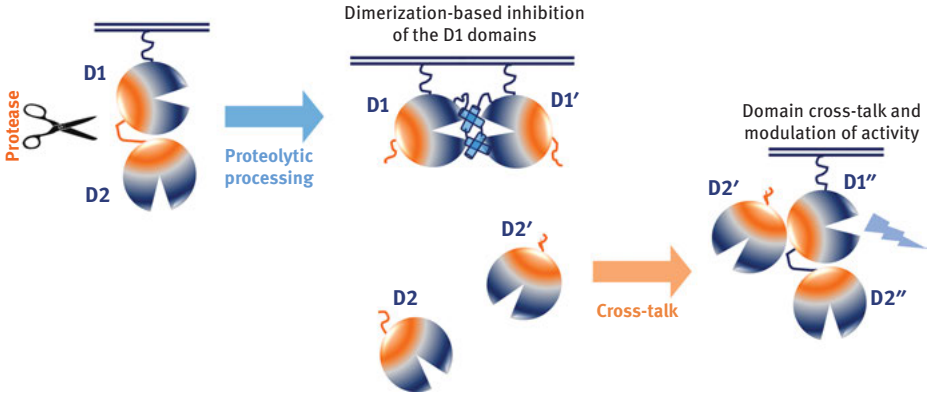


Figure 5.11: Receptor cross-talk and activity modulation of the D1 domains by the D2 domains of same or different double-domain receptor PTPs.

cell (Figure 5.11). This model also suggests a novel tyrosine phosphatase modulation in *trans* wherein different double-domain tyrosine phosphatases could modulate each other via their D2 domains.

References

[1] Andersen JN, Jansen PG, Echwald SM, Mortensen OH, Fukada T, Del Vecchio R et al. A genomic perspective on protein tyrosine phosphatases: gene structure, pseudogenes, and genetic disease linkage. FASEB J. 2004;18(1):8–30.

[2] Andersen JN, Mortensen OH, Peters GH, Drake PG, Iversen LF, Olsen OH et al. Structural and evolutionary relationships among protein tyrosine phosphatase domains. Mol Cell Biol. 2001;21(21):7117–36.

[3] Moretti DM, Ahuja LG, Nunes RD, Cudischevitch CO, Daumas-Filho CRO, Medeiros-Castro P et al. Molecular analysis of Aedes aegypti classical protein tyrosine phosphatases uncovers an ortholog of mammalian PTP-1B implicated in the control of egg production in mosquitoes. PLOS ONE. 2014;9(8):e104878.

[4] Madan LL, Goutam K, Gopal B. Inter-domain interactions influence the stability and catalytic activity of the bi-domain protein tyrosine phosphatase PTP99A. Biochim Biophys Acta. 2012; 1824(8):983–90.

[5] Madan LL, Gopal B. Conformational basis for substrate recruitment in protein tyrosine phosphatase 10D. Biochemistry. 2011;50(46):10114–25.

[6] Wang Y, Pallen CJ. The receptor-like protein tyrosine phosphatase HPTP alpha has two active catalytic domains with distinct substrate specificities. EMBO J. 1991;10(11):3231–7.

[7] Wu L, Buist A, den Hertog J, Zhang ZY. Comparative kinetic analysis and substrate specificity of the tandem catalytic domains of the receptor-like protein-tyrosine phosphatase alpha. J Biol Chem. 1997;272(11):6994–7002.

[8] Charbonneau H, Tonks NK, Walsh KA, Fischer EH. The leukocyte common antigen (CD45): a putative receptor-linked protein tyrosine phosphatase. Proc Natl Acad Sci U S A. 1988; 85(19):7182–6.

[9] Nam HJ, Poy F, Krueger NX, Saito H, Frederick CA. Crystal structure of the tandem phosphatase domains of RPTP LAR. Cell. 1999;97(4):449–57.
[10] Barr AJ, Ugochukwu E, Lee WH, King ON, Filippakopoulos P, Alfano I et al. Large-scale structural analysis of the classical human protein tyrosine phosphatome. Cell. 2009;136(2):352–63.
[11] Nam HJ, Poy F, Saito H, Frederick CA. Structural basis for the function and regulation of the receptor protein tyrosine phosphatase CD45. J Exp Med. 2005;201(3):441–52.
[12] Greer SF, Wang Y, Raman C, Justement LB. CD45 function is regulated by an acidic 19-amino acid insert in domain II that serves as a binding and phosphoacceptor site for casein kinase 2. J Immunol. 2001;166(12):7208–18.
[13] Wang Y, Guo W, Liang L, Esselman WJ. Phosphorylation of CD45 by casein kinase 2. Modulation of activity and mutational analysis. J Biol Chem. 1999;274(11):7454–61.
[14] Felberg J, Johnson P. Stable interdomain interaction within the cytoplasmic domain of CD45 increases enzyme stability. Biochem Biophys Res Commun. 2000;271(2):292–8.
[15] Streuli M, Krueger NX, Thai T, Tang M, Saito H. Distinct functional roles of the two intracellular phosphatase like domains of the receptor-linked protein tyrosine phosphatases LCA and LAR. EMBO J. 1990;9(8):2399–407.
[16] Madan LL, Veeranna S, Shameer K, Reddy CCS, Sowdhamini R, Gopal B. Modulation of catalytic activity in multi-domain protein tyrosine phosphatases. PLOS ONE. 2011;6(9):e24766.
[17] Hayami-Noumi K, Tsuchiya T, Moriyama Y, Noumi T. Intra- and intermolecular interactions of the catalytic domains of human CD45 protein tyrosine phosphatase. FEBS Lett. 2000;468(1):68–72.
[18] Felberg J, Johnson P. Characterization of recombinant CD45 cytoplasmic domain proteins. Evidence for intramolecular and intermolecular interactions. J Biol Chem. 1998; 273(28):17839–45.
[19] Sonnenburg ED, Bilwes A, Hunter T, Noel JP. The structure of the membrane distal phosphatase domain of RPTPalpha reveals interdomain flexibility and an SH2 domain interaction region. Biochemistry. 2003;42(26):7904–14.
[20] Krueger NX, Streuli M, Saito H. Structural diversity and evolution of human receptor-like protein tyrosine phosphatases. EMBO J. 1990;9(10):3241–52.
[21] Pils B, Schultz J. Evolution of the multifunctional protein tyrosine phosphatase family. Mol Biol Evol. 2004;21(4):625–31.
[22] Flint AJ, Tiganis T, Barford D, Tonks NK. Development of “substrate-trapping” mutants to identify physiological substrates of protein tyrosine phosphatases. Proc Natl Acad Sci. 1997; 94(5):1680–5.
[23] Krueger NX, Reddy RS, Johnson K, Bateman J, Kaufmann N, Scalice D et al. Functions of the ectodomain and cytoplasmic tyrosine phosphatase domains of receptor protein tyrosine phosphatase Dlar in vivo. Mol Cell Biol. 2003;23(19):6909–21.
[24] Buist A, Zhang YL, Keng YF, Wu L, Zhang ZY, den Hertog J. Restoration of potent protein-tyrosine phosphatase activity into the membrane-distal domain of receptor protein-tyrosine phosphatase alpha. Biochemistry. 1999;38(3):914–22.
[25] Lim KL, Kolatkar PR, Ng KP, Ng CH, Pallen CJ. Interconversion of the kinetic identities of the tandem catalytic domains of receptor-like protein-tyrosine phosphatase PTPalpha by two point mutations is synergistic and substrate-dependent. J Biol Chem. 1998;273(44):28986–93.
[26] Lim KL, Ng CH, Pallen CJ. Catalytic activation of the membrane distal domain of protein tyrosine phosphatase epsilon, but not CD45, by two point mutations. Biochim Biophys Acta. 1999; 1434(2):275–83.
[27] Tan X, Stover DR, Walsh KA. Demonstration of protein tyrosine phosphatase activity in the second of two homologous domains of CD45. J Biol Chem. 1993;268(10):6835–8.
[28] Madan LL, Veeranna S, Shameer K, Reddy CC, Sowdhamini R, Gopal B. Modulation of catalytic activity in multi-domain protein tyrosine phosphatases. PLoS One. 2011;6(9):e24766.

[29] Tsujikawa K, Kawakami N, Uchino Y, Ichijo T, Furukawa T, Saito H et al. Distinct functions of the two protein tyrosine phosphatase domains of LAR (leukocyte common antigen-related) on tyrosine dephosphorylation of insulin receptor. Mol Endocrinol. 2001; 15(2):271–80.
[30] Itoh M, Streuli M, Krueger NX, Saito H. Purification and characterization of the catalytic domains of the human receptor-linked protein tyrosine phosphatases HPTP beta, leukocyte common antigen (LCA), and leukocyte common antigen-related molecule (LAR). J Biol Chem. 1992;267(17):12356–63.
[31] Cho H, Krishnaraj R, Itoh M, Kitas E, Bannwarth W, Saito H et al. Substrate specificities of catalytic fragments of protein tyrosine phosphatases (HPTP beta, LAR, and CD45) toward phosphotyrosylpeptide substrates and thiophosphotyrosylated peptides as inhibitors. Protein Sci. 1993;2(6):977–84.
[32] Cho H, Ramer SE, Itoh M, Kitas E, Bannwarth W, Burn P et al. Catalytic domains of the LAR and CD45 protein tyrosine phosphatases from Escherichia coli expression systems: purification and characterization for specificity and mechanism. Biochemistry. 1992;31(1):133–8.
[33] Kashio N, Matsumoto W, Parker S, Rothstein DM. The second domain of the CD45 protein tyrosine phosphatase is critical for interleukin-2 secretion and substrate recruitment of TCR-zeta in vivo. J Biol Chem. 1998;273(50):33856–63.
[34] Konishi N, Tsujikawa K, Yamamoto H, Ishida E, Nakamura M, Shimada K et al. Overexpression of leucocyte common antigen (LAR) P-subunit in thyroid carcinomas. Br J Cancer. 2003; 88(8):1223–8.
[35] Salmeen A, Barford D. Functions and mechanisms of redox regulation of cysteine-based phosphatases. Antioxid Redox Signal. 2005;7(5–6):560–77.
[36] Mahadev K, Zilbering A, Zhu L, Goldstein BJ. Insulin-stimulated hydrogen peroxide reversibly inhibits protein-tyrosine phosphatase 1b in vivo and enhances the early insulin action cascade. J Biol Chem. 2001;276(24):21938–42.
[37] Lee SR, Kwon KS, Kim SR, Rhee SG. Reversible inactivation of protein-tyrosine phosphatase 1B in A431 cells stimulated with epidermal growth factor. J Biol Chem. 1998;273(25):15366–72.
[38] Meng TC, Fukada T, Tonks NK. Reversible oxidation and inactivation of protein tyrosine phosphatases in vivo. Mol Cell. 2002;9(2):387–99.
[39] Barrett WC, DeGnore JP, Keng YF, Zhang ZY, Yim MB, Chock PB. Roles of superoxide radical anion in signal transduction mediated by reversible regulation of protein-tyrosine phosphatase 1B. J Biol Chem. 1999;274(49):34543–6.
[40] Li S, Whorton AR. Regulation of protein tyrosine phosphatase 1B in intact cells by S-nitrosothiols. Arch Biochem Biophys. 2003;410(2):269–79.
[41] Blanchetot C, Tertoolen LG, den Hertog J. Regulation of receptor protein-tyrosine phosphatase alpha by oxidative stress. EMBO J. 2002;21(4):493–503.
[42] Persson C, Sjoblom T, Groen A, Kappert K, Engstrom U, Hellman U et al. Preferential oxidation of the second phosphatase domain of receptor-like PTP-alpha revealed by an antibody against oxidized protein tyrosine phosphatases. Proc Natl Acad Sci U S A. 2004;101(7):1886–91.
[43] van der Wijk T, Blanchetot C, Overvoorde J, den Hertog J. Redox-regulated rotational coupling of receptor protein-tyrosine phosphatase alpha dimers. J Biol Chem. 2003;278(16):13968–74.
[44] Toledano-Katchalski H, Tiran Z, Sines T, Shani G, Granot-Attas S, den Hertog J et al. Dimerization in vivo and inhibition of the nonreceptor form of protein tyrosine phosphatase epsilon. Mol Cell Biol. 2003;23(15):5460–71.
[45] Weiss A, Schlessinger J. Switching signals on or off by receptor dimerization. Cell. 1998; 94(3):277–80.
[46] Bilwes AM, den Hertog J, Hunter T, Noel JP. Structural basis for inhibition of receptor protein-tyrosine phosphatase-alpha by dimerization. Nature. 1996;382(6591):555–9.

[47] Desai DM, Sap J, Schlessinger J, Weiss A. Ligand-mediated negative regulation of a chimeric transmembrane receptor tyrosine phosphatase. Cell. 1993;73(3):541–54.
[48] Dornan S, Sebestyen Z, Gamble J, Nagy P, Bodnar A, Alldridge L et al. Differential association of CD45 isoforms with CD4 and CD8 regulates the actions of specific pools of p56lck tyrosine kinase in T cell antigen receptor signal transduction. J Biol Chem. 2002;277(3):1912–8.
[49] Takeda A, Wu JJ, Maizel AL. Evidence for monomeric and dimeric forms of CD45 associated with a 30-kDa phosphorylated protein. J Biol Chem. 1992;267(23):16651–9.
[50] Xu Z, Weiss A. Negative regulation of CD45 by differential homodimerization of the alternatively spliced isoforms. Nat Immunol. 2002;3(8):764–71.
[51] Jiang G, den Hertog J, Hunter T. Receptor-like protein tyrosine phosphatase alpha homodimerizes on the cell surface. Mol Cell Biol. 2000;20(16):5917–29.
[52] Majeti R, Bilwes AM, Noel JP, Hunter T, Weiss A. Dimerization-induced inhibition of receptor protein tyrosine phosphatase function through an inhibitory wedge. Science. 1998; 279(5347):88–91.
[53] Madan LL, Gopal B. Addition of a polypeptide stretch at the N-terminus improves the expression, stability and solubility of recombinant protein tyrosine phosphatases from Drosophila melanogaster. Protein Exp Purif. 2008;57(2):234–43.
[54] Xie Y, Massa SM, Ensslen-Craig SE, Major DL, Yang T, Tisi MA et al. Protein-tyrosine phosphatase (PTP) wedge domain peptides: a novel approach for inhibition of PTP function and augmentation of protein-tyrosine kinase function. J Biol Chem. 2006;281(24):16482–92.
[55] Blanchetot C, Tertoolen LG, Overvoorde J, den Hertog J. Intra- and intermolecular interactions between intracellular domains of receptor protein-tyrosine phosphatases. J Biol Chem. 2002;277(49):47263–9.
[56] Blanchetot C, den Hertog J. Multiple interactions between receptor protein-tyrosine phosphatase (RPTP) alpha and membrane-distal protein-tyrosine phosphatase domains of various RPTPs. J Biol Chem. 2000;275(17):12446–52.
[57] Johnson KG, Van Vactor D. Receptor protein tyrosine phosphatases in nervous system development. Physiol Rev. 2003;83(1):1–24.
[58] Schaapveld RQ, Schepens JT, Bachner D, Attema J, Wieringa B, Jap PH et al. Developmental expression of the cell adhesion molecule-like protein tyrosine phosphatases LAR, RPTPdelta and RPTPsigma in the mouse. Mech Dev. 1998;77(1):59–62.
[59] Wallace MJ, Fladd C, Batt J, Rotin D. The second catalytic domain of protein tyrosine phosphatase delta (PTP delta) binds to and inhibits the first catalytic domain of PTP sigma. Mol Cell Biol. 1998;18(5):2608–16.

6 The non-receptor protein tyrosine phosphatases: Part I

6.1 Classification of non-receptor protein tyrosine phosphatases

The protein tyrosine phosphatase (PTP) superfamily of signaling enzymes includes about 107 genes in the human genome [1, 2]. These are further subdivided into 17 subtypes that include eight receptor-bound forms and nine non-receptor PTPs (Figure 6.1). This subclassification depends on the presence of various accessory domains that are present alongside the active site cysteine-based tyrosine phosphatase domains in these proteins. These domains include the phosphotyrosine-binding SH2 domains [3], the FERM (4.1 protein, Ezrin, Radixin and Moesin) domains, PDZ domains and also proline-rich regions. These domains not only modulate the activity of the tyrosine phosphatase domains but they also allow for their subcellular targeting. This, in turn, allows for the high spatial and temporal specificity achieved by these enzymes in the needs of the cell.

Much like in the case of double-domain PTPs, related members of the non-receptor PTP subtypes are hypothesized to have been formed by gene duplication [4, 5]. These include PTP1B and T-cell protein tyrosine phosphatase (TCPTP) of the NT1 subtype and also the NT2 subtype members SHP1 and SHP2. While these pairs of genes share a high sequence identity; their physiological roles, specificity and activity are distinct and account for their non-redundant functions in the cell. The non-receptor PTPs are also characterized by the presence of various isoforms that allow for their participation at various stages of a signaling pathway. These include isoforms that allow certain tyrosine phosphatase forms to shuttle in-and-out of the nucleus or be targeted to the cytoplasmic face of the endoplasmic reticulum. Maneuvering of subcellular location of the non-receptor PTPs allows them to expand their substrate pool and show a targeted signaling effect in the need of the cell.

6.2 The NT1 subclass

The NT1 subclass of non-receptor PTPs includes the gene products of *PTPN1* (called as PTP1B) and *PTPN2* (called as TCPTP). These two tyrosine phosphatases share about 74% sequence identity and are reported to be ubiquitously expressed. PTP1B was first isolated from human placenta [6], while TCPTP was identified from T-cells' peripheral cDNA library using primers from the PTP1B sequence [7]. Both PTP1B and TCPTP contain a single and highly homologous PTP domain, followed by a long cytoplasmic C-tail (Figure 6.2). The C-tail of these proteins contains two tandem proline-rich motifs that serve as the docking site for SH3-domain containing proteins. PTP1B also contains hydrophobic motifs in its C-tail that allow it to localize on

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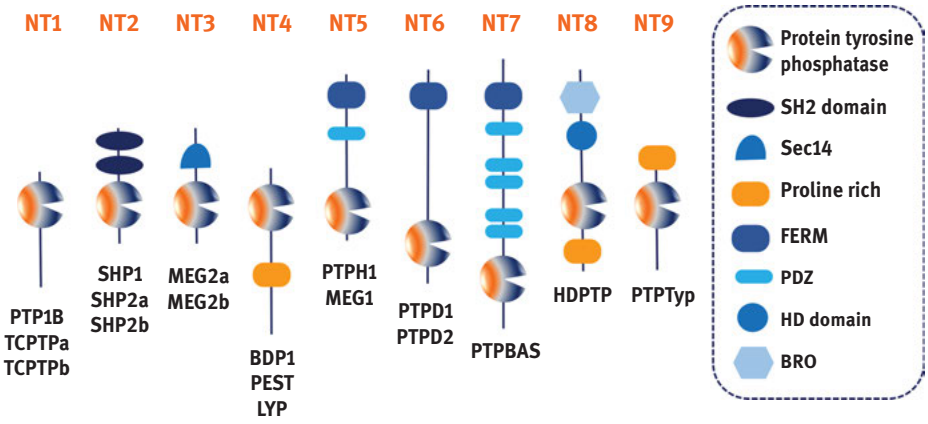

Figure 6.1: Various subtypes of the non-receptor PTPs.

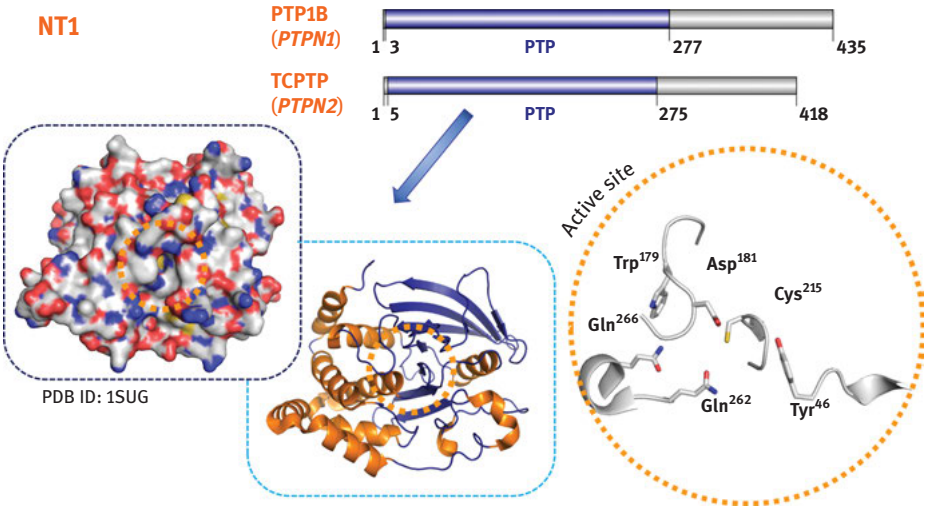

Figure 6.2: Domain organization and molecular architecture of the NT1 subtype of non-receptor PTPs PTP1B and TCPTP. Surface electrostatics of the catalytic domain and the arrangement of the active site residues for this subtype are well studied.

the cytoplasmic surface of the endoplasmic reticulum [8]. The cleavage of this hydrophobic segment localizes PTP1B to the cytoplasm and is also reported to modulate its substrate specificity [9]. Two splice variants are also reported for PTP1B whose relative levels are dependent on growth factor or insulin-induced signals [10, 11]. Correspondingly, TCPTP harbors a nuclear localization signal in its C-tail that allows it to shuttle in-and-out of the nucleus [12]. Two distinct splice variants of TCPTP decide its subcellular location. The larger 48.5 kDa form (also called TC48) is localized to the

endoplasmic reticulum, while the smaller 45 kDa (also called TC45) is localized in the nucleus. Mitotic stimulation of cells allows for translocation of TC45 into the cytoplasm form the nucleus and changes its substrate pool in the needs of the cell [13, 14]. The physiological role of PTP1B and TCPTP is hence critical in both spatial and temporal contexts and is carefully regulated in cells. Localization-based modulation of tyrosine phosphatase activity is also known as the "Zip-code" model, wherein certain sequences serve as the "codes" for certain locations [15].

6.3 PTP1B: Structure and catalytic function

PTP1B forms the prototype of the PTP superfamily and was the first tyrosine phosphatase to be purified and characterized [16]. PTP1B was named so based on the elution peak "B" of the ion-exchange chromatogram that showed its purification. The PTP1B catalytic domain was subsequently sequenced and its amino acid composition was known in 1989 [17]. PTP1B was also the first one to be crystallized and its structure serves as the fundamental example of tyrosine phosphatase structure and catalytic mechanism [18]. The human *PTPN1* gene is mapped to the region 20q13.13 and spans over 74 kb and has an unusual large first exon [19]. This *PTPN1* locus is also linked to Type II diabetes [20], fat mass and energy intake [21] and is frequently amplified in breast cancers [22]. *PTPN1* is speculated to have originated 600 million years ago and is conserved from Placozaons such as *Trichoplax adhaerens* to humans in a 1:1 orthology relationship [23]. Its evolutionary duplication to form the *PTPN2* gene coding for TCPTP is analyzed to have occurred before the fish evolutionary split. The evolution of the PTP1B gene through the 600 million years indicates a positive selection and high dependence for tyrosine phosphatase function in the context of the cell. Evolutionary selections include multiple regulatory measures to control its activity. The interactome of PTP1B is one of the biggest including 13 interacting partners and about 30 known substrates [24]. PTP1B interactome includes protein involved in cellular process of growth, division, signaling, proliferation, migration, intracellular contacts and also intracellular messengers [25].

The C-tail of PTP1B is reported to regulate both localization and catalytic efficiency of the enzyme. Truncation of 75 residues at the C-terminal of PTP1B by calpain proteases happens in response to gpIIb–IIIa integrin complex-mediated activation of platelets and forms a shorter 42 kDa version of PTP1B [9]. This truncation ensures the location of PTP1B in the cytoplasm by eliminating its binding to the endoplasmic reticulum. In addition, the truncated version of PTP1B is reported to have better catalytic efficiency than the longer version, indicating an inhibitory role of its C-tail. Interestingly, PTP1B is also one of the first proteins known to phosphorylated on its serine/threonine residues [26]. These phosphorylation sites are present in the C-tail and include Ser378 that is phosphorylated by protein kinase C and Ser386

that is phosphorylated by Ser/Thr kinase p34cdc2:Cyclin B complex. PTP1B is also phosphorylated at Ser352 in a cell cycle-dependent manner. PTP1B is also reported to be phosphorylated on its tyrosine residues and functions to dephosphorylate its phosphotyrosines by a *trans*-intermolecular tyrosine phosphatase action [27]. About 53% of intracellular PTP1B is reported to be phosphorylated in response to insulin singling [28]. Phosphorylated tyrosines include Tyr66, Tyr152 and Tyr153 [29]. Their phosphorylation is correlated with increased phosphatase activity of PTP1B, thus creating a feedback loop to regulate insulin signaling [27]. However, in adipose tissues and skeletal muscle, insulin-induced phosphorylation of PTP1B is associated with a decrease in its phosphatase activity [28]. This indicates that regulation and modulation of PTP1B is tissue specific and accounts for its ubiquitous presence being maneuvered by regulatory mechanism to achieve signaling specificity.

The catalytic PTP domain of PTP1B is perhaps the most well characterized by both structural and biochemical studies [18]. Its first crystal structure was solved with sodium tungstate as the site-directed active site cysteine inhibitor that also worked as a heavy atom. The structure showed a single globular domain formed by eight α-helices and 12 β-strands. About ten β-strands make a mixed β-sheet that spans the length of the globular domain (Figure 6.2). The active site cleft is lined by three motifs that include the WPD-loop containing the acid/base aspartate, the Q-loop containing the water-activating glutamines and the pY-loop that harbors a conserved tyrosine or phenylalanine that aids phosphotyrosine entry into the active site [30]. The base of the active site cleft has the catalytic cysteine in the P-loop. PTP1B catalytic domain contains all the ten motifs that define a PTP domain to the best compatibility with the consensus sequence [1]. Motif 9 is contained in the P-loop that includes the active site Cys215 of PTP1B and forms the nucleophile that chemically attacks the incoming phosphotyrosine [18, 31]. This motif and the active site cysteine are conserved during evolution from Placozaons to humans (Figure 6.2). The chemical environment of the active site lowers the p*K*a of the active site cysteine to ~5.4 pH units, allowing for the deprotonation of the cysteine. In the first step of the two-step catalytic mechanism, the ionized cysteine attacks the phosphotyrosine and forms a cysteinyl–phosphate intermediate [32]. The tyrosyl-leaving group of the substrate is protonated by the general acid Asp181 from the WPD-loop. The second step of catalysis required activation of a water molecule by Gln262 of the Q-loop and the simultaneous function of Asp181 as a base that extracts a proton from water. Activation of water and extraction of a proton allows for the hydrolysis of the cysteinyl–phosphate intermediate and regenerates the active site cysteine [33].

Various aspects of the PTP1B catalytic cycle and substrate binding have been reported through careful analysis of various crystal structures. Substrate recognition has been explained by the structure of a mutant form of PTP1B with a peptide containing the autophosphorylation site of the epidermal growth factor receptor [34] (Figure 6.3). The PTP1B mutant in the complex has its active site Cys215

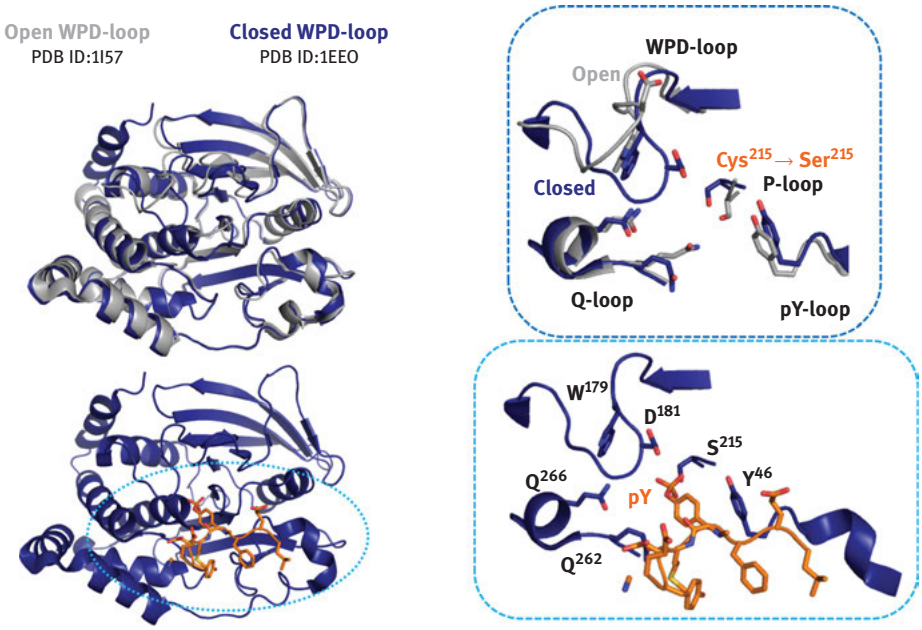

Figure 6.3: The opening and closing of the WPD-loop of PTP1B. Binding of the epidermal growth receptor-derived phosphopeptide at the active site of PTP1B.

substituted for a serine residue. This allows the mutant to bind the substrate to in the absence of any nucleophilic attack on the phosphotyrosine. This complex provides insights into the mode of substrate binding on the especially on the surface of PTP1B. The structure shows how the WPD-loop closes on the active site cleft to seal the phosphotyrosine into the PTP1B-active site. A large conformational change in the WPD-loop allows the Phe182 from motif 8 to interact with the phosphotyrosine and help stabilize it in the active site chamber. This complex represents the mode of "induced fit" wherein the enzyme undergoes a conformational change to accommodate the incoming substrate or ligand. The cysteinyl–phosphate intermediate has also been crystallized using carefully designed PTP1B mutants [35]. Mutation of the Q-loop Gln262 to an alanine removes its ability to hydrolyze the cysteinyl–phosphate at the active site. The structure of the mutant (Figure 6.4) allows for visualization of the intermediate and also confirms the role of Gln262 and Asp181 in the catalytic cycle. The structure also shows how the WPD-loop closes over the phosphotyrosine substrate and blocks the entry of extraneous phosphoacceptors. Another structure of PTP1B has orthovanadate at the active site that mimics a pentavalent phosphorus transition state. This structure shows the hydrogen bonding interactions between the phosphorus transition state (as orthovanadate) and the critical catalytic residues Gln262 and Asp181 [35].

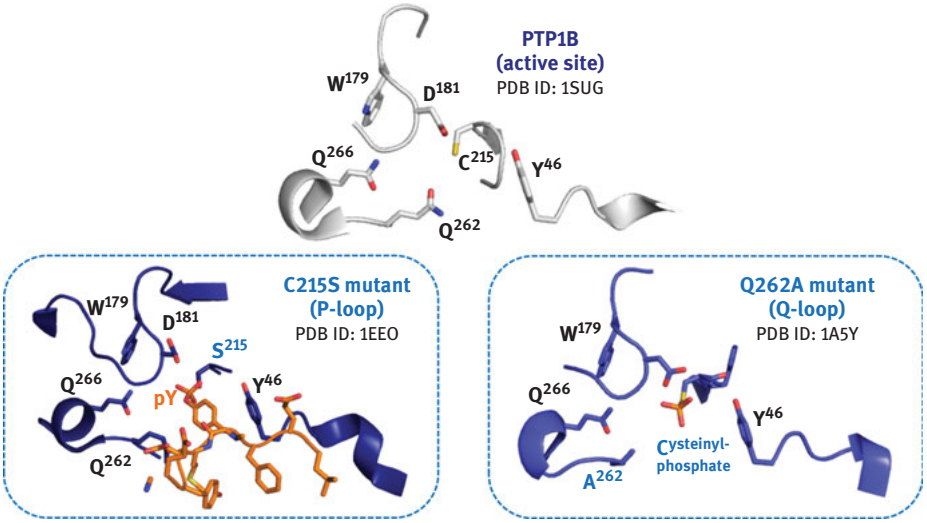

Figure 6.4: Strategies for making the "substrate-tap" of the PTP domain. Mutation of the active site cysteine of the P-loop traps the phosphopeptide bound to the active site. Mutation of the Q-loop glutamine abrogates the second step of catalysis to trap the cysteinyl–phosphate at the active site.

6.3.1 PTP1B: Substrate identification

PTP1B interacts with a wide variety of substrate proteins and binding partners that find their functions in cellular processes (Figure 6.5). This diverse set of PTP1B substrates includes receptor protein tyrosine kinases like the insulin receptor and the epidermal growth factor receptor [29, 36, 37], the JAK-signal transducer and activator of transcription (STAT) signal transducers [38, 39], cortactin protein that regulates cytoskeletal dynamics [40], STAM2 protein that regulates receptor tyrosine kinase sorting in the endosome [41] and also the PKR-like eukaryotic initiation factor 2α kinase [42]. Substrate identification for PTP1B has been largely aided by the knowledge of its catalytic mechanism and use of specific point mutations to block active site mechanics. Initially the active site cysteine to serine mutation was used [43], but this mutant was found to be useful only in cases of tight PTP1B: substrate associations. This cysteine to serine mutant proved ineffective in exploring and isolating PTP1B complexes from cell lysates. Subsequently, a better "substrate-trap" mutant was created by mutating the WPD-loop general acid/base Asp181 to alanine [43, 44]. The WPD-loop mutation approach proved especially successful where upon expression the mutant bound the physiological substrates in a dead-end complex. These PTP1B:substrate traps were then analyzed by immunoprecipitation. The role of PTP1B in dephosphorylating the epidermal growth factor receptor was elucidated using the PTP1B Asp181 to alanine mutant [44]. Cell-based assays showed that the PTP1B mutant colocalized with the

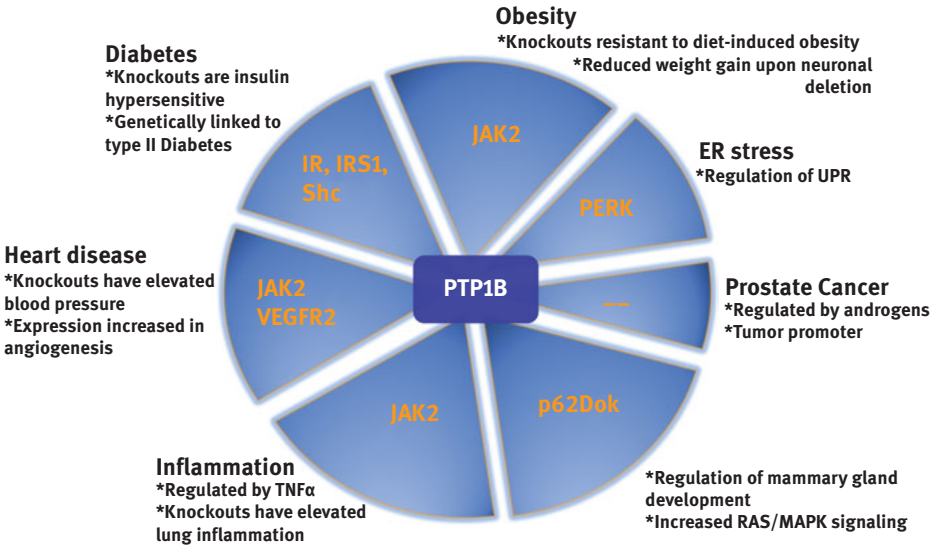

Figure 6.5: The important physiological roles of PTP1B.

endogenously expressed epidermal growth factor receptors in the punctate structures of COS cells. Further studies demonstrated that dephosphorylation of epidermal growth factor receptor by PTP1B required their endocytosis and actually occurred on specific sites on the endoplasmic reticulum that were represented by the punctate structures [45]. These punctate structures serve as dephosphorylation compartments that function as sorting sites for downregulating receptor tyrosine kinases. This suggested a crucial role of PTP1B in terminating ligand–receptor interactions of receptor tyrosine kinases rather than simply controlling their basal activity.

The single Asp181 to alanine substrate trap mutant has now been improved by using double mutants C215S/D181A and D181A/Q262A. These double mutants have been used to identify substrate libraries for PTP1B [46]. Recently, another PTP1B double mutant has also been reported as an improved substrate trap that has allowed for understanding the role of PTP1B in the biosynthesis and maturation of the insulin receptor [47]. The double mutant uses the Tyr46 to phenylalanine substitute in its pY-loop in addition to the Asp181 to alanine replacement. This tyrosine guards the entry of substrate into the active site of PTP1B is crucial for recognition of the phosphotyrosine residues [30]. Mutation of the tyrosine to a phenylalanine increases the aromaticity of the guarding residue and allows for stronger recognition of physiological substrates. In these studies, the PTP1B Y46F/D181A double mutant could effectively recover more insulin receptor in immunoprecipitation and showed better signal in bioluminescence energy transfer experiments.

6.3.2 PTP1B: Regulation of function

The prominent position of PTP1B in regulating multiple signaling processes requires its tight and precise regulation. This regulation of PTP1B has been extensively reported at both the transcriptional and post-translational levels [48]. Expression of PTP1B is under the regulation of Sp family of proteins, early growth response-1 protein, Y box-binding protein-1 and also nuclear factor kappa B (NF-κB) [49–51]. PTP1B transcriptional control is reported to also be context dependent. For example, upon androgen stimulation of prostate cells, the androgen receptor gets recruited to the *PTPN1* promoter and enhances the recruitment of RNA polymerase to the transcription site [52]. At the posttranslational level, PTP1B activity is regulated by various modifications including phosphorylation, oxidation, sumoylation, nitrosylation and also proteolytic processing. Phosphorylation of serine and tyrosine residues of PTP1B occurs in response to insulin receptor and epidermal growth factor receptor stimulation [29, 53]. Mass spectroscopy of PTP1B has allowed for the identification of several phosphorylation sites [54, 55]. Some phosphorylations are reported to enhance PTP1B activity, while some are inhibitory. However, given the complexity of multiple phosphorylations, the combinatorial role of these phosphorylations remains elusive. PTP1B is also reported to undergo sumoylation on Lys335 and Lys347 [56]. Sumoylated PTP1B shows decreased catalytic activity and is seen to localize on the endoplasmic reticulum accumulating in the perinuclear space. PTP1B interacts with an inner nuclear membrane protein called emerin and controls its tyrosine phosphorylation status [57]. PTP1B sumoylation allows for increased tyrosine phosphorylation that triggers the onset of mitosis. In this way PTP1B post-translational modification allows cells to manipulate its activity toward specific substrate depending on the needs of the cell.

Another crucial regulator of tyrosine phosphorylation-based signaling is the production and role of reactive oxygen species (ROS). PTPs including PTP1B are sensitive to ROS because of their active site cysteine [58, 59]. The most important consequence of ROS-based modification of PTP1B is implicated in cytokine crosstalk and immune signaling [60]. The function of ROS-mediated signaling is to inhibit PTP1B, thus enhancing phosphotyrosine levels for an optimal signaling response. Oxidative modification of PTP1B can be transient if the active site cysteine is converted to a sulfenamide but is irreversible if the cysteine is oxidized to sulfinic ($S–O_2H$) or sulfonic ($S–O_3H$) forms [61, 62]. The low p*K*a of the active site cysteine allows it to form a thiolate form at neutral pH. Harsh oxidizing agents like pervanadate oxidize this cysteine thiolate to sulfinic or sulfonic forms (Figure 6.6), while milder and more physiologically relevant agents like peroxidase allow its conversion to sulfenic acid. Interestingly, the sulfenic acid form is susceptible to further oxidation to form the sulfinic and sulfonic forms, but this is averted by a chemical change in the PTP1B-active site. The structure of the oxidized PTP1B with H_2O_2 demonstrates that the sulfenic acid intermediate of the active site is rapidly converted to sulfenyl–amide where the sulfur atom of the active site cysteine makes a covalent bond with the main

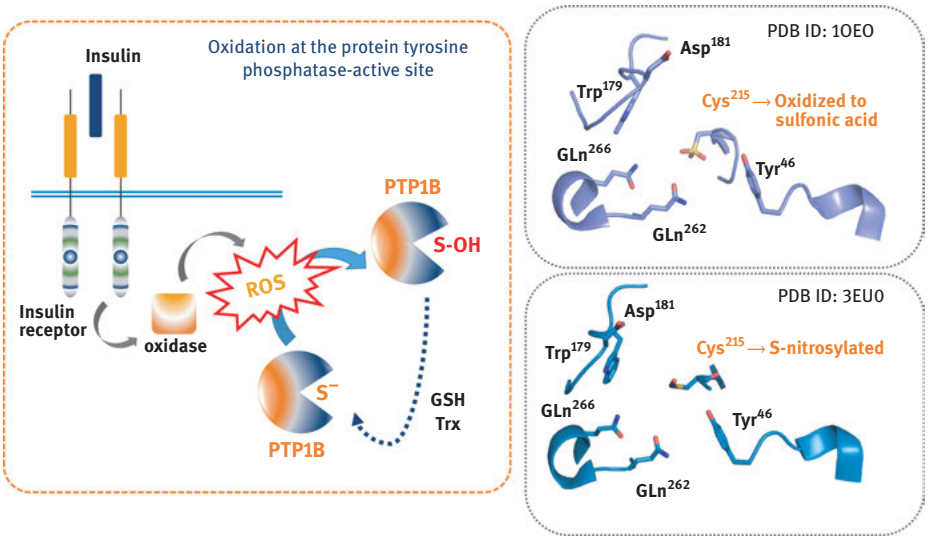

Figure 6.6: Role of ROS in regulation and abrogation of PTP activity.

chain nitrogen atom of the adjacent Ser216 [61]. This makes a five-atom structured ring at the active site and induced various other conformational changes in the PTP domain. The phosphotyrosine recognition motif containing pY-loop flips out to become more solvent exposed. As a consequence, the phosphotyrosine recognition residue, namely, Tyr46 becomes more susceptible to phosphorylation and is also unable to recruit the substrate efficiently. At the same time, conformational changes at the active site allow for exposing the sulfenyl–amide bond to reducing agents in the solvent. The sulfenyl–amide form prevents the oxidation of the cysteine to irreversible sulfinic and sulfonic forms and also provides ways to recycle the thiolate by providing access to reducing agents like glutathione. Reversible oxidation of PTP1B has been reported under physiological conditions for insulin receptor and epidermal growth factor receptor-induced signaling [63, 64]. Antibodies have been generated against the oxidized form of PTP1B that recognize and stabilize the oxidized conformation. These "intrabodies" are speculated to provide novel ways for PTP1B-based therapeutics [65]. Additionally, the active site cysteine of PTP1B is also reported to undergo nitric oxide (NO)-mediated S-nitrosylation that plays a protective role toward ROS-induced irreversible oxidation [66].

6.3.3 PTP1B: Role in disease

The earliest identified role of PTP1B included its ability to antagonize insulin signaling when injected into *Xenopus* oocytes [67]. Consequently, vanadate used

as an anit-diabetes drug was identified as a PTP1B inhibitor and showed the direct role of PTP1B in balancing insulin-mediated glucose metabolism [68]. Since these discoveries, a variety of reports substantiate the role of PTP1B in antagonizing insulin signaling. These reports include studies on expression and activity levels of various PTPs in models of diabetes and obesity [69, 70]. Recent studies of quantitative trait loci in *PTPN1* gene in humans show alterations in the 3′UTR of the gene that stabilizes its mRNA and is correlated with insulin resistance [71]. Mutations in the coding region of *PTPN1* remove a phosphorylation site in the C-terminal region of PTP1B and are correlated with type II diabetes [72, 73]. A landmark paper showed that PTP1B null mice appear healthy with slightly lower glucose levels and also lower insulin levels than wild-type mice. PTP1B knockout and heterozygous mice were resistant to weight gain and showed sensitivity to insulin even on a high-fat diet [74]. These reports allowed for pharmaceutical efforts develop PTP1B inhibitors as drugs for obesity and type II diabetes [75]. Consequently, PTP1B antisense oligonucleotides were seen to normalize glucose levels and improve insulin signaling in diabetic mice [76].

Integrated crystallographic and biochemical studies have provided a molecular basis for specificity of PTP1B toward the insulin receptor. Interaction between the activation loop of the insulin receptor and PTP1B shows the role of tandem phosphotyrosine in the sequence E/D–pY-pY–R/K to be optimal for PTP1B [77]. PTP1B is reported to bind about 70-folds better to peptides with tandem phosphotyrosine residues as compared to the ones having a single phosphotyrosine. This report has also allowed researchers to investigate and predict specific PTP1B physiological substrates that may harbor this sequence consensus. The strategy allowed for the identification of JAK2 and TYK2 protein kinases as novel substrates of PTP1B [39]. It is now known that PTP1B attenuates leptin signaling via the Janus Kinase. Mice deficient in PTP1B and leptin (a metabolic hormone) show increased metabolic rate, less adipose tissue and reduced weight gain [78]. These studies have provided insights into the role of PTP1B in controlling diet-induced obesity. PTP1B functions as a negative regulator of leptin signaling in the brain wherein mice with a proopiomelanocortin-specific deletion of PTP1B respond better to leptin and have increased energy expenditure [79]. Deletion of PTP1B in leptin receptor expressing neurons in mice leads to a reduction in their body weight on regular as well as high-fat diets. In addition, comparison of adiposity in leptin-expressing neuron deletions of PTP1B models versus general PTP1B knockout mice indicate a leptin-independent role of PTP1B in regulating metabolism that remains undiscovered [80]. The role of leptin in control of cardiovascular function has also allowed researchers to explore the functioning of PTP1B as a risk factor in heart disease. PTP1B-deficient mice are reported to display higher blood pressure that is further increased by leptin infusion [81]. Deletion of PTP1B affects vascular dysfunction in obese mice by modulation of peripheral insulin resistance [82]. PTP1B also plays a role in angiogenesis by regulation of the vascular endothelial growth

factor receptor (VEGFR2) [83]. PTP1B reduces the cellular phosphorylation levels of VE-cadherin and stabilizes the cell–cell junctions.

6.3.4 PTP1B: Role in cancer

As the role of protein tyrosine kinases like Src and Abl emerged as oncogenic proteins, the role of PTP1B was automatically suggestive of being a tumor suppressor. Indeed, cell culture experiments showed that PTP1B was able to decrease tumorigenecity in cells transformed with proto-oncogenes *v-Src* and *neu* [84, 85]. After these initial reports, it later became clear that the role of PTP1B in cancer was specific in the context of the tissue or cellular cancer. PTP1B deficient mice do not have an increased predisposition for cancer and do not develop spontaneous cancers [86]. Initial work on understanding the role of PTP1B in the cellular context of cancer used experiments were PTP1B knockout mice were crossed with P53 gene knockout mice. P53 gene knockout mice are reported to develop spontaneous tumors like T-cell lymphomas [87]. Progeny of crosses between P53 knockouts and PTP1B knockouts showed earlier tumor development and decreased rates of survival, with a shift in propensity to develop B-cell lymphomas as opposed to T-cell lymphomas. This report hinted toward a vital role of PTP1B in regulation of hematopoietic function. The double knockout mice were also seen to have elevated B-cells in the bone marrow and lymph nodes, indicating a direct role of PTP1B in B-cell development [88]. Other reports have suggested that PTP1B is expressed in about 58% of diffuse large B-cell lymphomas, 62% of peripheral T-cell lymphomas and about 33% of marginal zone lymphomas. PTP1B expression correlates with the expressions of BCL2 and MUM1 that serve as lymphoma-specific molecular markers. These studies have also reported the role of PTP1B in regulating interleukin (IL-4) induced STAT6 signaling where STAT6 is the direct substrate of PTP1B [89].

PTP1B regulates specific and important pathways of prostate cancer including those of platelet-derived growth factor receptor (PDGFR), c-Met, EGFR, IGF-R1, Src, JAKs and also MAPK signaling [90, 91]. PTP1B levels are elevated in human prostate cancer tissue and are reported to be directly regulated by androgen receptors. It is reported to enhance prostate cell migration and metastasis by dephosphorylating a yet unknown substrate protein [52]. PTP1B is also reported to be overexpressed in about 72.4% of human breast cancer tissue samples [92]. It is able to initiate transformation in breast tissue when expressed alone under the MMTV promoter. A significant delay in transformation is observed in PTP1B knockout mutations introduced in EBRB2 mouse model. This delay in cancer growth has been attributed to a decrease in MAPK signaling linked to the p62dok and RAS interaction complex [93]. In this way, signaling cascade of ErbB2–PTP1B–Src is reported to control cancer in human breast epithelial cells [94]. PTP1B is also reported to function in an

autonomous manner to delay Her2 tumors in mammary epithelial cells [95]. In addition, PTP1B does not participate in tumor growth or maintenance after it is established. PTP1B deletion allows for early differentiation of the mammary gland, thus allowing decreased numbers of Her2 transformed cells [96]. Recently, in contradiction to its role as a tumor promoter, PTP1B has been reported to be associated with estrogen receptor expression and is speculated to be a predictor of improved survival in breast cancer [97]. These studies indicate that PTP1B function may be contextual and specific to certain cell and tissue types and may also vary in tissues in certain physiological scenarios. However, additional work is required to dissect the complex mechanisms that underlie this seeming ambiguity in the role of PTP1B as a tumor promoter versus a tumor suppressor.

6.3.5 PTP1B as a therapeutic target

PTP1B knockout mice models suggest its crucial role in obesity and insulin resistance and make it one of the most promising therapeutic targets. Anti-sense oligonucleotides for PTP1B have been reported to significantly reduce levels of phosphate in the liver but not in muscle and thus help in enhancing insulin sensitivity [76]. PTP1B inhibitor design has been a lucrative project for various academic and industrial laboratories. Several crystal structures of PTP1B have been reported in combination with various substrates or inhibitors. The highly conserved active site of PTPs exemplifies the challenges faced by these laboratories in designing specific and potent drugs for the specialized targeting of PTP1B. Efforts are focused on identifying unique molecular interaction pockets on the surface of PTP1B that could help in inhibitor design. Specifically, binding of tandem phosphotyrosine residues from the insulin receptor to PTP1B has allowed for the identification of a second shallow grove on the PTP1B surface (Figure 6.7) [77]. A peptide from the activation loop of insulin receptor with two phosphotyrosine residues pTyr1162 and pTyr1163 is solved in complex with PTP1B. The catalytic center of PTP1B is occupied by pTyr1162 that serves as the substrate, while pTyr1163 is held in place in another shallow grove by interactions with the positively charged Arg24 and Arg254 in the enzyme. This site was initially identified as a "second aryl-binding site" and provided hints to the design of bidentate inhibitors that could span both the catalytic and allosteric pockets [98].

The second aryl-binding site has been explored as a potent site for allosteric modulation and inhibition of PTP1B. This strategy to design antagonists of PTP1B uses tandem phosphotyrosine-containing peptides to gradually reduce their peptide character to yield a small-molecule inhibitor [99, 100]. However, the high-positive charge at the secondary bindings introduces a crucial challenge in PTP1B inhibitor design. The high surface charge of the pockets of PTP1B requires that the binding ligand also should be heavily charged. While compounds have

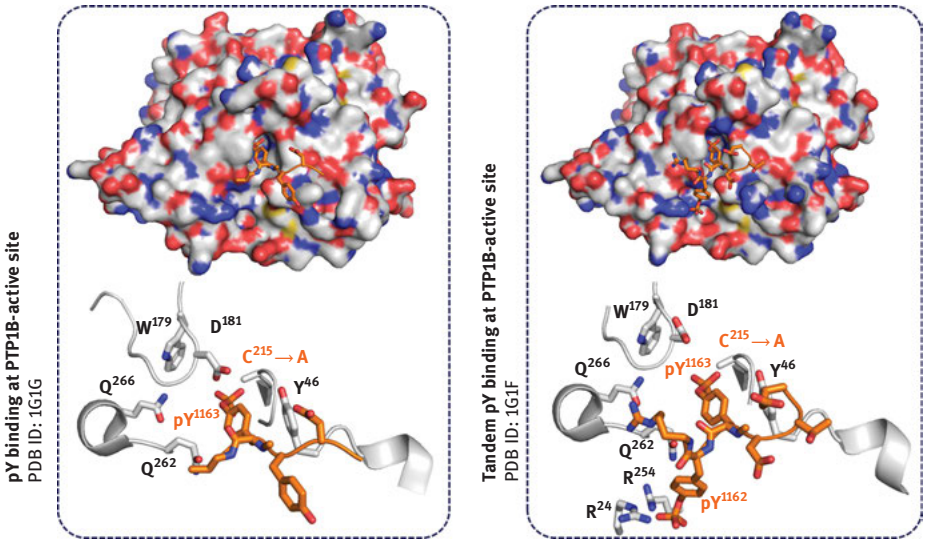

Figure 6.7: Mode of tandem biphosphotyrosine binding at the PTP1B-active site. The second phosphotyrosine-binding pocket is unique to PTP1B.

been generated that can bind the two sites of PTP1B simultaneously with nanomolar potency, they have limited ability to cross the plasma membrane due to their high charge. These compounds while being effective inhibitors are very poor drugs because of their limited physiological adaptation [101]. Another class of bidentate PTP1B inhibitors has been created by using two phosphotyrosine mimetics that use an alternate conformation to bind PTP1B [102, 103]. These inhibitors make molecular connections with Arg47 that decides the substrate specificity of PTP1B. This arginine is poorly conserved in the PTP domain and allows for accommodation of negative charge at the N-terminal of the substrate phosphotyrosine [104, 105]. The residue adjacent to Arg47 is Asp48 that is the target for making PTP1B specific small-molecule inhibitors. This approach uses optimization of a compound 2-(oxalylamino)-benzoic acid toward increasing its specificity and efficacy toward PTP1B [106]. 2-(Oxalylamino)-benzoic acid is a competitive, reversible inhibitor of the PTP domain [107]. Introduction of nitrogen facilitates its interaction with Asp48 of PTP1B and simultaneously disengages the compound from interacting with other PTPs that usually have an asparagine at the said position. Similarly a nonconserved Gly259 has been explored as a gateway to the second phosphotyrosine binding site that is unique to PTP1B. The channel created by the small Gly259 allows the interaction of a bulky derivative of 2-(oxalylamino)-benzoic acid to bind PTP1B, an interaction that is prevented in other PTPs due to the presence of a large hydrophobic residue [108]. These steric hindrance-based drug designs illustrate the potential for successful specific inhibitor design of PTPs (Figure 6.8) [109].

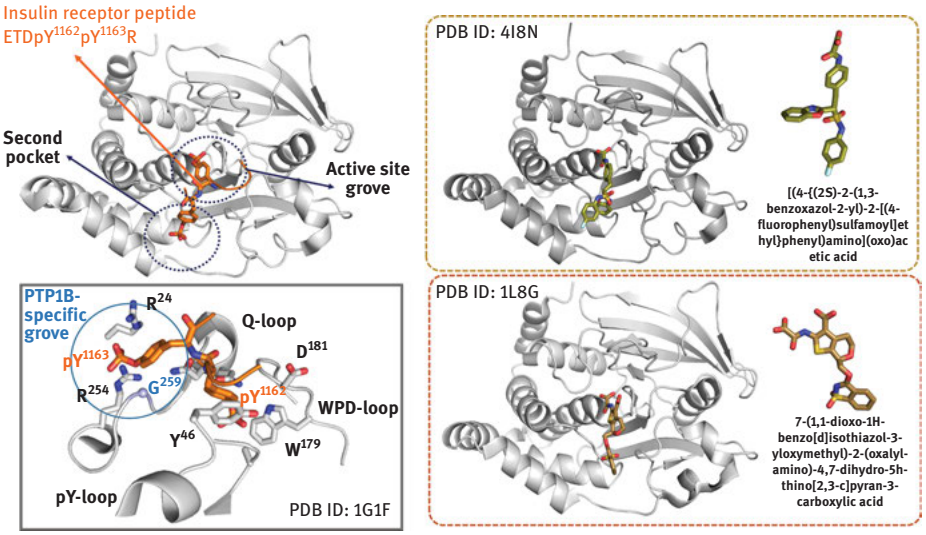

Figure 6.8: Harnessing the second-aryl binding site of PTP1B for designing of specific inhibitors.

6.4 T-Cell protein tyrosine phosphatase

The human *PTPN2* gene codes for the T-cell protein tyrosine phosphatase (TCPTP) that alongside PTP1B belongs to the NT1 subtype of non-receptor PTPs. The *PTPN2* gene maps to chromosome 18p11.3-p11.2, is about 100 kb and contains 10 exons [7]. TCPTP is ubiquitously expressed in the human body and its highest levels are found in the hematopoietic tissues. Transcription of TCPTP is tightly regulated in accordance cell cycle events such that it's expression levels peak at the G1 phase. The *PTPN2* gene promoter contains a suppressor element that allows for inhibition of its expression when the cells switch from the G to the S phase of the cell cycle [110]. This promoter also harbors regulatory sites that respond to the biological action of c-myc, PEA3, NF-κB and APF. Two isoforms of TCPTP are created by alternate splicing of ten exons of the *PTPN2* gene. Exons 1–7 code for the conserved PTP domain of about 272 amino acid residues and that shares 74% identity with the catalytic domain of PTP1B. Alternate splicing of exons 8–10 allows for making two isoforms of TCPTP that have distinct C-terminal regions [111]. This C-terminal part of TCPTP is inhibitory to the catalytic domain and controls its activity by autoinhibition [112]. Limited proteolysis of TCPTP releases a core 33 kDa enzyme that has higher activity and is inhibited by the external addition of the non-catalytic C-terminal of the shorter isoform [113]. This shorter isoform of TCPTP has about 397amino acids and is 45 kDa in size. The C-terminal region of this major isoform contains a bipartite nuclear localization signal that allows this TCPTP to shuttle between the nucleus and the cytosol [111]. The less abundant isoform of TCPTP has 415 amino acids and is 48 kDa in size. The longer C-terminal of this isoform has binding sites for p23 and p25 proteins that target this TCPTP to the endoplasmic reticulum [114].

Like in all PTPs, the active site cysteine of TCPTP forms its functional yet most vulnerable chemical environment. Cys216 of TCPTP is located in the conserved [I/V] HCxxGxxR[S/T] of the P-loop. This cysteine is reported to be sensitive to oxidation and is inactivated by ROS released by insulin signaling [115]. This interplay between insulin and TCPTP signaling is especially crucial in the maintenance of optimal metabolism in peripheral tissues including the lungs and liver [116]. TCPTP activity is also regulated by post-translational phosphorylation by the cyclin-dependent protein kinases. Ser304 of the 45 kDa isoform (but not the 48 kDa isoform) is phosphorylated by CDK1/cyclin-B1 in a cell cycle-dependent pattern [117]. Phosphorylation of Ser304 increases in a cell cycle-dependent manner as cells progress from the G2 to mitotic phase. As this phosphorylation site lies far from the active site, it is not reported to affect the catalytic activity of cellular localization of TCPTP. This makes the role of phosphorylation of Ser304 in the cell cycle elusive and complicated. Evidently more research is required to understand the role of such accessory phosphorylations in tyrosine phosphatase signaling. Isoform-specific phosphorylations also require further clarifications.

6.4.1 Biological role of TCPTP

TCPTP finds its main biological function in lymphoid cells where it has an anti-inflammatory role in suppressing the response initiated by the proinflammatory cytokines. The proinflammatory cytokines include IL-2 and IFN-γ that play a major role in macrophage activation. TCPTP dephosphorylates JAK1 and JAK3 tyrosine kinases that function as the pathway hubs for cytokine receptors of IL-2 and IFN-γ [118]. In addition, both the nuclear and cytosolic TCPTP are reported to suppress IFN-γ-mediated gene expression by dephosphorylation of the STAT1 protein [119]. The 45 kDa nuclear isoform of TCPTP plays a major in regulation of JAK downstream effectors including STAT1, STAT3, STAT5a/b, all of whom it can directly use as substrate. Dephosphorylation of STAT3 is critical for suppressing the signaling pathways of the pleiotropic cytokine IL-6 that participates in hematopoiesis and inflammatory response [120]. TCPTP regulates prolactin signaling by dephosphorylating STAT5a/b [121]. In the lymphoid immune cells, TCPTP binds to TRAF2 that targets TCPTP to the Src kinase and allows for the subsequent inhibition of Erk-dependent signaling. In this way, TCPTP suppresses TNF-α-mediated signaling in immune cells by directly engaging with a component of the TNF-α signaling pathway [122].

A robust and ubiquitous expression profile of TCPTP allows it to control various signaling pathways in different tissues. In the liver and muscles, TCPTP serves as the negative regulator of the insulin-signaling pathway [123]. Proliferation of hematopoietic tissues is regulated by TCPTP by the dephosphorylation of growth factor receptors including those of EGF and PDGF [124]. TCPTP downregulates colony-stimulating factor (CSF-1)-based signaling in myeloid cells. TCPTP dephosphorylates pTyr807 of the CSF-1 receptor and inhibits the recruitment of Src family of protein kinases. This

switches off the Src and Erk signaling pathways that are responsible for the regulation of differential in a variety of cell types [125, 126]. TCPTP also inhibits CSF-1 signaling by another mechanism. TCPTP directly dephosphorylates $p52^{Shc}$ that is the direct substrate of CSF-1 and serves as an adaptor protein that associates with Grb2 for mediating macrophage differentiation. TCPTP dephosphorylates $p52^{Shc}$ at its pTyr239 and inhibits its association with Grb2 [13, 126]. In addition, TCPTP dephosphorylates and downregulates Src kinases and therefore directly inhibits macrophage development [127].

6.5 TCPTP and PTP1B

Both PTP1B and TCPTP are members of the NT1 subtype of non-receptor PTPs and their catalytic domains share a very high sequence identity. Their ubiquitous expressions in various tissue types explain their crucial biological function. To understand the exact physiological roles of PTP1B viz.-a-viz. TCPTP, null mutant mice have been generated and studied for their distinct phenotypes [74, 75, 128]. The "substrate trap" approach has been used delineate the direct interaction pools of PTP1B and TCPTP. A brief description of their distinct substrates is provided in Table 6.1. Both PTP1B and TCPTP serve as regulators of cytokine signaling. Both participate in limiting the signaling by cytokines by regulating the JAK/STAT pathway but have distinct interacting partners. Substrate traps of PTP1B pull down the JAK2 and TYK2 proteins, while traps of TCPTP interact with JAK1 and JAK3 [39, 118]. In transfected COS7 cells, TCPTP recognizes STAT1, STAT3 and STAT5a/b, while PTP1B interacts with STAT5a and STAT5b [119–121, 129].

Table 6.1: Some common and unique substrates of PTP1B and TCPTP identified from "substrate trap" experiments.

	PTP1B	TCPTP
Common substrates	Epidermal Growth Factor Receptor (EGFR) Platelet Derived Growth Factor Receptor (PDGFR) Insulin Receptor (IR) STAT5a/b	
Unique Substrates	Insulin Receptor Substrate – 2 (IRS-2) Insulin-like Growth Factor Receptor (IRS1-R) Janus Kinase 2 (JAK2) Tyrosine Kinase 2 (TYK2) p62DOK p210Bcr-Abl Src p130cas	Janus Kinase 1 (JAK1) Janus Kinase 3 (JAK3) STAT1 STAT3 p52Shc

TCPTP plays a major role in the development of the lymphoid tissue and major defects have been reported in the lymphoid lineage of TCPTP-deficient mice [128].

Hematopoietic abnormalities in TCPTP null mice are attributed to increased and uncontrolled proinflammatory action of cytokines. The B-cells are reported to be specifically affected as indicated by their impaired response to lipopolysaccharides [128]. While the same phenotype is not reported for PTP1B null mice, PTP1B's role is ascertained to be critical in high endoplasmic reticulum -containing cells that include the B-cells. PTP1B is responsible for potentiating inositol-requiring enzyme-based endoplasmic reticulum stress signaling. As this pathway is effected, impaired JNK activation and corresponding XBP-1 splicing are seen in PTP1B-deficient fibroblast cells [130].

Both TCPTP and PTP1B play similar and major roles in control of cellular proliferation that is under the effect of growth factor receptors including EGF and PDGF. PTP1B functions as a major dephosphorylating enzyme for both EGFR and PDGFR. Both EGFR and PDGFR are reported to be hyperphosphorylated in PTP1B-deficient fibroblasts [131]. Substrate-trapping mutants of PTP1B and fluorescence imaging of overexpressed PTP1B demonstrate colocalization and substrate interaction of growth factor receptors with PTP1B [45]. TCPTP-deficient fibroblasts have shown the role of TCPTP in regulating signaling of PDGFRβ by dephosphorylation of pTyr1021 [132]. TCPTP also dephosphorylates the EGFR and inhibits the EGF facilitated association of Grb2 and $p52^{SHC}$ [132]. PTP1B and TCPTP are also the most important tyrosine phosphatases that control insulin signaling. Disruption of PTP1B gene in mice leads to tissue-specific elevated insulin sensitivity and provides resistance to obesity and diabetes [75]. As this action of PTP1B is reported to be tissue specific, there is a valid speculation for the role of TCPTP in complementing this action of PTP1B in tissues. A better understanding of their complementary role requires generation of mice doubly deficient in both PTP1B and TCPTP. Alternatively, tissue-specific knockout mosaics of PTP1B and TCPTP may elaborate on their complementary or compensatory roles in regulating metabolism and glucose homeostasis.

References

[1] Andersen JN, Mortensen OH, Peters GH, Drake PG, Iversen LF, Olsen OH, et al. Structural and evolutionary relationships among protein tyrosine phosphatase domains. Mol Cell Biol. 2001;21(21):7117–36.

[2] Andersen JN, Jansen PG, Echwald SM, Mortensen OH, Fukada T, Del Vecchio R, et al. A genomic perspective on protein tyrosine phosphatases: gene structure, pseudogenes, and genetic disease linkage. FASEB J. 2004;18(1):8–30.

[3] Heldin CH. SH2 domains: elements that control protein interactions during signal transduction. Trends Biochem Sci. 1991;16(12):450–2.

[4] Böhmer F, Szedlacsek S, Tabernero L, Östman A, den Hertog J. Protein tyrosine phosphatase structure–function relationships in regulation and pathogenesis. FEBS J. 2013;280 (2):413–31.

[5] Hendriks WJAJ, Elson A, Harroch S, Pulido R, Stoker A, den Hertog J. Protein tyrosine phosphatases in health and disease. FEBS J. 2013;280(2):708–30.

[6] Tonks NK, Diltz CD, Fischer EH. Purification of the major protein-tyrosine-phosphatases of human placenta. J Biol Chem. 1988;263(14):6722–30.
[7] Cool DE, Tonks NK, Charbonneau H, Walsh KA, Fischer EH, Krebs EG. cDNA isolated from a human T-cell library encodes a member of the protein-tyrosine-phosphatase family. Proc Natl Acad Sci U S A. 1989;86(14):5257–61.
[8] Frangioni JV, Beahm PH, Shifrin V, Jost CA, Neel BG. The nontransmembrane tyrosine phosphatase PTP-1B localizes to the endoplasmic reticulum via its 35 amino acid C-terminal sequence. Cell. 1992;68(3):545–60.
[9] Frangioni JV, Oda A, Smith M, Salzman EW, Neel BG. Calpain-catalyzed cleavage and subcellular relocation of protein phosphotyrosine phosphatase 1B (PTP-1B) in human platelets. EMBO J. 1993;12(12):4843–56.
[10] Shifrin VI, Neel BG. Growth factor-inducible alternative splicing of nontransmembrane phosphotyrosine phosphatase PTP-1B pre-mRNA. J Biol Chem. 1993;268(34):25376–84.
[11] Sell SM, Reese D. Insulin-inducible changes in the relative ratio of PTP1B splice variants. Mol Genet Metab. 1999;66(3):189–92.
[12] Lorenzen JA, Dadabay CY, Fischer EH. COOH-terminal sequence motifs target the T cell protein tyrosine phosphatase to the ER and nucleus. J Cell Biol. 1995;131(3):631–43.
[13] Tiganis T, Bennett AM, Ravichandran KS, Tonks NK. Epidermal growth factor receptor and the adaptor protein p52Shc are specific substrates of T-cell protein tyrosine phosphatase. Mol Cell Biol. 1998;18(3):1622–34.
[14] Tiganis T, Kemp BE, Tonks NK. The protein-tyrosine phosphatase TCPTP regulates epidermal growth factor receptor-mediated and phosphatidylinositol 3-kinase-dependent signaling. J Biol Chem. 1999;274(39):27768–75.
[15] Mauro LJ, Dixon JE. “Zip codes” direct intracellular protein tyrosine phosphatases to the correct cellular “address”. Trends Biochem Sci. 1994;19(4):151–5.
[16] Tonks NK, Diltz CD, Fischer EH. Characterization of the major protein-tyrosine-phosphatases of human placenta. J Biol Chem. 1988;263(14):6731–7.
[17] Charbonneau H, Tonks NK, Kumar S, Diltz CD, Harrylock M, Cool DE, et al. Human placenta protein-tyrosine-phosphatase: amino acid sequence and relationship to a family of receptor-like proteins. Proc Natl Acad Sci U S A. 1989;86(14):5252–6.
[18] Barford D, Flint AJ, Tonks NK. Crystal structure of human protein tyrosine phosphatase 1B. Science. 1994;263(5152):1397–404.
[19] Brown-Shimer S, Johnson KA, Lawrence JB, Johnson C, Bruskin A, Green NR, et al. Molecular cloning and chromosome mapping of the human gene encoding protein phosphotyrosyl phosphatase 1B. Proc Natl Acad Sci U S A. 1990;87(13):5148–52.
[20] Bowden DW, Sale M, Howard TD, Qadri A, Spray BJ, Rothschild CB, et al. Linkage of genetic markers on human chromosomes 20 and 12 to NIDDM in Caucasian sib pairs with a history of diabetic nephropathy. Diabetes. 1997;46(5):882–6.
[21] Dong C, Wang S, Li WD, Li D, Zhao H, Price RA. Interacting genetic loci on chromosomes 20 and 10 influence extreme human obesity. Am J Hum Genet. 2003;72(1):115–24.
[22] Tanner MM, Tirkkonen M, Kallioniemi A, Isola J, Kuukasjarvi T, Collins C, et al. Independent amplification and frequent co-amplification of three nonsyntenic regions on the long arm of chromosome 20 in human breast cancer. Cancer Res. 1996; 56(15):3441–5.
[23] Feldhammer M, Uetani N, Miranda-Saavedra D, Tremblay ML. PTP1B: a simple enzyme for a complex world. Crit Rev Biochem Mol Biol. 2013;48(5):430–45.
[24] Ferrari E, Tinti M, Costa S, Corallino S, Nardozza AP, Chatraryamontri A, et al. Identification of new substrates of the protein-tyrosine phosphatase PTP1B by Bayesian integration of proteome evidence. J Biol Chem. 2011;286(6):4173–85.

[25] Mertins P, Eberl HC, Renkawitz J, Olsen JV, Tremblay ML, Mann M, et al. Investigation of protein-tyrosine phosphatase 1B function by quantitative proteomics. Mol Cell Proteomics. 2008; 7(9):1763–77.
[26] Flint AJ, Gebbink MF, Franza BR, Jr., Hill DE, Tonks NK. Multi-site phosphorylation of the protein tyrosine phosphatase, PTP1B: identification of cell cycle regulated and phorbol ester stimulated sites of phosphorylation. EMBO J. 1993;12(5):1937–46.
[27] Dadke S, Kusari A, Kusari J. Phosphorylation and activation of protein tyrosine phosphatase (PTP) 1B by insulin receptor. Mol Cell Biochem. 2001;221(1–2):147–54.
[28] Tao J, Malbon CC, Wang HY. Insulin stimulates tyrosine phosphorylation and inactivation of protein-tyrosine phosphatase 1B in vivo. J Biol Chem. 2001;276(31):29520–5.
[29] Bandyopadhyay D, Kusari A, Kenner KA, Liu F, Chernoff J, Gustafson TA, et al. Protein-tyrosine phosphatase 1B complexes with the insulin receptor in vivo and is tyrosine-phosphorylated in the presence of insulin. J Biol Chem. 1997;272(3):1639–45.
[30] Madan LL, Gopal B. Conformational basis for substrate recruitment in protein tyrosine phosphatase 10D. Biochemistry. 2011;50(46):10114–25.
[31] Jia Z, Barford D, Flint AJ, Tonks NK. Structural basis for phosphotyrosine peptide recognition by protein tyrosine phosphatase 1B. Science. 1995;268(5218):1754.
[32] Guan KL, Dixon JE. Evidence for protein-tyrosine-phosphatase catalysis proceeding via a cysteine-phosphate intermediate. J Biol Chem. 1991;266(26):17026–30.
[33] Zhang ZY, Wang Y, Dixon JE. Dissecting the catalytic mechanism of protein-tyrosine phosphatases. Proc Natl Acad Sci U S A. 1994;91(5):1624–7.
[34] Jia Z, Barford D, Flint AJ, Tonks NK. Structural basis for phosphotyrosine peptide recognition by protein tyrosine phosphatase 1B. Science. 1995;268(5218):1754–8.
[35] Pannifer AD, Flint AJ, Tonks NK, Barford D. Visualization of the cysteinyl-phosphate intermediate of a protein-tyrosine phosphatase by x-ray crystallography. J Biol Chem. 1998;273(17):10454–62.
[36] Seely BL, Staubs PA, Reichart DR, Berhanu P, Milarski KL, Saltiel AR, et al. Protein tyrosine phosphatase 1B interacts with the activated insulin receptor. Diabetes. 1996;45(10):1379–85.
[37] Ishino Y, Zhu C, Harris DL, Joyce NC. Protein tyrosine phosphatase-1B (PTP1B) helps regulate EGF-induced stimulation of S-phase entry in human corneal endothelial cells. Mol Vis. 2008;14:61–70.
[38] Gu F, Dube N, Kim JW, Cheng A, Ibarra-Sanchez Mde J, Tremblay ML, et al. Protein tyrosine phosphatase 1B attenuates growth hormone-mediated JAK2-STAT signaling. Mol Cell Biol. 2003;23(11):3753–62.
[39] Myers MP, Andersen JN, Cheng A, Tremblay ML, Horvath CM, Parisien JP, et al. TYK2 and JAK2 are substrates of protein-tyrosine phosphatase 1B. J Biol Chem. 2001;276(51):47771–4.
[40] Stuible M, Dube N, Tremblay ML. PTP1B regulates cortactin tyrosine phosphorylation by targeting Tyr446. J Biol Chem. 2008;283(23):15740–6.
[41] Stuible M, Abella JV, Feldhammer M, Nossov M, Sangwan V, Blagoev B, et al. PTP1B targets the endosomal sorting machinery: dephosphorylation of regulatory sites on the endosomal sorting complex required for transport component STAM2. J Biol Chem. 2010;285(31):23899–907.
[42] Krishnan N, Fu C, Pappin DJ, Tonks NK. H2S-Induced sulfhydration of the phosphatase PTP1B and its role in the endoplasmic reticulum stress response. Sci Signal. 2011;4(203):ra86.
[43] Blanchetot C, Chagnon M, Dube N, Halle M, Tremblay ML. Substrate-trapping techniques in the identification of cellular PTP targets. Methods. 2005;35(1):44–53.
[44] Flint AJ, Tiganis T, Barford D, Tonks NK. Development of "substrate-trapping" mutants to identify physiological substrates of protein tyrosine phosphatases. Proc Natl Acad Sci U S A. 1997;94(5):1680–5.
[45] Haj FG, Verveer PJ, Squire A, Neel BG, Bastiaens PI. Imaging sites of receptor dephosphorylation by PTP1B on the surface of the endoplasmic reticulum. Science. 2002;295(5560):1708–11.

[46] Xie L, Zhang YL, Zhang ZY. Design and characterization of an improved protein tyrosine phosphatase substrate-trapping mutant. Biochemistry. 2002;41(12):4032–9.
[47] Boubekeur S, Boute N, Pagesy P, Zilberfarb V, Christeff N, Issad T. A new highly efficient substrate-trapping mutant of Protein Tyrosine Phosphatase 1B (PTP1B) reveals full autoactivation of the insulin receptor precursor. J Biol Chem. 2011;286(22):19373–80.
[48] Yip SC, Saha S, Chernoff J. PTP1B: a double agent in metabolism and oncogenesis. Trends Biochem Sci. 2010;35(8):442–9.
[49] Fukada T, Tonks NK. The reciprocal role of Egr-1 and Sp family proteins in regulation of the PTP1B promoter in response to the p210 Bcr-Abl oncoprotein-tyrosine kinase. J Biol Chem. 2001;276(27):25512–9.
[50] Fukada T, Tonks NK. Identification of YB-1 as a regulator of PTP1B expression: implications for regulation of insulin and cytokine signaling. EMBO J. 2003;22(3):479-93.
[51] Zabolotny JM, Kim YB, Welsh LA, Kershaw EE, Neel BG, Kahn BB. Protein-tyrosine phosphatase 1B expression is induced by inflammation in vivo. J Biol Chem. 2008;283(21):14230–41.
[52] Lessard L, Labbe DP, Deblois G, Begin LR, Hardy S, Mes-Masson AM, et al. PTP1B is an androgen receptor-regulated phosphatase that promotes the progression of prostate cancer. Cancer Res. 2012;72(6):1529–37.
[53] Liu F, Chernoff J. Protein tyrosine phosphatase 1B interacts with and is tyrosine phosphorylated by the epidermal growth factor receptor. Biochem J. 1997;327 (Pt 1):139–45.
[54] Iliuk AB, Martin VA, Alicie BM, Geahlen RL, Tao WA. In-depth analyses of kinase-dependent tyrosine phosphoproteomes based on metal ion-functionalized soluble nanopolymers. Mol Cell Proteomics. 2010;9(10):2162–72.
[55] Rigbolt KT, Prokhorova TA, Akimov V, Henningsen J, Johansen PT, Kratchmarova I, et al. System-wide temporal characterization of the proteome and phosphoproteome of human embryonic stem cell differentiation. Sci Signal. 2011;4(164):rs3.
[56] Dadke S, Cotteret S, Yip SC, Jaffer ZM, Haj F, Ivanov A, et al. Regulation of protein tyrosine phosphatase 1B by sumoylation. Nat Cell Biol. 2007;9(1):80–85.
[57] Yip SC, Cotteret S, Chernoff J. Sumoylated protein tyrosine phosphatase 1B localizes to the inner nuclear membrane and regulates the tyrosine phosphorylation of emerin. J Cell Sci. 2012;125(Pt 2):310–6.
[58] Lohse DL, Denu JM, Santoro N, Dixon JE. Roles of aspartic acid-181 and serine-222 in intermediate formation and hydrolysis of the mammalian protein-tyrosine-phosphatase PTP1. Biochemistry. 1997;36(15):4568–75.
[59] Trumpler A, Schlott B, Herrlich P, Greer PA, Bohmer FD. Calpain-mediated degradation of reversibly oxidized protein-tyrosine phosphatase 1B. FEBS J. 2009;276(19):5622–33.
[60] Sharma P, Chakraborty R, Wang L, Min B, Tremblay ML, Kawahara T, et al. Redox regulation of interleukin-4 signaling. Immunity. 2008;29(4):551–64.
[61] Salmeen A, Andersen JN, Myers MP, Meng TC, Hinks JA, Tonks NK, et al. Redox regulation of protein tyrosine phosphatase 1B involves a sulphenyl-amide intermediate. Nature. 2003;423 (6941):769–73.
[62] van Montfort RL, Congreve M, Tisi D, Carr R, Jhoti H. Oxidation state of the active-site cysteine in protein tyrosine phosphatase 1B. Nature. 2003;423(6941):773–7.
[63] Mahadev K, Zilbering A, Zhu L, Goldstein BJ. Insulin-stimulated hydrogen peroxide reversibly inhibits protein-tyrosine phosphatase 1b in vivo and enhances the early insulin action cascade. J Biol Chem. 2001;276(24):21938–42.
[64] Lee SR, Kwon KS, Kim SR, Rhee SG. Reversible inactivation of protein-tyrosine phosphatase 1B in A431 cells stimulated with epidermal growth factor. J Biol Chem. 1998;273(25):15366–72.
[65] Haque A, Andersen JN, Salmeen A, Barford D, Tonks NK. Conformation-sensing antibodies stabilize the oxidized form of PTP1B and inhibit its phosphatase activity. Cell. 2011;147(1):185–98.

[66] Chen YY, Chu HM, Pan KT, Teng CH, Wang DL, Wang AH, et al. Cysteine S-nitrosylation protects protein-tyrosine phosphatase 1B against oxidation-induced permanent inactivation. J Biol Chem. 2008;283(50):35265–72.
[67] Tonks NK, Cicirelli MF, Diltz CD, Krebs EG, Fischer EH. Effect of microinjection of a low-Mr human placenta protein tyrosine phosphatase on induction of meiotic cell division in Xenopus oocytes. Mol Cell Biol. 1990;10(2):458–63.
[68] Khandelwal RL, Pugazhenthi S. In vivo effects of vanadate on hepatic glycogen metabolizing and lipogenic enzymes in insulin-dependent and insulin-resistant diabetic animals. Mol Cell Biochem. 1995;153(1–2):87–94.
[69] Goldstein BJ. Protein-tyrosine phosphatase 1B (PTP1B): a novel therapeutic target for type 2 diabetes mellitus, obesity and related states of insulin resistance. Curr Drug Targets Immune Endocr Metabol Disord. 2001;1(3):265–75.
[70] Kennedy BP, Ramachandran C. Protein tyrosine phosphatase-1B in diabetes. Biochem Pharmacol. 2000;60(7):877–83.
[71] Di Paola R, Frittitta L, Miscio G, Bozzali M, Baratta R, Centra M, et al. A variation in 3′ UTR of hPTP1B increases specific gene expression and associates with insulin resistance. Am J Hum Genet. 2002;70(3):806–12.
[72] Echwald SM, Bach H, Vestergaard H, Richelsen B, Kristensen K, Drivsholm T, et al. A P387L variant in protein tyrosine phosphatase-1B (PTP-1B) is associated with type 2 diabetes and impaired serine phosphorylation of PTP-1B in vitro. Diabetes. 2002;51(1):1–6.
[73] Mok A, Cao H, Zinman B, Hanley AJ, Harris SB, Kennedy BP, et al. A single nucleotide polymorphism in protein tyrosine phosphatase PTP-1B is associated with protection from diabetes or impaired glucose tolerance in Oji-Cree. J Clin Endocrinol Metab. 2002;87 (2):724–7.
[74] Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL, et al. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. Science. 1999;283(5407):1544–8.
[75] Klaman LD, Boss O, Peroni OD, Kim JK, Martino JL, Zabolotny JM, et al. Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. Mol Cell Biol. 2000;20(15):5479–89.
[76] Zinker BA, Rondinone CM, Trevillyan JM, Gum RJ, Clampit JE, Waring JF, et al. PTP1B antisense oligonucleotide lowers PTP1B protein, normalizes blood glucose, and improves insulin sensitivity in diabetic mice. Proc Natl Acad Sci U S A. 2002;99(17):11357–62.
[77] Salmeen A, Andersen JN, Myers MP, Tonks NK, Barford D. Molecular basis for the dephosphorylation of the activation segment of the insulin receptor by protein tyrosine phosphatase 1B. Mol Cell. 2000;6(6):1401–12.
[78] Cheng A, Uetani N, Simoncic PD, Chaubey VP, Lee-Loy A, McGlade CJ, et al. Attenuation of leptin action and regulation of obesity by protein tyrosine phosphatase 1B. Dev Cell. 2002;2 (4):497–503.
[79] Banno R, Zimmer D, De Jonghe BC, Atienza M, Rak K, Yang W, et al. PTP1B and SHP2 in POMC neurons reciprocally regulate energy balance in mice. J Clin Invest. 2010;120(3):720–34.
[80] Tsou RC, Zimmer DJ, De Jonghe BC, Bence KK. Deficiency of PTP1B in leptin receptor-expressing neurons leads to decreased body weight and adiposity in mice. Endocrinology. 2012;153 (9):4227–37.
[81] Belin de Chantemele EJ, Muta K, Mintz J, Tremblay ML, Marrero MB, Fulton DJ, et al. Protein tyrosine phosphatase 1B, a major regulator of leptin-mediated control of cardiovascular function. Circulation. 2009;120(9):753–63.
[82] Ali MI, Ketsawatsomkron P, Belin de Chantemele EJ, Mintz JD, Muta K, Salet C, et al. Deletion of protein tyrosine phosphatase 1b improves peripheral insulin resistance and vascular

function in obese, leptin-resistant mice via reduced oxidant tone. Circ Res. 2009;105(10): 1013–22.
[83] Nakamura Y, Patrushev N, Inomata H, Mehta D, Urao N, Kim HW, et al. Role of protein tyrosine phosphatase 1B in vascular endothelial growth factor signaling and cell-cell adhesions in endothelial cells. Circ Res. 2008;102(10):1182–91.
[84] Woodford-Thomas TA, Rhodes JD, Dixon JE. Expression of a protein tyrosine phosphatase in normal and v-src-transformed mouse 3T3 fibroblasts. J Cell Biol. 1992;117(2):401–14.
[85] Brown-Shimer S, Johnson KA, Hill DE, Bruskin AM. Effect of protein tyrosine phosphatase 1B expression on transformation by the human neu oncogene. Cancer Res. 1992;52(2): 478–82.
[86] Julien SG, Dube N, Hardy S, Tremblay ML. Inside the human cancer tyrosine phosphatome. Nat Rev Cancer. 2011;11(1):35–49.
[87] Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA, Jr., Butel JS, et al. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature. 1992;356(6366):215–21.
[88] Dube N, Bourdeau A, Heinonen KM, Cheng A, Loy AL, Tremblay ML. Genetic ablation of protein tyrosine phosphatase 1B accelerates lymphomagenesis of p53-null mice through the regulation of B-cell development. Cancer Res. 2005;65(21):10088–95.
[89] Lu X, Malumbres R, Shields B, Jiang X, Sarosiek KA, Natkunam Y, et al. PTP1B is a negative regulator of interleukin 4-induced STAT6 signaling. Blood. 2008;112(10):4098–108.
[90] Chang YM, Kung HJ, Evans CP. Nonreceptor tyrosine kinases in prostate cancer. Neoplasia. 2007;9(2):90–100.
[91] Mimeault M, Batra SK. Recent advances on multiple tumorigenic cascades involved in prostatic cancer progression and targeting therapies. Carcinogenesis. 2006;27(1):1–22.
[92] Wiener JR, Kerns BJ, Harvey EL, Conaway MR, Iglehart JD, Berchuck A, et al. Overexpression of the protein tyrosine phosphatase PTP1B in human breast cancer: association with p185c-erbB-2 protein expression. J Natl Cancer Inst. 1994;86(5):372–8.
[93] Julien SG, Dube N, Read M, Penney J, Paquet M, Han Y, et al. Protein tyrosine phosphatase 1B deficiency or inhibition delays ErbB2-induced mammary tumorigenesis and protects from lung metastasis. Nat Genet. 2007;39(3):338–46.
[94] Arias-Romero LE, Saha S, Villamar-Cruz O, Yip SC, Ethier SP, Zhang ZY, et al. Activation of Src by protein tyrosine phosphatase 1B Is required for ErbB2 transformation of human breast epithelial cells. Cancer Res. 2009;69(11):4582–8.
[95] Balavenkatraman KK, Aceto N, Britschgi A, Mueller U, Bence KK, Neel BG, et al. Epithelial protein-tyrosine phosphatase 1B contributes to the induction of mammary tumors by HER2/Neu but is not essential for tumor maintenance. Mol Cancer Res. 2011;9(10): 1377–84.
[96] Milani ES, Brinkhaus H, Dueggeli R, Klebba I, Mueller U, Stadler M, et al. Protein tyrosine phosphatase 1B restrains mammary alveologenesis and secretory differentiation. Development. 2013;140(1):117–25.
[97] Soysal S, Obermann EC, Gao F, Oertli D, Gillanders WE, Viehl CT, et al. PTP1B expression is an independent positive prognostic factor in human breast cancer. Breast Cancer Res Treat. 2013;137(2):637–44.
[98] Puius YA, Zhao Y, Sullivan M, Lawrence DS, Almo SC, Zhang ZY. Identification of a second aryl phosphate-binding site in protein-tyrosine phosphatase 1B: a paradigm for inhibitor design. Proc Natl Acad Sci U S A. 1997;94(25):13420–5.
[99] Groves MR, Yao ZJ, Roller PP, Burke TR, Jr., Barford D. Structural basis for inhibition of the protein tyrosine phosphatase 1B by phosphotyrosine peptide mimetics. Biochemistry. 1998; 37(51):17773–83.

[100] Larsen SD, Barf T, Liljebris C, May PD, Ogg D, O'Sullivan TJ, et al. Synthesis and biological activity of a novel class of small molecular weight peptidomimetic competitive inhibitors of protein tyrosine phosphatase 1B. J Med Chem. 2002;45(3):598–622.
[101] Szczepankiewicz BG, Liu G, Hajduk PJ, Abad-Zapatero C, Pei Z, Xin Z, et al. Discovery of a potent, selective protein tyrosine phosphatase 1B inhibitor using a linked-fragment strategy. J Am Chem Soc. 2003;125(14):4087–96.
[102] Asante-Appiah E, Patel S, Dufresne C, Roy P, Wang Q, Patel V, et al. The structure of PTP-1B in complex with a peptide inhibitor reveals an alternative binding mode for bisphosphonates. Biochemistry. 2002;41(29):9043–51.
[103] Jia Z, Ye Q, Dinaut AN, Wang Q, Waddleton D, Payette P, et al. Structure of protein tyrosine phosphatase 1B in complex with inhibitors bearing two phosphotyrosine mimetics. J Med Chem. 2001;44(26):4584–94.
[104] Sarmiento M, Puius YA, Vetter SW, Keng YF, Wu L, Zhao Y, et al. Structural basis of plasticity in protein tyrosine phosphatase 1B substrate recognition. Biochemistry. 2000;39(28): 8171–9.
[105] Sun JP, Fedorov AA, Lee SY, Guo XL, Shen K, Lawrence DS, et al. Crystal structure of PTP1B complexed with a potent and selective bidentate inhibitor. J Biol Chem. 2003;278(14): 12406–14.
[106] Iversen LF, Andersen HS, Branner S, Mortensen SB, Peters GH, Norris K, et al. Structure-based design of a low molecular weight, nonphosphorus, nonpeptide, and highly selective inhibitor of protein-tyrosine phosphatase 1B. J Biol Chem. 2000;275(14):10300–7.
[107] Andersen HS, Iversen LF, Jeppesen CB, Branner S, Norris K, Rasmussen HB, et al. 2-(oxalylamino)-benzoic acid is a general, competitive inhibitor of protein-tyrosine phosphatases. J Biol Chem. 2000;275(10):7101–8.
[108] Peters GH, Iversen LF, Branner S, Andersen HS, Mortensen SB, Olsen OH, et al. Residue 259 is a key determinant of substrate specificity of protein-tyrosine phosphatases 1B and alpha. J Biol Chem. 2000;275(24):18201–9.
[109] Iversen LF, Andersen HS, Moller KB, Olsen OH, Peters GH, Branner S, et al. Steric hindrance as a basis for structure-based design of selective inhibitors of protein-tyrosine phosphatases. Biochemistry. 2001;40(49):14812–20.
[110] Wee C, Muise ES, Coquelet O, Ennis M, Wagner J, Lemieux N, et al. Promoter analysis of the murine T-cell protein tyrosine phosphatase gene. Gene. 1999;237(2):351–60.
[111] Kamatkar S, Radha V, Nambirajan S, Reddy RS, Swarup G. Two splice variants of a tyrosine phosphatase differ in substrate specificity, DNA binding, and subcellular location. J Biol Chem. 1996;271(43):26755–61.
[112] Zander NF, Cool DE, Diltz CD, Rohrschneider LR, Krebs EG, Fischer EH. Suppression of v-fms-induced transformation by overexpression of a truncated T-cell protein tyrosine phosphatase. Oncogene. 1993;8(5):1175–82.
[113] Hao L, Tiganis T, Tonks NK, Charbonneau H. The noncatalytic C-terminal segment of the T cell protein tyrosine phosphatase regulates activity via an intramolecular mechanism. J Biol Chem. 1997;272(46):29322–9.
[114] Gupta V, Swarup G. Evidence for a role of transmembrane protein p25 in localization of protein tyrosine phosphatase TC48 to the ER. J Cell Sci. 2006;119(Pt 9):1703–14.
[115] Meng TC, Buckley DA, Galic S, Tiganis T, Tonks NK. Regulation of insulin signaling through reversible oxidation of the protein-tyrosine phosphatases TC45 and PTP1B. J Biol Chem. 2004;279(36):37716–25.
[116] Galic S, Hauser C, Kahn BB, Haj FG, Neel BG, Tonks NK, et al. Coordinated regulation of insulin signaling by the protein tyrosine phosphatases PTP1B and TCPTP. Mol Cell Biol. 2005;25(2): 819–29.

[117] Bukczynska P, Klingler-Hoffmann M, Mitchelhill KI, Lam MH, Ciccomancini M, Tonks NK, et al. The T-cell protein tyrosine phosphatase is phosphorylated on Ser-304 by cyclin-dependent protein kinases in mitosis. Biochem J. 2004;380(Pt 3):939–49.
[118] Simoncic PD, Lee-Loy A, Barber DL, Tremblay ML, McGlade CJ. The T cell protein tyrosine phosphatase is a negative regulator of janus family kinases 1 and 3. Curr Biol. 2002;12(6): 446–53.
[119] ten Hoeve J, de Jesus Ibarra-Sanchez M, Fu Y, Zhu W, Tremblay M, David M, et al. Identification of a nuclear Stat1 protein tyrosine phosphatase. Mol Cell Biol. 2002;22(16):5662–8.
[120] Yamamoto T, Sekine Y, Kashima K, Kubota A, Sato N, Aoki N, et al. The nuclear isoform of protein-tyrosine phosphatase TC-PTP regulates interleukin-6-mediated signaling pathway through STAT3 dephosphorylation. Biochem Biophys Res Commun. 2002;297(4):811–7.
[121] Aoki N, Matsuda T. A nuclear protein tyrosine phosphatase TC-PTP is a potential negative regulator of the PRL-mediated signaling pathway: dephosphorylation and deactivation of signal transducer and activator of transcription 5a and 5b by TC-PTP in nucleus. Mol Endocrinol. 2002;16(1):58–69.
[122] Zheng Y, Schlondorff J, Blobel CP. Evidence for regulation of the tumor necrosis factor alpha-convertase (TACE) by protein-tyrosine phosphatase PTPH1. J Biol Chem. 2002;277 (45):42463–70.
[123] Galic S, Klingler-Hoffmann M, Fodero-Tavoletti MT, Puryer MA, Meng TC, Tonks NK, et al. Regulation of insulin receptor signaling by the protein tyrosine phosphatase TCPTP. Mol Cell Biol. 2003;23(6):2096–108.
[124] Sarhan AR, Patel TR, Creese AJ, Tomlinson MG, Hellberg C, Heath JK, et al. Regulation of platelet derived growth factor signaling by Leukocyte Common Antigen-related (LAR) protein tyrosine phosphatase: a quantitative phosphoproteomics study. Mol Cell Proteomics. 2016;15(6): 1823–36.
[125] Csar XF, Wilson NJ, McMahon KA, Marks DC, Beecroft TL, Ward AC, et al. Proteomic analysis of macrophage differentiation. p46/52(Shc) Tyrosine phosphorylation is required for CSF-1-mediated macrophage differentiation. J Biol Chem. 2001;276(28):26211–7.
[126] Stanley ER, Berg KL, Einstein DB, Lee PS, Pixley FJ, Wang Y, et al. Biology and action of colony – stimulating factor-1. Mol Reprod Dev. 1997;46(1):4–10.
[127] Gobert Gosse S, Bourgin C, Liu WQ, Garbay C, Mouchiroud G. M-CSF stimulated differentiation requires persistent MEK activity and MAPK phosphorylation independent of Grb2-Sos association and phosphatidylinositol 3-kinase activity. Cell Signal. 2005;17(11):1352–62.
[128] You-Ten KE, Muise ES, Itie A, Michaliszyn E, Wagner J, Jothy S, et al. Impaired bone marrow microenvironment and immune function in T cell protein tyrosine phosphatase-deficient mice. J Exp Med. 1997;186(5):683–93.
[129] A cytosolic protein-tyrosine phosphatase PTP1B specifically dephosphorylates and deactivates prolactin-activated STAT5a and STAT5b. J Biol Chem. 2011;286(12):10888.
[130] Gu F, Nguyen DT, Stuible M, Dube N, Tremblay ML, Chevet E. Protein-tyrosine phosphatase 1B potentiates IRE1 signaling during endoplasmic reticulum stress. J Biol Chem. 2004;279(48): 49689–93.
[131] Ostman A, Bohmer FD. Regulation of receptor tyrosine kinase signaling by protein tyrosine phosphatases. Trends Cell Biol. 2001;11(6):258–66.
[132] Persson C, Savenhed C, Bourdeau A, Tremblay ML, Markova B, Bohmer FD, et al. Site-selective regulation of platelet-derived growth factor beta receptor tyrosine phosphorylation by T-cell protein tyrosine phosphatase. Mol Cell Biol. 2004;24(5):2190–201.

7 The non-receptor protein tyrosine phosphatases: Part II

The previous chapter describes the classification of the non-receptor protein tyrosine phosphatases (PTPs) into various subtypes. All the 18 subtypes of this large superfamily of signaling enzymes have their own demarcated role in human physiology and health (Table 7.1). Key mutations that compromise their activity lead to various diseases and make these proteins lucrative targets for drug discovery efforts.

7.1 The NT2 subtype of non-receptor PTPs

The NT2 subtype of non-receptor PTPs includes two gene products, SHP1 and SHP2, coded by genes *PTPN6* and *PTPN11*, respectively. Both proteins are characterized by the presence of tandem Src homology 2 (SH2) domains that serve as phosphotyrosine-binding modules [1, 2]. SHP1 is expressed in low levels in the human epithelial cells and expressed at all stages of maturation of the hematopoietic stem cells [3]. SHP2 protein is expressed ubiquitously alongside SHP1 [1]. The differential expression of SHP1 and SHP2 is often used to ascertain their biological role in human physiology. Many cells express good amounts of both SHP1 and SHP2 but the lack of function of each protein is reported to lead to different manifestations. Both SHP1 and SHP2 have a conserved PTP domain that is the bigger domain in their polypeptide chain. Their PTP domain is characterized by a well-formed active site with a fully functional nucleophilic cysteine that sits in the VHCSAGIGRTG P-loop motif. This PTP is flanked by the tandem SH2 domains at the N-terminus and a C-terminal tail that contains various phosphorylation sites (Figure 7.1). The SH2 domains of the proteins regulate their intracellular location and also catalytic function. In the resting state, the SH2 domains fold over the catalytic PTP domain and repress its activity [4]. The C-terminal tail of the proteins also plays a regulatory role in maintaining required signaling levels of the SHP1 and SHP2 activities [5]. Two tyrosines in the C-terminal of these proteins are phosphorylation sites that are used in response to various cellular signals for controlling their activity. In SHP1 these tyrosines are Y536 and Y564, and in SHP2 these tyrosines are Y542 and Y580 (Figure 7.1) [6, 7].

7.1.1 Structure and regulation of SHPs

The SHPs contain two tandem SH2 domains N-terminal to their PTP domain. SHP1 and SHP2 are highly similar in sequence covering their PTP domains. The comparable structures of their phosphatase domains and their similar enzymatic properties suggest a comparable molecular mechanism for their overall regulation. The overall

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Table 7.1: Eighteen subtypes of non-receptor PTPs.

Gene name	Subtype	Chromosome location	Protein name
PTPN1	NT1	20q13.13	PTP1B
PTPN2	NT1	18p11.21	TCELLPTP, TC-PTP, TCPTP
PTPN3	NT5	9q31	PTPH1
PTPN4	NT5	2q14.2	PTPMEG
PTPN5	R7	11p15.1	STEP, PTPSTEP, STEP61
PTPN6	NT2	12p13.31	HCP, HCPH, PTP-1C, SHP-1, SHP1
PTPN7	R7	1q32.1	HEPTP, LC-PTP
PTPN9	NT3	15q24.2	MEG2
PTPN11	NT2	12q24.13	BPTP3, SH-PTP2, SHP-2, PTP2C, SHP2, NS1
PTPN12	NT4	7q11.23	PTPG1, PTP-PEST
PTPN13	NT7	4q21.3	PTP1E, PTP-BAS, PTPL1, PTP-BL
PTPN14	NT6	1q32.3–q41	PEZ, PTPD2
PTPN18	NT4	2q21.1	BDP1
PTPN20	NT9	10q11.22	bA42B19.1, DKFZP566K0524, bA142I17.1, CT126, PTPN20B, PTPN20A
PTPN21	NT6	14q31	PTPD1, PTPRL10
PTPN22	NT4	1p13.2	Lyp, Lyp1, Lyp2, PTPN8
PTPN23	NT8	3p21.31	DKFZP564F0923, KIAA1471, HD-PTP

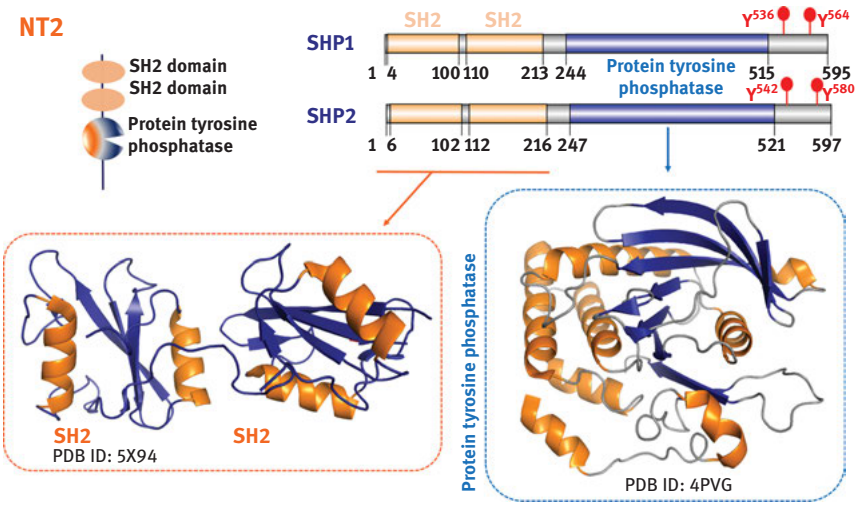

Figure 7.1: Domain organization of the SHPs. These proteins have two tandem SH2 domains that lie N-terminal to the protein tyrosine phosphatase domain.

identity between SHP1 and SHP2 is high and their N-terminal SH2 domain is the region of most variability. In the proposed basal state, SHP is speculated to be inactive where its N-terminal SH2 domain folds over to block access to the active

site of the phosphatase domain (Figure 7.2). Most importantly, residues D61 and E76 from the N-terminal SH2 domain occupy the peptide-binding pocket of the PTP cleft. The second C-terminal SH2 domain is unperturbed and has its phosphotyrosine-binding pocket available to incoming ligands.

Structural studies provide support for speculation of the C-terminal SH2 domain surveying the cell for binding partners to release the intramolecular inhibition. Further support for the intramolecular inhibition model comes from analysis of the N-terminal SH2 domain and phosphatase domain interface. Mutations at the domain–domain interface are linked to diseases including Noonan syndrome (NS) and juvenile myelomonocytic leukemia (JMML) [8]. Germline mutations in SHP2 are responsible for about ~50% of reported cases of NS and somatic mutations of SHP2 are seen in about 20–25% of JMML. These mutants have less inhibited and show higher basal levels of SHP catalytic activity [9]. The most common mutations of NS include D61G, D61A, E76D and E76K, all of which effect the domain–domain interface of the N-terminal SH2 domain and the catalytic phosphatase domain of SHPs (Figure 7.2).

The C-tail of SHPs plays a critical role in regulating their subcellular regulation. SHP1 localizes to the nucleus with the help of a functional nuclear localization signal in its C-tail [10]. The C-tail of SHP1 hence allows it to shuttle between the cytoplasm and the nucleus depending on the needs of the cell. SHP1 has been reported to be localized in the [11] nucleus of epithelial cells and in the cytoplasm of randomly growing hematopoietic cells [12]. The nuclear localization signal is located at the end of the C-tail of SHP1 (residues 576–595) and allows for bipartite localization of SHP1 in cytokine signaling. Upon cytokine stimulation, nuclear localization of SHP1 is induced albeit with delayed kinetics or a lag time of over an hour after stimulation. This study emphasizes the signaling role of SHP1 in the later events of cytokine signaling [12].

Intramolecular allostery is speculated to allow for activation of the SHPs probably via the C-terminal SH2 domain that is primed to bind an incoming phosphotyrosine. Two potential modes of activation have been postulated for SHPs (Figure 7.2). In the first mechanism, a phosphotyrosine containing binding partner engages the SH2 domains and allows for their conformational re-arrangement. In this model, the C-terminal SH2 domain is postulated to engage the binding partner first that would allow for a corresponding conformational change in the SHPs while simultaneously increasing the local concentration of phosphotyrosine around the N-terminal SH2 domain. This model finds support in various structural and biochemical studies. Elaborate reports are available for the binding of glycoprotein GP49B with SHP1 using this mechanism [13]. The second mechanism allows for a role of the C-terminal tail in providing inhibition relief. Phosphorylation of two conserved tyrosines, namely Y536 and Y564 in SHP1 and Y542 and Y580 in SHP2 allows for their intramolecular association with the SH2 domains. This mechanism would allow for the reverse contortion of the SHP molecule where the SH2 domain would fold over to interact with the tyrosine phosphorylated C-tail thereby releasing the phosphatase active site from inhibition (Figure 7.2). Support

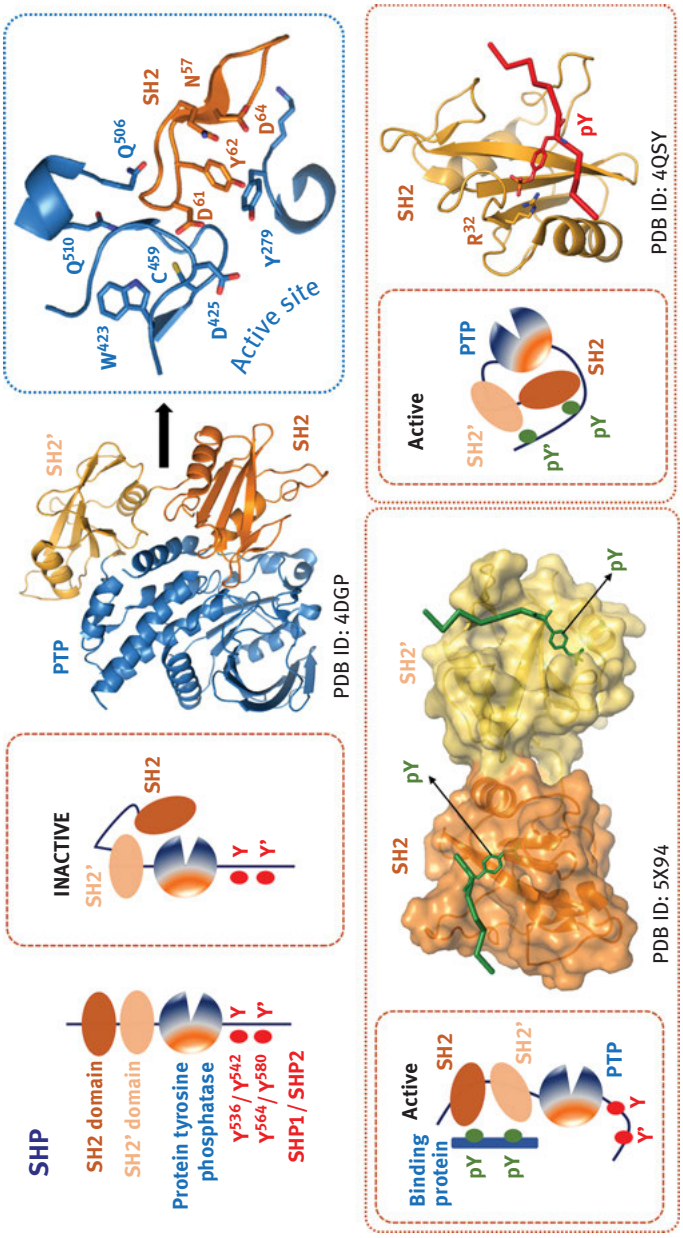

Figure 7.2: Mode of regulation of activity of SHPs by their SH2 domains.

for this model comes from biochemical studies where Y542 and Y580 of SHP2 were replaced with nonhydrolyzable phosphotyrosine mimetics. These engineered SHP2 molecules showed a higher basal activity about threefold higher than the nonphosphorylated SHP2 protein [14]. Mutagenesis and proteolysis resistance assays have allowed for the identification of binding modes of N-terminal SH2 domain with Y542 and C-terminal SH2 domain with Y580 (Figure 7.2) [15].

Several binding partners of the SHPs have been explored and are reported to contain one of more of the immunoreceptor tyrosine-based inhibitory motif (ITIMs). Binding partners include receptor tyrosine kinases, scaffolding adaptors such as IRS and DOS/GAB and various immune inhibitory receptors. The ITIMs have a consensus sequence [I/V/L]x**pY**xx[I/V/L] that harbors the phosphotyrosine in the center to bind the SH2 domains of SHPs [16]. Some ITIMs are reported to prefer one SH2 domain of SHPs over the other. Also, the exact mechanism of this preference is not clear as some binding partners bind both SHP1 and SHP2 while some show a preference for one over the other [17]. For example, mast cell function-associated antigen (MAFA)-based peptide binds both SHP1 and SHP2 [18]. MAFA contains an ITIM-like motif that contains a serine instead of a small aliphatic residue at two positions N-terminal of the phosphotyrosine. SHP1 is reported to bind tumor necrosis factor (TNF) family members via an alternate Ax**pY**xxL motif [19]. Peptide library-based approaches have been used to characterize the binding specificity of SHPs [20]. Interestingly, this binding specificity of the SH2 domains of SHPs overlaps with those of other signaling proteins including Socs family of ubiquitin ligases and inositol phosphatase Ship [21–23].

7.1.2 Physiological role of SHP1

Genetic models of SHP deficiency have allowed for the exploration of their physiological function. The SHP1 deficiency is generically referred to as *me* (standing for *motheaten*). The *motheaten* phenotype gets its name from the patchy hair loss seen in SHP1-deficient mice due to sterility of their dermal abscesses. Mouse models have two naturally occurring *me* mutations. The *motheaten* (me) allele results from a splicing mutation in the SHP1 gene (*PTPN6*) leading to frameshift at the 5′-end of the coding sequence [24, 25]. This results in loss of protein expression such that mutant mice (me/me) are essentially SHP-1 null animals. These mice have severe defects in their hematopoietic lineages and die within 2–3 weeks of birth [26]. The second naturally occurring allele is called as *motheaten variable* (me^v) that encodes two different kinds of aberrant SHP1 proteins. One of these proteins has a small deletion, while the other has a small insertion in the PTP domain. The me^v/me^v mice suffer less as compared to me/me strains. These mice remain alive anywhere between 9 and 12 weeks compared to just 2–3 weeks of survival for the me/me mice.

SHP1 is reported to regulate integrin such that bone marrow macrophages deficient in SHP1 are known to hyperadhere to both β1 and β2 integrin ligands [27]. An SHP1-binding protein SHPS1 along with PirB protein allows for this regulatory function. SHPS1's rapid tyrosyl phosphorylation allows for the recruitment of SHP1 [28]. SHPS1 is also able to complex with other proteins of the integrin signaling pathway including various adaptors such as Skaphom/R, SLAP130/Fyb and ADAP (adhesion and degranulation promoting adapter protein) [29, 30]. Skaphom, ADAP and Pyk2 are reported to become phosphorylated on their tyrosine residues upon adhesion. It is hence speculated that not only do these proteins constitutively associate with SHPS1, but they also serve as direct substrates for SHP1 tyrosine phosphatase.

Some of the earliest cell biology-based experiments have shown that SHP1 binds erythropoietin receptor (EpoR) and also dephosphorylates Jak2 kinases that are reported to associate with these receptors [31]. Genetic studies show that mutants with truncations in EpoR show erythrocytosis and that the deleted region of the receptor corresponds to binding sites for SHP1 and Soc3 [32]. As the binding sites for SHP1 and Soc3 overlap for the most part, it is speculated that probably both proteins work in combination to regulate Jak2 [33]. Activated Jak2 is reported to be phosphorylated on Tyr1007 and Tyr1008. As the doubly phosphorylated Jak2 would be unable to bind Soc3, the current model suggests that SHP1 associates with Jak2 first and dephosphorylates pTyr1008. The single phosphorylation on Tyr1007 now allows Jak2 to associate with Soc3 that primes it for degradation.

SHP1 is also reported to be a modulator of TNF and Fas death receptors [19]. Activation of these receptors allows for the phosphorylation of a cytoplasmic AxYxxL motif in the receptor tails. Activation of the death receptor antagonizes the effect of the cytokine signaling via the pYxxL phosphotyrosine motif. In this context, the "me" neutrophils are resistant to death receptor stimulation. This also corresponds to increased levels of phosphorylated form of Lyn upon cytokine stimulation of these neutrophils. These reports indicate a crucial interplay between the death receptor and cytokines being mediated by Lyn, and that this role of Lyn is dependent on its tyrosine dephosphorylation by SHP1.

7.1.3 SHP2 signaling and function

Much like SHP1, SHP2 plays a crucial role in integrin signaling. SHP2 is involved in integrin-mediated cell migration and spreading, a process that also requires signaling from protein kinase Erk [34, 35]. Cells with mutated copy of SHP2 show enhanced levels of activated small G-protein Rho and have increased stress fibers [36]. Again, like SHP1, SHP2 is reported to interact with SHPS1 protein that is phosphorylated on its tyrosine residues following integrin activation [37]. SHP2 also allows for integration of integrin signaling with signaling by the receptor tyrosine kinases [38]. Stimulation of receptor tyrosine kinase EphA2 leads to SHP2-mediated dephosphorylation of focal adhesion

kinase (Fak) that eventually results in breaking or inhibiting integrin signaling. However, this interplay between Fak and SHP2 is reported to vary in various cell types, and various discrepancies exist in reports by various research groups. Some authors report enhanced tyrosine phosphorylation of Fak in a dominant-negative SHP2 environment [39], while some do not [35]. Some fibroblast cell-based studies are also divergent. Some reports suggest that there is a decreased Fak dephosphorylation upon inhibition of adhesion [34]. Then, fibroblasts that lack the SHP2 interacting region of SHPS1 cytoplasmic domain show defective migration and decreased Rho activation [40]. Another report clearly suggests that SHP2 is crucial for the integrin-mediated phosphorylation of Src and Fak proteins [41].

SHP2 is reported to be indispensable in receptor tyrosine kinase and cytokine signaling. For example, in some cell types growth factor receptor-based activation of Erk requires the presence of active SHP2 [42]. Cells having dominant-negative SHP2 are unable to activate Ras signaling [43]. The current model for SHP2-mediated activation of Ras/Erk pathway is the "adapter model" [44] (Figure 7.3). Signaling by the receptor tyrosine kinases is initiated by ligand binding that activates these receptors leading to autophosphorylation of their multiple tyrosine residues. These phosphotyrosines in turn serve as recruitment sites for SH2 and PTB domain containing proteins that mediate downstream signaling. Ras is recruited by the adapter protein Grb2, or sometimes also by Shc. Grb2 also recruits the guanine nucleotide exchange factor (GEF) that activates Ras by promoting simultaneous GDP release and GTP binding. Ras is switched off by GTPase activating protein (GAPs) that promote hydrolysis of Ras-GTP to Ras-GDP. These GAPs are also recruited to the cytoplasmic tails of receptor tyrosine kinases by their SH2 domains. Here, SHP2 plays the role of

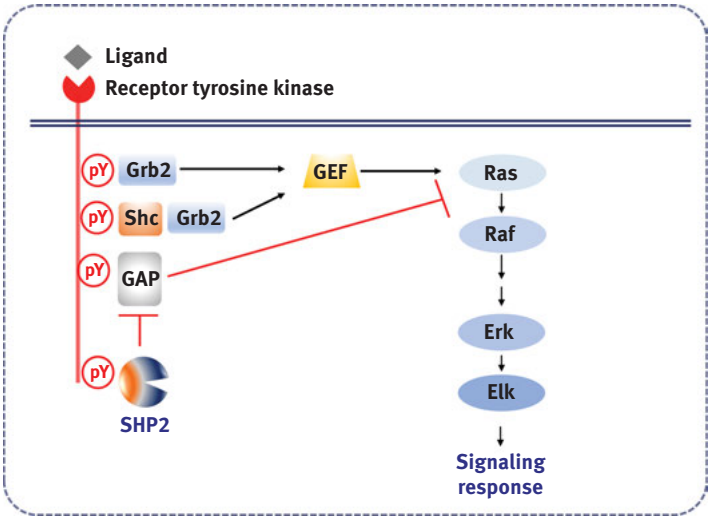

Figure 7.3: The "Adaptor model" of Erk activation by SHPs.

dephosphorylating the phosphotyrosines of the receptors that serve as Ras-GAP binding sites. SHP2 binds directly to some receptor tyrosine kinases like the platelet-derived growth factor receptor to directly dephosphorylate the Ras-Gap binding site [45]. Binding and dephosphorylation of other receptor tyrosine kinases is mediated by adapter proteins, including FRS2, Gab/DOS and the insulin receptor substrate family of adapter proteins [46]. In this way, SHP2 prevents recruitment of GAPs on the receptors to maintain continued Ras signaling.

SHP2 regulates Ras signaling also via the Src family of kinases (SFKs). Expression of GAP1–SHP2 fusion constructs in cells results in enhanced Src signaling, which suggests that SHP2 directly serves as the tyrosine phosphatase for dephosphorylating the inhibitory C-tail tyrosine phosphorylation of SFKs. Alternatively, it is possible that SHP2 inhibits recruitment of Csk kinase (that phosphorylates the SFK C-tails) to the membrane by dephosphorylating PAG/CBP (Figure 7.4) [47, 48]. In this model, SHP2 is also speculated to work in conjunction with receptor PTPα that is the major phosphatase for SFKs [49]. It is speculated that while PTPα accounts for activating of SFKs following growth factor stimulation, SHP2 plays a crucial role in maintaining the pools of the active SFKs in the cell.

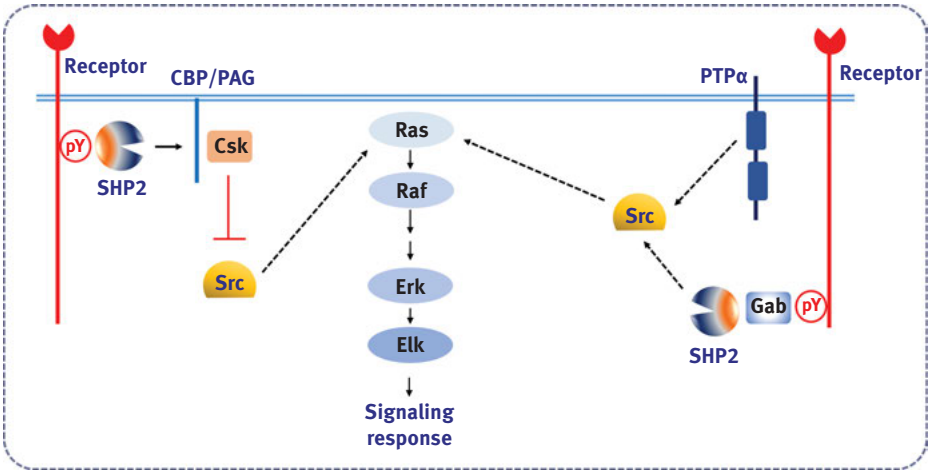

Figure 7.4: Mode of regulation of Erk pathway by SHPs via the Src family of kinases.

7.2 The NT3 subtype of non-receptor PTPs

The NT3 non-receptor PTPs include the cytosolic PTP-MEG2 that is coded by the *PTPN9* gene. This *PTPN9* gene was first reported and originally cloned from megakaryocytes [50], but was later reported to be expressed in various mammalian cells and tissue types. *PTPN9* gene codes for a 65-kDa protein that contains a single PTP domain along with unique N-terminal domain (Figure 7.5). The unique N-terminal

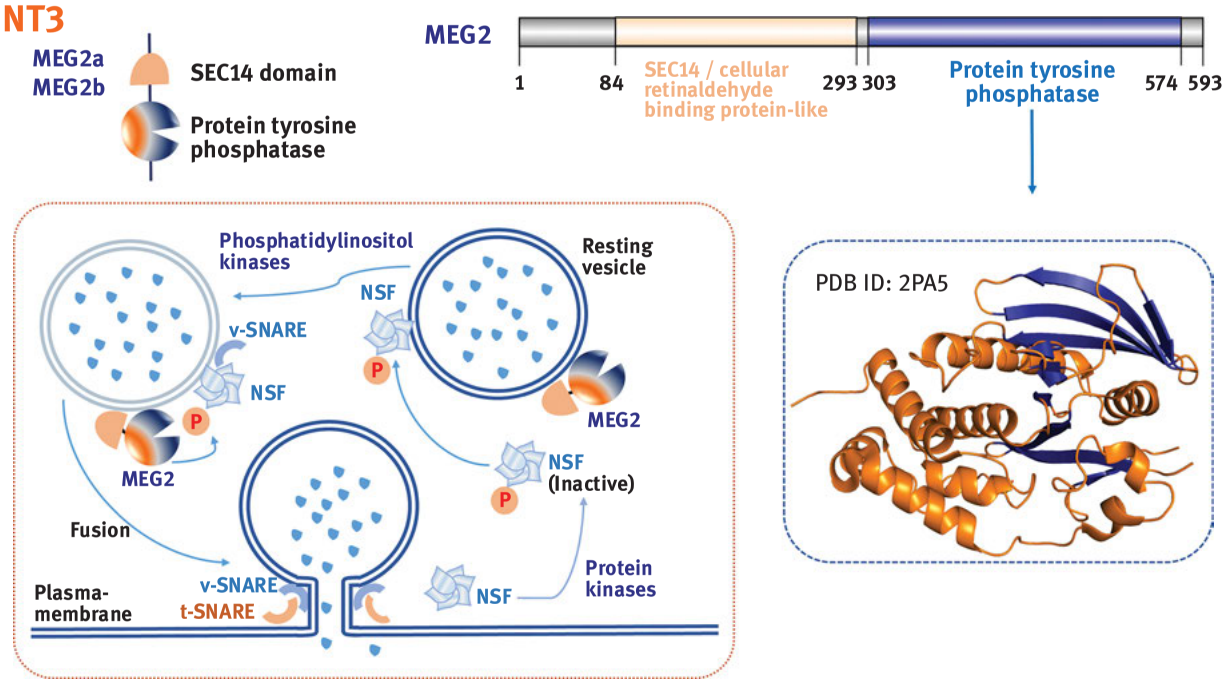

Figure 7.5: Domain organization and catalytic domain architecture of the MEG protein tyrosine phosphatases. PTP-MEG plays an important role in vesicle formation, maturation and fusion with the cell membrane.

domain is about 24% identical to SEC14p phosphoinositol-binding protein from yeast and 28% identical to retinaldehyde-binding protein [50]. This unique N-terminal domain of PTP-MEG2 binds phosphatidylinositol (3,4,5)-triphosphate (PIP_3) and allows for its localization to the membranes of secretory vesicles [51, 52]. Reports show that exogenously expressed PTP-MEG2 predominantly localizes to the intracellular secretory vesicles in mast cells. PTP-MEG2 when exogenously expressed in neutrophils localizes to the nascent phagosomes [51, 52].

Biological activity of PTP-MEG2 is predominantly crucial for the regulation of vesicle formation and fusion [53]. PTP-MEG2 also participates in regulation of insulin signaling in hepatocytes [54] and vascular endothelial growth factor receptor signaling in endothelial cells [55]. Overactivity of PTP-MEG2 in erythroid progenitors is associated with polycythemia vera, a blood disorder where bone marrow makes excessive red blood cells [56]. Physiological substrates of PTP-MEG2 include the epithelial growth factor receptor and the vascular endothelial growth factor receptor. In the secretory vesicles PTP-MEG2 dephosphorylates the *N*-ethylmaleimide sensitive factor (NSF) at a critical phosphotyrosine position (Tyr83) that allows for its activation [53]. NSF regulates the disassembling of the soluble NSF attachment protein receptor complexes that allow for vesicle fusion with the plasma membrane (Figure 7.5). Specific phosphatidylinositols engage the N-terminal unique domain of PTP-MEG2 and affect its activity. PIP_3, phosphatidylinositol (4,5)-bisphosphate (PIP_2) and phosphatidylserine enhance the activity of PTP-MEG2. Inhibition of PIP_3 biosynthesis is detrimental to PTP-MEG2 signaling [57].

PTP-MEG2 deficiency is reported to be embryonic lethal. Some studies using irradiated mice being reconstituted with PTP-MEG2-deficient fetal liver cells demonstrate that these animals have severely deficient lymphocytes and platelets that may have matured normally [58]. Electron microscope images and analysis of T cells deficient in PTP-MEG2 show increased electron dense particles that correlate with decreased populations of matured secretory vesicles. Animals reconstituted with PTP-MEG2-deficient fetal cells show abnormal T-cell function with diminished T-cell receptor-induced secretion of cytokines such as interleukin (IL)-2, IL-6 and interferon-γ [59]. These animals also demonstrate diminished thrombin-induced platelet aggregation due to impaired platelet granule release by vesicle secretion [60].

7.3 The NT4 subtype of non-receptor PTPs

The NT4 subtype of nonreceptor PTPs includes three member phosphatases. These are PTP-PEST (proline, glutamate, serine and threonine), BDP1 (brain-derived phosphatase) and Lyp (lymphoid phosphatase) coded by the *PTPN12, PTPN18* and *PTPN22* genes, respectively [61]. Collectively these three tyrosine phosphatases are also called the PEST family of PTPs as they contain regions rich in the proline, glutamate, serine and threonine sequence. These proteins are characterized by an N-terminal classical PTP domain that is followed by proline-rich regions in the C-terminal part (Figure 7.6). These

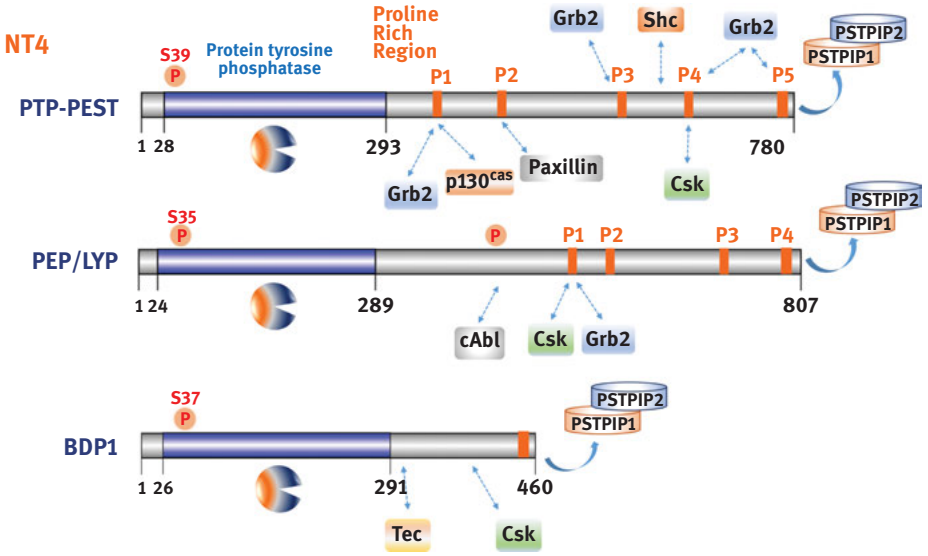

Figure 7.6: Domain organization of the NT4 subtype of non-receptor protein tyrosine phosphatases.

proline-rich regions allow these tyrosine phosphatases to engage with other proteins of the cellular signaling machinery. The interdomain regions between the tyrosine phosphatase domain and the PEST-rich region are reported to be crucial for the regulation of their catalytic activity [62].

7.3.1 The PTP-PEST

The *PTPN12* gene is reported to be a tumor suppresser whose gene product PTP-PEST allows for the regulation of epidermal growth factor receptor (EGFR)/HER2 signaling pathways [63]. PTP-PEST also functions to regulate signaling monitoring cell invasion and morphogenesis [64]. Physiological substrates of PTP-PEST mediate immune response, neuronal signaling and also cell motility. PTP-PEST deficiency leads to embryonic lethality [65]; therefore, most studies on this tyrosine phosphatase use substrate trapping mutants of the catalytic domain or the PTP-PEST −/− fibroblasts [66]. Substrates of PTP-PEST identified using these biochemical and cell-culture-based assays include $p130^{cas}$, Sin, CasL, Paxillin, Shc, Pyk2, Vav2, p190RhoGAP and tyrosine kinases like cAbl [67, 68].

7.3.2 Structural architecture of PTP-PEST

The tyrosine phosphatase domain of PTP-PEST retains all the classic components of the PTP superfamily [69]. The domain has central β-sheet flanked by α-helices

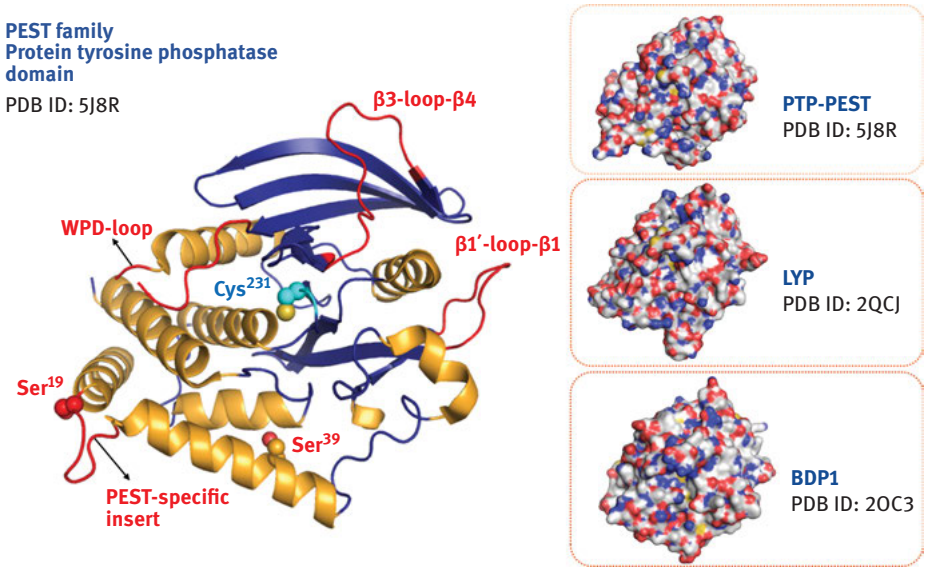

Figure 7.7: The catalytic domain of PEST family of protein tyrosine phosphatases showing the PEST family-specific secondary structural elements.

that allow for presentation of key structural loops around the active site. A PTP-PEST-specific linker is seen connecting the α1′ and α2′ helices (Figure 7.7). This unique linker has a sequence MKSPDHNG that serves as a substrate recognition site for the cyclin-dependent protein kinases. Phosphorylation of PTP-PEST at this unique serine residue by CDK2/cyclin has been demonstrated in biochemical assays using recombinant CDK2/cyclin proteins and CDK2-substrate antibodies as probes. Four regions in the PTP domain of PTP-PEST explain the diversity seen in the PEST family. Differences between Lyp, BDP1 and PTP-PEST are seen in the WPD-loop, the β1-loop-β2, β3-loop-β4 and also the α1′ helix (Figure 7.7). Distinct loops of these three phosphatases define their surface electrostatic potential and regulate their substrate preference. Mode of substrate binding to PTP-PEST has been explained by biochemical analysis of the interaction of the phosphatase with various phosphopeptides derived from the HER2 protein [69]. Peptides containing pTyr1196 and pTyr1248 demonstrate the requirement for acidic residues in the substrate at pY-4 and pY-1 positions. A hydrophobic residue is preferred at the pY+1 position. Four key residues on the surface of the PTP-PEST catalytic domain allow it to distinguish the different phosphosites on HER2. Arg36 defines the interaction between PTP-PEST and HER2 sites pTyr1112 and pTyr1248. Lys45 defines the interaction between PTP-PEST and HER2 sites pTyr1112 and pTyr1196. Lys142 defines PTP-PEST's specificity for site pTyr1248, while His274 specifies the interaction with pTyr1112.

Other than the PTP domain, PTP-PEST contains five proline-rich regions [70]. These regions allow PTP-PEST to interact with other proteins of the various signaling pathways. PTP-PEST binds the inhibitory protein tyrosine kinase Csk via its P4 proline-rich region [67, 71]. It binds the scaffold proteins $p130^{cas}$, Sin/Efs and CasL/Hef-1 using its P1 proline-rich region. The P2 proline-rich regions mediate binding to the LIM domains of paxillin, Hic-5 and leupaxin. The NPLH sequence of PTP-PEST allows its binding to the Shc PTB domains [72]. PTP-PEST also binds to the coiled-coil domains of cytoskeletal regulating proteins CD2BP1 [73].

7.3.3 PTP-PEST: Mode of physiological function

The importance of PTP-PEST function in immune cell regulation is described by its interaction with an adaptor protein called PSTPIP1 (Figure 7.6) [74]. Familial recurrent arthritis and Pyogenic Arthritis, Pyoderma gangrenosum and Acne (PAPA) syndrome are two autosomal dominant autoimmune disorders that result from the inability of PSTPIP1 to associate with PTP-PEST [75]. The gene locus of *PTPN12* gene also falls on chromosome 7q at a region linked to inflammatory bowel disease, though the exact role of PTP-PEST in the autoimmune disorder remains unexplored [76]. Studies on PTP-PEST in T cells have elucidated the role of its interaction with PSTPIP1 in detail. The SH3 domain of PSTPIP1 recruits the Abl tyrosine kinase and mediates its interaction with PTP-PEST. This allows PTP-PEST to regulate signaling induced or regulated by tyrosine kinases. PSTPIP1 also interacts with a critical regulator of actin polymerization, WASp [77]. Stimulation of the T-cell receptor causes Fyn tyrosine kinase-mediated phosphorylation of WASp at Tyr291. PSTPIP1-associated PTP-PEST dephosphorylates the phosphotyrosine on WASp and allows for reversal of T-cell receptor signaling and regulation of immune synapse formation [78].

In B cells, PTP-PEST overexpression inhibits the B-cell receptor-induced Erk activation that can be rescued by the overexpression of Ras proteins [79]. PTP-PEST is also reported to associate with Csk, paxillin, $p130^{cas}$ and Shc in the A20 B-cell line [80]. Pyk2, FAK, $p130^{cas}$ and Shc tyrosine phosphorylations are markedly reduced in cells overexpressing PTP-PEST. PTP-PEST is reported to directly inhibit B-cell receptor-induced IL-2 production. Overexpression of PTP-PEST in fibroblasts causes diminished integrin-growth factor signaling as adaptor proteins paxillin, $p130^{cas}$ and their binding partner FAK are direct substrates of PTP-PEST [81]. PTP-PEST also directly associates with Vav2 and p190RhoGAP proteins that are upstream regulators of the Rho GTPases [82].

7.3.4 The PTP-PEP/Lyp tyrosine phosphatase

The *PTPN22* gene coding for a PEST-domain-enriched PTP was first cloned in 1992 for the mouse spleen [3]. The gene was reported to be expressed exclusively in the

hematopoietic cells. The human analogue was discovered later and was called the lymphoid phosphatase or Lyp owing to its high expression in the thymus and spleen [83]. The structural architecture of Lyp, much like the NT4 subtype of non-receptor PTPs, is composed of three major regions/domains (Figure 7.6). The N-terminal region has a classic PTP domain where Cys227 and Glu195 from the conserved regions of the P-loop and the WPD-loop, respectively, form the active site [84]. An Arg263 to glutamine variant of this domain exhibits decreased catalytic activity [85]. A long interdomain region of about 300 residues connects the tyrosine phosphatase domain to the C-terminal region. A particular segment of this interdomain regions acts as an intramolecular inhibitor for the tyrosine phosphatase domain [62]. The C-terminal region/domain of Lyp has four proline-rich PEST sequences. Lyp is hence also known as PTP-PEP or the PEST-domain-enriched tyrosine phosphatase. The P1 proline-rich region of Lyp allows it to make a 1:1 stoichiometric complex with Csk protein kinase by engaging its SH3 domain [86]. This P1 region also allows Lyp to associate with TRAF3 in myeloid cells. The R620W mutation in the P1 region diminishes the affinity of Lyp for Csk and TRAF3 [87, 88]. Two phosphorylation sites have been reported for the Lyp phosphatase. These are Ser59 that is phosphorylated by protein kinase C and Tyr536 that is a substrate site for Lck tyrosine kinase [89].

Several Lyp isoforms find their description in literature. An alternatively spiced shorter isoform of Lyp has been reported from T cells [83]. This isoform called as Lyp2 is about 691 amino acids long and has a unique seven amino acid C-terminal region. Lyp2 is reported to be the more abundant isoform in the resting human peripheral blood T cells. It is also reported that while T-cell receptor stimulation upregulates expression of Lyp, the expression of Lyp2 is downregulated upon stimulation. An abnormal increased ratio of Lyp:Lyp2 transcripts is seen in peripheral blood mononuclear cells of patients with *PTPN22*-associated rheumatoid arthritis [90]. Human peripheral blood mononuclear cells also express another isoform of Lyp called as Lyp3. Lyp3 is unique in having a 28-amino acid deletion in its proline-rich region right after the Arg620 site [91]. A fourth biological isoform of Lyp has been cloned from human CD4+ T cells. This isoform called as PTPN22.6 has a unique eight-amino acid C-terminal sequence and lacks a part of the tyrosine phosphatase domain [92]. PTPN22.6 is reported to behave as a dominant negative when expressed with the wild-type Lyp in Jurkat cells.

7.3.5 Role of the tyrosine phosphatase domain of Lyp

Lyp is reported to augment T-cell receptor signaling by dephosphorylating the proteins that are phosphorylated upon T-cell receptor stimulation. These substrates of Lyp include TCR-CD3ζ and CD3ε, the GEF called Vav and the ATPase valosin containing protein (VCP) [84]. The substrate trap Glu195 to alanine mutant of Lyp can precipitate Fyn, TCR-CD3ζ and Zap-80 from the lysates of COS-1 cells [93].

Protease mapping of Fyn has revealed an Src family-specific dephosphorylation site for Lyp. Lyp dephosphorylates Fyn at phosphotyr420, Lck at phosphotyr394 and ZAP-70 at phosphotyr493. All these tyrosines lie in the activation segment of these tyrosine kinases, and their phosphorylation status is critical for their catalytic activity. Increased Lck phosphorylation is seen upon T-cell receptor stimulation in cells deficient in the *PTPN22* gene [94]. Correspondingly, treatment of Jurkat cells with Lyp inhibitors shows increase in the phosphorylation levels of Lck and TCR-CD3ζ [95].

Putative substrates of Lyp include c-casitas B-lineage (cCbl), BCR-Abl and Src kinase-associated protein of 55 kDa homologue (SKAP-HOM) proteins. Lyp can dephosphorylate and co-immunoprecipitate with cCbl from Jurkat cell and COS-1 cell lysates. Lysates of chronic myeloid leukemia cell line overexpressing Lyp show depressed levels of phosphorylated BCR-Abl [96]. Also, downstream substrates of BCR-Abl remain unphosphorylated in these cells in a Lyp-dependent manner. Direct interaction of cCbl and BCR-Abl with Lyp substrate trapping mutants remains unexplored. The lymphocyte adhesion regulating adaptor protein SKAP-HOM has been identified as a putative substrate for Lyp in a profiling assay [97]. A putative phosphotyrosine of SKAP-HOM lies in the preferred sequence YGEEpYDDLY for Lyp phosphatase. Direct physiological association between SKAP-HOM and Lyp remains to be verified.

7.3.6 Mode of action of Lyp

Lyp PTP associates strongly with the protein tyrosine kinase Csk. It is estimated that as much as 25–50% of Lyp is bound to about 5–6% of Csk in mammalian T cells [86]. Csk phosphorylates the inhibitory tyrosine in the C-tails of the Src family of tyrosine phosphatases and serves as a negative regulator of T-cell receptor signaling [98]. Molecular association of Lyp with Csk indicates a synergistic function that allows for an additional layer for regulation to T-cell receptor signaling [99]. In one of the signaling pathways, Csk would phosphorylate the C-tail Tyr505 of Lck, and Lyp would dephosphorylate the activation loop pTyr394 of Lck in parallel. This synergistic model of Lyp and Csk signaling is substantiated by studies comparing the wild-type Lyp with R620W variant of Lyp that is unable to associate with Csk and predisposes humans to various autoimmune disorders [100]. However, conflicting reports exist in literature regarding the mode of action and dynamic regulation of the Lyp/Csk complex. Three independent reports suggest that the stoichiometry of the Lyp/Csk remains unaltered, is increased or is even decreased upon T-cell receptor stimulation of Jurkat cells or primary human T cells [95, 101, 102]. Data suggest that Csk can promote Lck-mediated phosphorylation of Lyp at Tyr536 in the interdomain region. Phosphorylation of Lyp at Tyr536 is inhibitory to its phosphatase activity and accentuates T-cell receptor signaling. Overexpressed Y536F mutant of Lyp behaves as a gain of function and is more effective in inhibiting T-cell receptor signaling. These contrasting reports have provided for a “differential phosphorylation” model of Lyp

signaling wherein Lyp and Lck form a feedback loop. In this loop, while Lyp acts as a phosphatase to inactivate Lck, it is itself a substrate for Lck in a Csk-dependent mode. Csk-mediated phosphorylation of Lyp by Lck is inhibitory to its phosphatase function. Lyp variants like R620W that compromise the ability of Lyp to associate with Csk show decreased Lck-mediated phosphorylation and are uninhibited. These Lyp variants in turn allow for increased dephosphorylation of Lck and increasingly deactivate Lck. In this way, while these variants lose their ability to associate with Csk, they are still able to inhibit the downstream signaling of T-cell receptors mediated by the Lck or associated tyrosine kinases.

7.3.7 PTP HSCF/BDP1

The third member of the NT4 subtype of non-receptor PTPs is PTP HSCF (hematopoietic stem cell fraction), also known as BDP1 that is coded by the *PTPN18* gene. It is highly expressed in the bone marrow and hence finds its name as PTP HSCF [103]. PTP HSCF is also expressed in various tissues in the body including the brain. Hence it also finds a name as BDP1 [104]. Much like the other two NT4 tyrosine phosphatases, PTP HSCF has an N-terminal tyrosine phosphatase domain followed by a lone C-terminal region. PTP HSCF is albeit shorter than PTP-PEST and Lyp. Similar to PTP-PEST and Lyp, PTP HSCF associates and works with Csk kinase [105]. However, in contrast to PTP-PEST and Lyp, PTP HSCF associates with Csk through its C-terminal region using phosphotyrosine(s) sites Tyr354 and Tyr381 to engage with the SH2 domains of Csk. Binding of PTP HSCF with Csk is also linked to regulation of SFKs in immune signaling. PTP HSCF dephosphorylates the activating tyrosines of the activation loops of these kinases. However, much like the other PEST phosphatases, PTP HSCF is also a substrate for these kinases [105].

Overexpression of PTP HSCF in Ramos B cells is seen to directly augment B-cell receptor signaling [105]. Mutation of Tyr281 to a phenylalanine creates a phosphatase that is less effective in inhibiting B-cell receptor signaling [105, 106]. The Y281F mutation is suggested to affect the interaction of PTP HSCF with Tec kinase [106]. This interaction is mediated by the SH2 domain of Tec that can dock onto the phosphotyrosine(s) of PTP HSCF. While the exact mode of binding of Tec and PTP HSCF remains unclear, data suggest that four key tyrosines, namely Tyr281, Tyr303, Tyr354 and Tyr381, can independently effect their interaction.

7.4 The NT5 subtype of non-receptor PTPs

The NT5 subtype of non-receptor PTPs includes the phosphatases PTPMEG (also called PTP MEG1) and PTPH1. These are the products of genes *PTPN4* and *PTPN3* located at chromosomes 2q14.2 and 9q31, respectively. Structurally both phosphatases show a

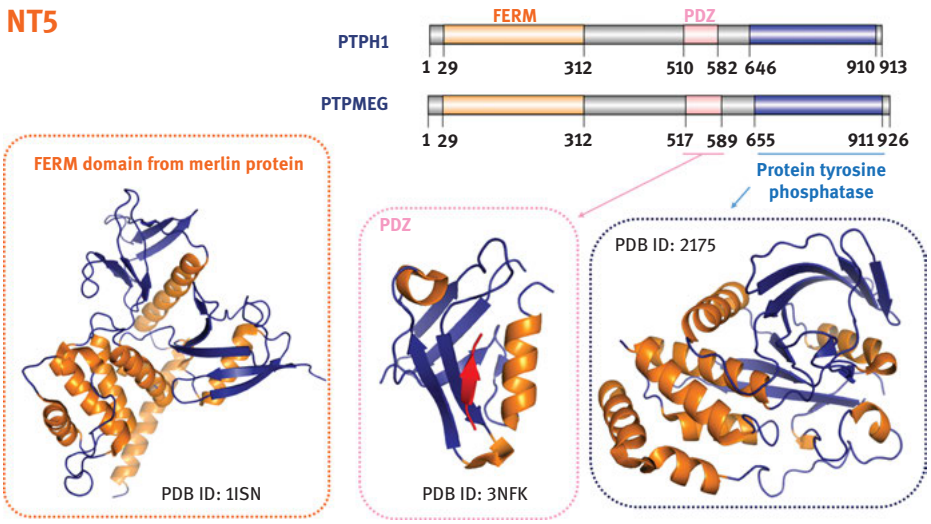

Figure 7.8: Domain architecture and molecular structures of various regions of the NT5 subtype of non-receptor protein tyrosine phosphatases.

common architecture and share 50% identity and 67% homology at the sequence level. Both PTPMEG and PTPH1 have an N-terminal FERM (band 4.1, ezrin, radixin and moesin) domain, a central PDZ domain and a C-terminal classical PTP domain (Figure 7.8). The FERM and PDZ domains of PTPMEG and PTPH1 allow them to interact with other components of the signaling machinery of the cell. PTPMEG and PTPH1 can associate with the plasma membrane by binding to phospholipids such as the phosphatidylinositol (4,5)-bisphosphate (PIP_2) [107].

PTPMEG1 was first cloned from a megakaryoblastic cell line while PTPH1 was identified from HeLa cells [108, 109]. Both PTPMEG and PTPH1 are ubiquitously expressed in the body with higher expression in the thalamus [110, 111]. PTPMEG is also reported to be highly expressed in the testes. Mutations in PTPH1 are reported in colorectal cancer and an altered PTPH1 expression is associated with esophageal tumors [112].

PTPMEG-deficient mice are reported to be mostly normal showing only subtle deficiencies in motor learning and cerebellar synaptic plasticity. T-cell function is reported to be intact in these PTPMEG-deficient mice [113, 114]. Similarly, PTPH1-deficient mice are also reported to show only subtle phenotypic variations and are largely normal. Data suggest that male mice deficient in PTPH1 show higher body mass and reduced working memory while females with PTPH1 deficiency show slight motor learning deficiencies [110, 115]. A role of PTPH1 has also been suggested in the pathways of pain perception [116]. The high degree of homology between PTPH1 and PTPMEG and the subtle phenotypes of their individual deficiencies are suggestive of their compensatory role in mammalian physiology. It is

likely that double-deficient animals may be required for better understanding of their biological functions.

Both PTPMEG and PTPH1 have been reported to bind and dephosphorylate the T-cell receptor ζ chain [117, 118]. Overexpression of PTPH1 in T-cell lines is reported to diminish the expression of IL-2 by inhibition of T-cell receptor signaling [119]. PTPMEG has been reported to bind ε and δ subunits of the glutamate receptor that functions in learning and memory [120]. Interaction of PTPH1 with the phosphorylated growth factor receptor has been demonstrated *in vitro* [121]. Other PTPH1 interacting proteins include the cardiac sodium channel $Na_v1.5$, the TNF alpha-convertase and the 14-3-3beta protein [122, 123]. Association of PTPH1 with p97/VCP inhibits cellular growth of fibroblast cells [124]. Association of PTPH1 with p38γ mitogen-activated protein kinase (MAPK) is reported to play role in Ras-dependent malignancies [125].

7.5 The NT6 subtype of non-receptor PTPs

The NT6 subtype of PTPs includes two members. These are the PTPD1 and PTPD2 proteins coded by *PTPN21* and *PTPN14* genes, respectively. Both proteins are characterized by the presence of N-terminal FERM domain that allows these proteins to interact with the cytoskeletal signaling machinery. Owing to the presence of the FERM domain, the NT5, NT6 and NT7 subtypes of non-receptor PTPs are also referred to as the cytoskeletal PTPs in scientific literature [107].

7.5.1 PTPD1 non-receptor PTP

PTPD1 is a cytosolic non-receptor PTP that has an N-terminal FERM domain, and the C-terminal harbors a classic PTP domain (Figure 7.9) [126]. The WPD-loop of the tyrosine phosphatase domain of PTPD1 substitutes the catalytic aspartate for a longer glutamate residue. This natural variation in its catalytic domain is speculated to weaken its enzymatic turnover rates while maintaining substrate specificity [127]. A long interdomain spacer region of about 580 amino acids connects the FERM domain to the tyrosine phosphatase domain. PTPD1 is a widely expressed tyrosine phosphatase [126] and is reported to be increased in various human cancers [128]. PTPD1 associates with kinesins KIF1C and KIF16C proteins that regulate vesicle transport of the Golgi network and also the reverse transport for the Golgi to the endoplasmic reticulum [129, 130]. PTPD1 regulates the signaling pathways of the Tec family of receptor protein kinases [131]. PTPD1 also works to activate Src kinase and accentuates signaling by the EGFR. PTPD1 is also reported to direct EGFR signaling into the nucleus and promote Erk1, Erk2-dependent gene transcription. PTPD1 is reported to associate with Src such that the PTPD1–Src

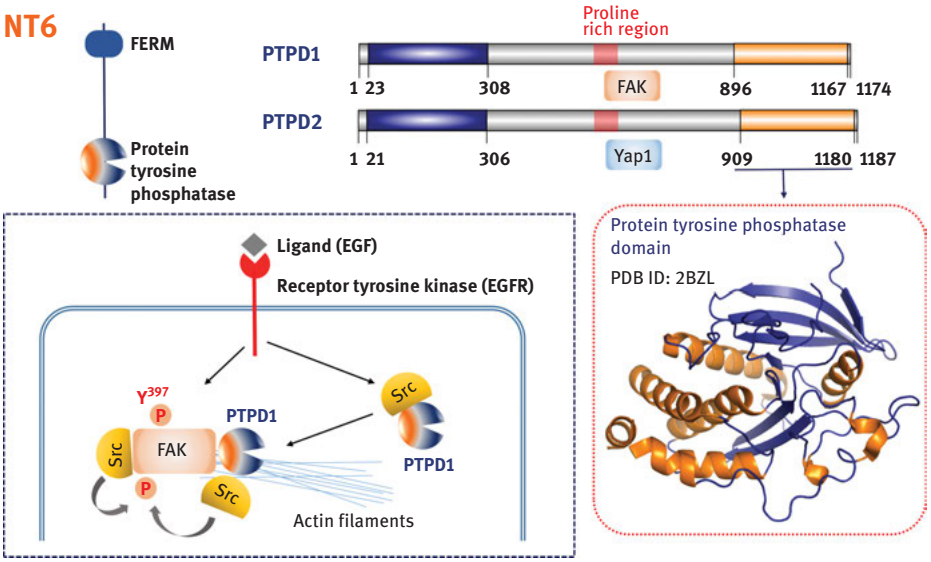

Figure 7.9: Structural features and physiological role of the NT6 subtype of non-receptor protein tyrosine phosphatases.

complex in turn associates with a protein kinase A anchoring protein AKAP121 to localize on the outer membrane of the mitochondria [132]. At the mitochondria, this complex helps to maintain ATP oxidative synthesis and also the mitochondrial membrane potential [133, 134].

EGFR, FAK and Src work in complementation to allow for cell scattering and migration [135]. PTPD1 supports this induced motility via the PTPD1–Src–FAK complex. PTPD1 constructs that are catalytically dead (lacking the active site Cys1108) or lacking the FERM domain both reduce cell motility. As cell motility plays a crucial role in tumor invasion, it is logical how elevated levels of PTPD1 are associated with various cancers [136]. Accordingly, overexpression on catalytically dead PTPD1 C11008S mutant in breast cancer MCF-7 cells is reported to inhibit extracellular matrix invasion.

EGFR stimulation is transiently associated with membrane remodeling wherein more phosphoinositides including PIP_2 get incorporated into the membrane. This membrane remodeling is essential from the formation of endocytic structures and also allows for the recruitment of various actin regulating proteins to the membrane for cytoskeletal rearrangement around the vesicles [137, 138]. PTPD1 forms a direct complex with actin, Src kinase and the FAK at the adhesion plaques, essentially via its FERM domain [139]. A model for the working of the PTPD1–Src–FAK complex at focal adhesions has been suggested based on cell-based assays and immunoprecipitation studies using PTPD1-transfected HEK293 cells [139]. PTPD1 and Src form a stable complex that is lost if PTPD1 is missing its FERM domain (PTPD1 Δ1-325).

The FERM domain contains the motifs Y^{158}ESQ and the Y^{217}GEE that are suggested to be the binding sites for the SH2 domains upon phosphorylation. PTPD1 immunoprecipitates with FAK even as a FERM domain deletion mutant (PTPD1 Δ1-325) but not the extended deletion mutant containing a sequence C-terminal to the FERM domain (PTPD1 Δ1-581). PTPD1 residues 329–587 contain the proline-rich motif P^{565}PPPYPPPRP^{574} that is suggested to be the binding site for FAK. The current model suggests that PTPD1 recruits Src close to FAK in response to EGFR stimulation [139]. The PTPD1-mediated Src–FAK complex can then trigger phosphorylation-based signaling of various cytoskeletal regulatory proteins including p130cas, p190RhoGAP and paxillin [140, 141].

7.5.2 PTPD2 non-receptor PTP

The second member of the NT6 subtype of non-receptor is PTPD2 that was first identified as a cytoskeletal-associated phosphatase [142]. The protein was called as PEZ or the phosphatase with ezrin domain. PTPD2 is also characterized to have an N-terminal FERM domain that is connected to the C-terminal tyrosine phosphatase domain via a long 600 amino acid spacer region (Figure 7.9). PTPD2 is a developmentally regulated tyrosine phosphatase whose deficiency leads to lymphedema in mice and humans. Loss of function of PTPD2 is associated with various tumors including those of the liver and the breast [112, 143]. The tumor suppressor function of PTPD2 is suggested to be independent of its phosphatase activity although substrate proteins have been discovered for this phosphatase [144]. PTPD2 overexpression in HeLa cells leads to decreased cell-matrix adhesion due to alterations in the actin cytoskeleton [145]. PTPD2 directly associates with and removes the phosphate of pTyr128 of the scaffold protein p130cas [146]. PTPD2 localizes to adherence junctions of confluent epithelial cells and regulates these junctions via the dephosphorylation of β-catenin [147]. PTPD2 promotes the transition of epithelial-to-mesenchymal cells via the autocrine action of transforming growth factor β [148].

Data suggest that expression levels of PTPD2 may be regulated by cell density and these in turn correlate with the density-dependent translocation of PTPD2 substrate Yap1 from the nucleus into the cytoplasm [144]. In low-density cells, PTPD2 is actively degraded by ubiquitination. The E3 ligase that tags PTPD2; $CRL2^{LRR}$ complex is regulated in response to increase in cell density. In this way, a model has been suggested for "cell-density-$CRL2^{LRR}$-PTPD2-Yap1" signaling that regulates Yap1 function. Yap1 or Yes-associated protein is a transcriptional coactivator that associates with the TEAD family of transcription factors [149]. Yap1 promotes cell proliferation and its overexpression leads to loss of contact inhibition in cells, thus aiding their oncogenic transformation [150, 151]. The middle spacer region of PTPD2 has a conserved PPxY motif (residues 567–570) that engages the WW domain of YAP1. Cell density-dependent association of PTPD2 and Yap1 allows for its translocation into the

cytosol of confluent cells. In this way PTPD2 regulates the oncogenic potential of Yap1 by targeting it away from the nucleus and depriving it of its transcriptional coactivator function. PTPD2 also uses its PPxY to bind the WW domains of another protein known as Kibra. Association of PTPD2 and Kibra allows for the activation of LATS1 kinase that also results in the cytosolic targeting of Yap1 [152].

PTPD2 is also reported to inhibit surface representation of receptors and release of secretory vesicles [144]. PTPD2 regulates cell trafficking by dephosphorylating two key proteins, PRKCD and RIN1. PRKCD is responsible for maintaining a balance between recycling of receptors and their degradation [153]. Phosphorylation of PRKCD on Tyr374 promotes receptor recycling and allows for their enhanced surface presentation. Dephosphorylation of PRKCD by PTPD2 shifts this balance away from surface representation to degradation of receptors. Accordingly, PTPD2 also dephosphorylates RIN1 and inhibits its association with Abl kinase to allow for a steady rate of micropinocytosis. RIN1 is phosphorylated on Tyr36 by Abl which upon phosphorylation also creates an interaction site on RIN1 for the SH2 domains of Abl itself [154]. Signals from phosphorylated RIN1–Abl complexes and RIN1 complexes with Rab5 (that downregulates growth factor receptors) compete with each other and decide the receptor population presented on the cell surface. A stable RIN1–Abl complex promotes inhibition of micropinocytosis and clathrin-independent internationalization of receptors to the lysosome [155]. PTPD2-mediated dephosphorylation of RIN1 relieves this inhibition and promotes receptor degradation.

7.6 The NT7 subtype of non-receptor PTPs

The NT7 subtype of non-receptor PTPs is formed by PTP-BAS coded by the *PTPN13* gene. PTP-BAS is also a cytoskeletal tyrosine phosphatase as it contains an N-terminal FERM domain that allows it to bind PIP_2 [156]. PTP-BAS is unique in having five PDZ domains that lie in between the N-terminal FERM domain and the C-terminal tyrosine phosphatase domain. These PDZ domains allow PTP-BAS to contact other signaling proteins and participate in their biological pathways.

The first PDZ1 domain of PTP-BAS mediates its interaction with the transcription factor inhibitory protein IkappaBalpha and the PIP_2 binding adaptors PLEKHAI/TAPP1 and PLEKHA2/TAPP2 [157, 158]. Interaction with TAPP1 and TAPP2 allows for the membrane localization of PTP-BAS in response to elevated H_2O_2 levels that lead to PIP_2 production. The second PDZ2 domain mediates PTP-BAS binding to the small adaptor protein RIL and the Zyxin-related protein TRIP6/ZRP-1 [159, 160]. Two splice variants of PTP-BAS differ in their PDZ2 domains. One isoform of PTP-BAS has an insertion of five amino acids in its PDZ2 domain that allows for a differential binding of the two isoforms to the tumor suppressor APC (Adenomatous Polyposis Coli) protein [161]. The region between the PDZ1 and PDZ2 domains allows PTP-BAS binding to PTEN (phosphatase and tensin homologue) that is a phosphatase acting on PIP_3 to release PIP_2 [162]. The PDZ2,

PDZ3 and PDZ4 domains of PTP-BAS allow its association with the Fas receptor/death receptor [163–165]. PTP-BAS is hence also known as FAP-1 or the Fas-associated phosphatase. A conserved cysteine in the C-terminal region of PRK2 protein kinase binds the PDZ3 domain of PTP-BAS [166]. PTP-BAS also binds the C-terminal region of the nerve growth factor receptor via its PDZ3 domain [167]. PDZ4 mediates the binding of PTP-BAS with the RhoGAPs ARHGAP29/PARG1 and the LIM domain containing adaptor protein CRIP2 [168, 169]. The complement component (3b/4b) receptor 1 associates with PTP-BAS by engaging its PDZ2, PDZ3 and PDZ5 domains [170].

PTP-BAS contains a classic PTP domain (Figure 7.10). This C-terminal phosphatase domain is similar to that of the NT1 subtype PTP1B phosphatase in having an accessible second phosphotyrosine binding site [171]. Gly2449 of PTP-BAS provides entry to a tandem phosphotyrosine into a secondary site close to the active site, much like Gly259 of PTP1B (Figure 7.10). Biochemical studies using the EGFR pathway substrate 15 (Eps15) have allowed for the exploration of how PTP-BAS phosphatase domain recruits its substrate [127]. Eps15 functions as a scaffolding adaptor protein that regulates endocytosis and trafficking of growth factor receptors [172]. EPS15 is phosphorylated on Tyr549 by the protein kinase activity of growth factor receptors [173]. Dephosphorylation of this residue by tyrosine phosphatases promotes endocytosis and degradation of growth factors, eventually inhibiting cell proliferation [174]. Peptide derived from the phosphorylation site of Eps15 (EPS15$^{846-854}$) has been used in biochemical experiments with PTP1B and other FERM domain containing tyrosine

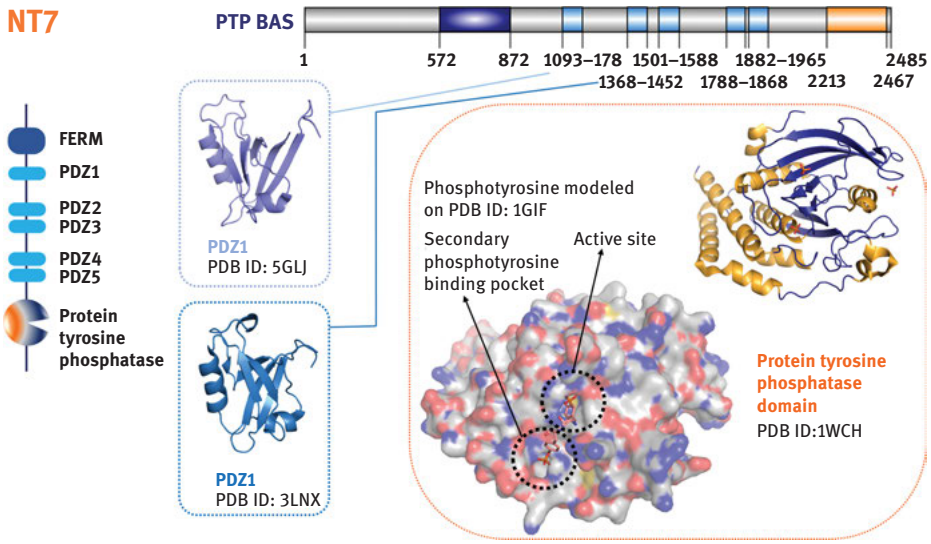

Figure 7.10: Domain architecture and catalytic domain of NT7 subtype of non-receptor protein tyrosine phosphatases. The catalytic domain of PTP-BAS shows a second aryl-binding pocket as seen in the case of the NT1 subtype PTP1B.

phosphatases PTPH1, PTPMEG, PTPD2 and PTP-BAS. The EPS15 $^{846\text{-}854}$ peptide binds the catalytic domain of PTP-BAS with a higher affinity as compared to PTP1B. A key histidine residue His2379 in the WPD-loop of PTP-BAS allows for this stronger interaction with EPS15$^{846-854}$. PTP-BAS also has an additional aspartate residue in its *WPD*-loop, which alongside the catalytic aspartate allows for better substrate recruitment of EPS15$^{846-854}$. In this way the WPD-loop of PTP-BAS allows for threefold better Michaelis Menten constant (K_M) for EPS15$^{846-854}$ when compared to the catalytic domain of PTPH1 [127].

PTP-BAS is a tumor suppressor and also has pro-apoptotic properties. It functions to counteract the activity of tyrosine kinases and inactivates Src kinase by dephosphorylating its activation loop pTyr419 [175]. PTP-BAS is reported to play a crucial role in breast cancer cells where it participates in apoptotic signaling by inhibiting the insulin receptor substrate-1/phosphatidylinositol-3-kinase pathway [176]. PTP-BAS expression is regulated by STAT3, by transcription factor EWS-FLI1 and also by miR-200C [177–179]. PTP-BAS is epigenetically regulated by methylation of a bidirectional promoter. This methylation disrupts its tumor suppressor functions in various lymphomas and cancers [180]. PTP-BAS is commonly expressed in ovarian cancers where it is associated with death receptor resistance [181]. Substrates of PTP-BAS include the Src family of tyrosine kinases, Her2/ErB2, insulin response substrate-1, p85beta and also glycogen synthase kinase beta [175, 182–185]. PTP-BAS inhibits MEK and Erk phosphorylation, but their direct binding to PTP-BAS is not reported [186]. PTP-BAS regulates quiescence of hematopoietic stem cells in conjunction with β-catenin [187].

7.7 The NT8 subtype of non-receptor PTPs

The NT8 subtype of non-receptor PTPs includes HD-PTP that is coded by the *PTPN23* gene. Its uniqueness lies in the presence of a BRO1 domain that allows HD-PTP to participate in endosomal protein sorting [188]. HD-PTP has three more unique regions other than its BRO1 domain and the PTP domain. These include the ALIX_LYPXL_bnd domain that is next to the BRO1 domain and two proline-rich regions (Figure 7.11). The central proline-rich region has multiple dispersed histidine residues and is also called the histidine domain (hence the name HD-PTP). The second proline-rich domain that lies C-terminal to the PTP domain has a disordered secondary structure and contains a PEST motif [188, 189]. The PTP domain of HD-PTP is speculated to be mostly inactive due to the presence of a critical serine residue in its phosphate binding or the P-loop [190]. Reverse mutation of this critical serine to an alanine to match the consensus of a classic P-loop is reported to induce activity in the phosphatase. Some studies show that Src, β-catenin and E-cadherin could be direct substrates of HD-PTP [191]. However, another study shows that HD-PTP was unable to modulate the phosphorylation status of Src in both *in vivo* and *in vitro* assays [192]. The tyrosine phosphatase domain of HD-PTP is absent in its *Caenorhabditis elegans* homologue known as Ego2 or Y53H1C [193].

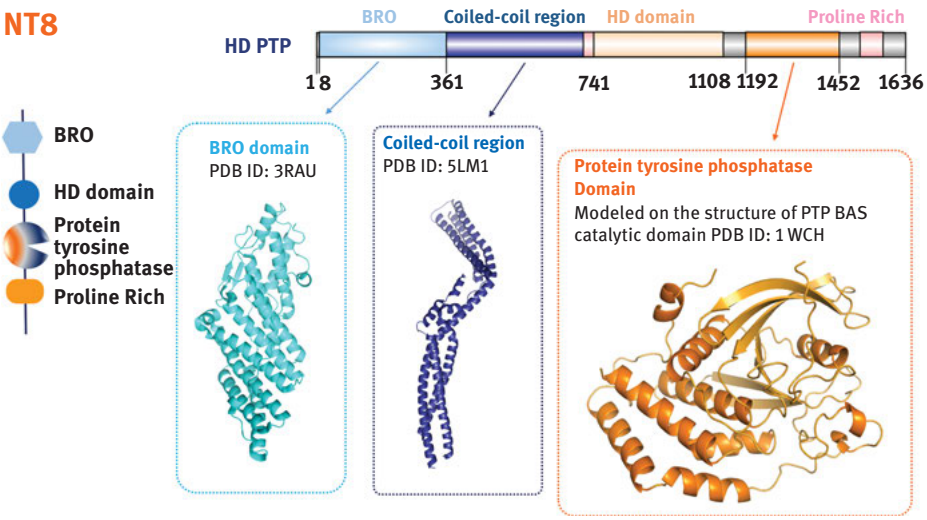

Figure 7.11: Domain architecture and catalytic domain of NT8 subtype of non-receptor protein tyrosine phosphatases.

HD-PTP plays a central role in endosomal sorting, apoptosis and also cell adhesion. Its BRO1 domain allows it to bind charged multivesicular body protein 4B or CHMP4B that is a key component for the endosomal sorting complex required for transport complex (ESRTC)-III required for the sorting of cell surface receptors [188, 194]. HD-PTP binds the signal transducing adaptor molecule STAM2 using two sites. Its BRO1 domain binds the central core of STAM2 while its histidine domain binds the SH3 domain of STAM2 [188]. The histidine domain of HD-PTP also allows it to bind TSG101 that is a component of ESRTC-I functioning in the sorting of ubiquitinated cargo proteins [194]. The same histidine domain of HD-PTP can also bind to endophilin A, another protein involved in receptor endocytosis [194].

HD-PTP regulates cell adhesion and migration by modulating the spatial distribution of Rab4 proteins [195]. HD-PTP also functions as a negative regulator of the Fak. Whether Fak is a direct substrate for HD-PTP is unclear [196]. HD-PTP is a tumor suppressor in testicular germ cell tumors [197]. It is a widely expressed tyrosine phosphatase whose intracellular levels are regulated by proteasomal degradation in response to fibroblast growth factor but not the vascular endothelial growth factor [198]. In T24 bladder carcinoma cells, HD-PTP is degraded by calpains using a calcium-dependent mechanism [199].

7.8 The NT9 subtype of non-receptor PTPs

The NT9 subtype of non-receptor PTPs includes Typ that is coded by the *PTPN20* gene. Typ was first identified from mouse testis cDNA library and hence got the name testis-

specific tyrosine phosphatase or Typ [200]. The human *PTPN20* gene is one of the most alternatively spliced genes that can code for about 16 potential isoforms of the protein Typ [201]. These alternate forms include variants with different N-terminal regions or even complete deletions of their tyrosine phosphatase domain (Figure 7.12). Two other examples of the alternate splicing of tyrosine phosphatases leading to generation of constructs lacking phosphatase domains include the case of PTPζ and the striatal-enriched tyrosine phosphatase (STEP) [202, 203]. While the alternate spliced products of these tyrosine phosphatases are reported to be secreted out of the cells as competitive molecules for ligand binding (see Chapter 4), the role of the alternate forms of Typ is unclear. Also, these tyrosine phosphatase domain "null" constructs are reported to be expressed in insignificant amounts in various cells.

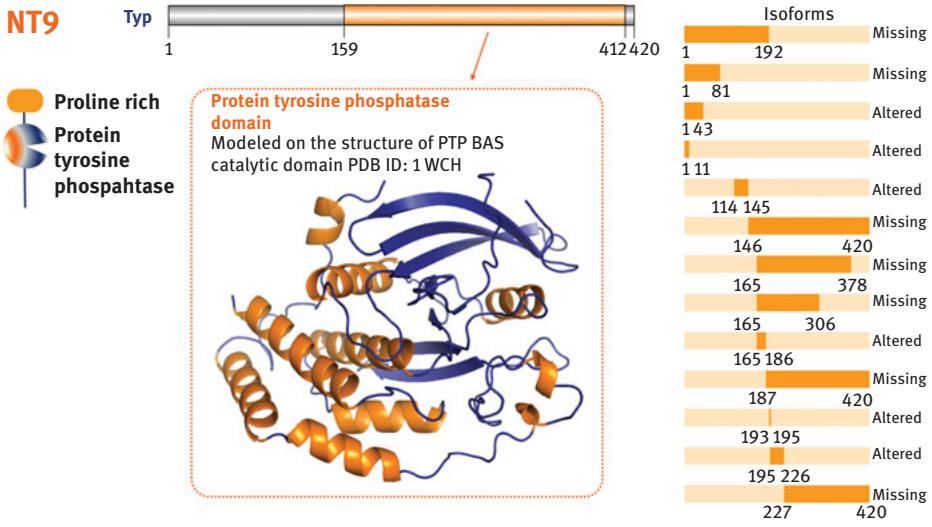

Figure 7.12: NT9 is the most spliced protein tyrosine phosphatase subtype.

The 420 amino acid long Typ (human isoform hPTPN20a) has a single tyrosine phosphatase domain in its C-terminal region. Much like the mouse homologue, human Typ is also expressed maximally in the testes and at much lower levels in other tissues. The human and the mouse homologues are about 66% identical and their tyrosine phosphatase domains have about 74% identity in amino acid sequence. The N-terminal region of human Typ contains a PEST domain region (residues 19–31, 54–76 and 87–104) and also multiple threonine residues that can serve as phosphorylation sites. The C-terminal PTP domain has a well-formed active site with a nucleophilic cysteine. Tyrosine phosphatase activity of Typ has been confirmed in biochemical experiments using tyrosyl phosphorylated reduced carboxyamidomethylated and maleylated lysozyme as substrate [201].

Immunofluorescence microscopy has been used to assess the subcellular localization of human Typ. The phosphatase localizes to the nucleus and its overall distribution in cells is dependent on the integrity of the microtubule networks. Human Typ localizes to all organelles that depend on the microtubule network including the Golgi, endoplasmic reticulum and the endosomes [204–206]. Localization of Typ to the nucleus in the absence of any nuclear localization signal is suggested to be possible via microtubule-dependent trafficking as seen in the case of p53 and parathyroid hormone proteins [207, 208]. Under growth factor-stimulated signaling or under conditions of osmotic stress, Typ re-localizes to be targeted specifically to the sites of dynamic actin polymerization. The role of human Typ in modulation of actin dynamics is unclear and its exact substrate(s) remains unknown.

7.9 The PTPN5 RR subfamily of PTPs

A unique family of PTPs includes both receptor and non-receptor members. Members of this family include tyrosine phosphatases that engage the MAPKs via their kinase-interacting motif or KIM domain that has a consensus sequence $(R/K)_{2\text{-}3}\text{-}X_{2\text{-}6}\text{-}\phi_A\text{-}X\text{-}\phi_B$, where ϕ is a hydrophobic amino acid (Figure 7.13) [209, 210]. They bind and dephosphorylate the regulatory phosphotyrosine of Erk 1/2 to inactivate them [211].

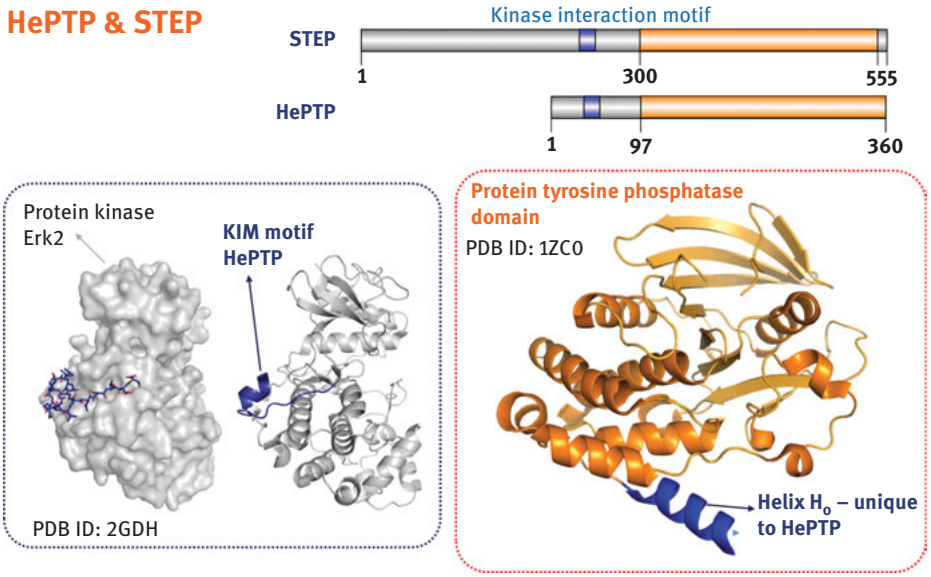

Figure 7.13: The unique PTPN5 RR subfamily of protein tyrosine phosphatases. Unique elements include the presence of a kinase interaction motif (KIM) and an extra α-helical region (H_0) at the N-terminus of the protein tyrosine phosphatase catalytic domain.

Human members of this subfamily include STEP, the hematopoietic tyrosine phosphatase (HePTP, also called leukocyte protein tyrosine phosphatase) and the receptor PTPRR (also called PTP-SL) [209]. PTP-SL is discussed along with the receptor PTPs in Chapter 4 (Section 4.8). All three members of this subfamily are expressed in the human thymus, brain and the spleen.

STEP and HePTP are coded by the *PTPN5* and *PTPN7* genes, respectively. They are both cytosolic tyrosine phosphatases, although isoforms of STEP are predicted to have transmembrane regions (but no signal peptide) as seen in bioinformatics-based analysis of their sequences. Both proteins have an N-terminal KIM domain that is connected to a C-terminal tyrosine phosphatase domain. The catalytic domain of HePTP is unique in having a variant of the conserved phosphotyrosine recognition loop where a conserved asparagine is substituted for an aspartate (KDRY instead of KNRY) (Figure 7.13) [212, 213]. Catalytic domains of HePTP are characterized by an extra alpha helix (Ho) that is absent in STEP and other classic tyrosine phosphatase domains (Figure 7.13) [212].

STEP gets its name from its high expression in the striatum as compared to other areas of the brain [214]. STEP is reported to be important for the development of cognition, morphological changes of the brain and also long-term memory [215, 216]. STEP dephosphorylates the N-methyl-D-aspartate (NMDA) glutamate receptors and promotes their endocytosis [217, 218]. Abnormalities in STEP function are correlated with Alzheimer's disease and also schizophrenia [217, 219]. Alongside Erk, STEP also functions to regulate the activity of Fyn by dephosphorylating its activation loop pTyr420 (but not the inhibitory pTyr531 on the C-tail). Interaction between STEP and Fyn is highly specific as the substrate trap mutants of STEP fail to show binding to other members of the Src family of tyrosine kinases [220]. This unique interaction uses the KIM and proline-rich regions of STEP and the SH2 domain and unique N-terminal region of Fyn. STEP also dephosphorylates the pTyr789 of PTPα and regulates the PTPα/Fyn pathway [221].

Expression of HePTP is under the control of mitogenic stimuli that are trigged in relation to cell cycle events [222, 223]. HePTP is hence reported to be highly expressed in myeloid malignancies [224, 225]. HePTP promotes the functional activity and translocation of Erk 1/2 and p53 [226, 227]. However, this interaction is specific to be non-conformal with the Jnk mitotic kinases [228, 229]. HePTP uses residues of its KIM domain for a reciprocal interaction with Erk. Erk phosphorylates resides Thr45 and Ser72 of HePTP that in turn dephosphorylates the pTyr185 of Erk. Phosphorylation of Ser23 of HePTP in its KIM domain allows for the regulation of its interaction with Erk via protein kinase A signaling [230].

References

[1] Feng GS, Hui CC, Pawson T. SH2-containing phosphotyrosine phosphatase as a target of protein-tyrosine kinases. Science. 1993;259(5101):1607–11.

[2] Plutzky J, Neel BG, Rosenberg RD. Isolation of a src homology 2-containing tyrosine phosphatase. Proc Natl Acad Sci U S A. 1992;89(3):1123–7.
[3] Matthews RJ, Bowne DB, Flores E, Thomas ML. Characterization of hematopoietic intracellular protein tyrosine phosphatases: description of a phosphatase containing an SH2 domain and another enriched in proline-, glutamic acid-, serine-, and threonine-rich sequences. Mol Cell Biol. 1992;12(5):2396–405.
[4] Dechert U, Adam M, Harder KW, Clark-Lewis I, Jirik F. Characterization of protein tyrosine phosphatase SH-PTP2. Study of phosphopeptide substrates and possible regulatory role of SH2 domains. J Biol Chem. 1994;269(8):5602–11.
[5] Zhao Z, Bouchard P, Diltz CD, Shen SH, Fischer EH. Purification and characterization of a protein tyrosine phosphatase containing SH2 domains. J Biol Chem. 1993;268(4): 2816–20.
[6] Lorenz U, Ravichandran KS, Pei D, Walsh CT, Burakoff SJ, Neel BG. Lck-dependent tyrosyl phosphorylation of the phosphotyrosine phosphatase SH-PTP1 in murine T cells. Mol Cell Biol. 1994;14(3):1824–34.
[7] Bennett AM, Tang TL, Sugimoto S, Walsh CT, Neel BG. Protein-tyrosine-phosphatase SHPTP2 couples platelet-derived growth factor receptor beta to Ras. Proc Natl Acad Sci U S A. 1994;91 (15):7335–9.
[8] Kratz CP, Niemeyer CM, Castleberry RP, Cetin M, Bergstrasser E, Emanuel PD, et al. The mutational spectrum of PTPN11 in juvenile myelomonocytic leukemia and Noonan syndrome/ myeloproliferative disease. Blood. 2005;106(6):2183–5.
[9] O'Reilly AM, Pluskey S, Shoelson SE, Neel BG. Activated mutants of SHP-2 preferentially induce elongation of Xenopus animal caps. Mol Cell Biol. 2000;20(1):299–311.
[10] Craggs G, Kellie S. A functional nuclear localization sequence in the C-terminal domain of SHP-1. J Biol Chem. 2001;276(26):23719–25.
[11] Ram PA, Waxman DJ. Interaction of growth hormone-activated STATs with SH2-containing phosphotyrosine phosphatase SHP-1 and nuclear JAK2 tyrosine kinase. J Biol Chem. 1997;272 (28):17694–702.
[12] Yang W, Tabrizi M, Yi T. A bipartite NLS at the SHP-1 C-terminus mediates cytokine-induced SHP-1 nuclear localization in cell growth control. Blood Cells Mol Dis. 2002;28(1):63–74.
[13] Wang LL, Blasioli J, Plas DR, Thomas ML, Yokoyama WM. Specificity of the SH2 domains of SHP-1 in the interaction with the immunoreceptor tyrosine-based inhibitory motif-bearing receptor gp49B. J Immunol. 1999;162(3):1318–23.
[14] Lu W, Gong D, Bar-Sagi D, Cole PA. Site-specific incorporation of a phosphotyrosine mimetic reveals a role for tyrosine phosphorylation of SHP-2 in cell signaling. Mol Cell. 2001;8 (4):759–69.
[15] Barford D, Neel BG. Revealing mechanisms for SH2 domain mediated regulation of the protein tyrosine phosphatase SHP-2. Structure. 1998;6(3):249–54.
[16] Ravetch JV, Lanier LL. Immune inhibitory receptors. Science. 2000;290(5489):84–9.
[17] Neel BG, Gu H, Pao L. The 'Shp'ing news: SH2 domain-containing tyrosine phosphatases in cell signaling. Trends Biochem Sci. 2003;28(6):284–93.
[18] Philosof-Oppenheimer R, Hampe CS, Schlessinger K, Fridkin M, Pecht I. An immunoreceptor tyrosine-based inhibitory motif, with serine at site Y-2, binds SH2-domain-containing phosphatases. Eur J Biochem. 2000;267(3):703–11.
[19] Daigle I, Yousefi S, Colonna M, Green DR, Simon HU. Death receptors bind SHP-1 and block cytokine-induced anti-apoptotic signaling in neutrophils. Nat Med. 2002;8(1):61–7.
[20] Beebe KD, Wang P, Arabaci G, Pei D. Determination of the binding specificity of the SH2 domains of protein tyrosine phosphatase SHP-1 through the screening of a combinatorial phosphotyrosyl peptide library. Biochemistry. 2000;39(43):13251–60.

[21] Schmitz J, Weissenbach M, Haan S, Heinrich PC, Schaper F. SOCS3 exerts its inhibitory function on interleukin-6 signal transduction through the SHP2 recruitment site of gp130. J Biol Chem. 2000;275(17):12848–56.
[22] Nicholson SE, De Souza D, Fabri LJ, Corbin J, Willson TA, Zhang JG, et al. Suppressor of cytokine signaling-3 preferentially binds to the SHP-2-binding site on the shared cytokine receptor subunit gp130. Proc Natl Acad Sci U S A. 2000;97(12):6493–8.
[23] Vely F, Olivero S, Olcese L, Moretta A, Damen JE, Liu L, et al. Differential association of phosphatases with hematopoietic co-receptors bearing immunoreceptor tyrosine-based inhibition motifs. Eur J Immunol. 1997;27(8):1994–2000.
[24] Shultz LD, Schweitzer PA, Rajan TV, Yi T, Ihle JN, Matthews RJ, et al. Mutations at the murine motheaten locus are within the hematopoietic cell protein-tyrosine phosphatase (Hcph) gene. Cell. 1993;73(7):1445–54.
[25] Tsui HW, Siminovitch KA, de Souza L, Tsui FW. Motheaten and viable motheaten mice have mutations in the haematopoietic cell phosphatase gene. Nat Genet. 1993;4 (2):124–9.
[26] Shultz LD, Sidman CL. Genetically determined murine models of immunodeficiency. Annu Rev Immunol. 1987;5:367–403.
[27] Roach TI, Slater SE, White LS, Zhang X, Majerus PW, Brown EJ, et al. The protein tyrosine phosphatase SHP-1 regulates integrin-mediated adhesion of macrophages. Curr Biol. 1998;8 (18):1035–8.
[28] Timms JF, Swanson KD, Marie-Cardine A, Raab M, Rudd CE, Schraven B, et al. SHPS-1 is a scaffold for assembling distinct adhesion-regulated multi-protein complexes in macrophages. Curr Biol. 1999;9(16):927–30.
[29] Peterson EJ, Woods ML, Dmowski SA, Derimanov G, Jordan MS, Wu JN, et al. Coupling of the TCR to integrin activation by Slap-130/Fyb. Science. 2001;293(5538):2263–5.
[30] Griffiths EK, Krawczyk C, Kong YY, Raab M, Hyduk SJ, Bouchard D, et al. Positive regulation of T cell activation and integrin adhesion by the adapter Fyb/Slap. Science. 2001;293 (5538):2260–3.
[31] Klingmuller U, Lorenz U, Cantley LC, Neel BG, Lodish HF. Specific recruitment of SH-PTP1 to the erythropoietin receptor causes inactivation of JAK2 and termination of proliferative signals. Cell. 1995;80(5):729–38.
[32] Zang H, Sato K, Nakajima H, McKay C, Ney PA, Ihle JN. The distal region and receptor tyrosines of the Epo receptor are non-essential for in vivo erythropoiesis. EMBO J. 2001;20(12):3156–66.
[33] Sasaki A, Yasukawa H, Shouda T, Kitamura T, Dikic I, Yoshimura A. CIS3/SOCS-3 suppresses erythropoietin (EPO) signaling by binding the EPO receptor and JAK2. J Biol Chem. 2000;275 (38):29338–47.
[34] Yu DH, Qu CK, Henegariu O, Lu X, Feng GS. Protein-tyrosine phosphatase Shp-2 regulates cell spreading, migration, and focal adhesion. J Biol Chem. 1998;273(33):21125–31.
[35] Inagaki K, Noguchi T, Matozaki T, Horikawa T, Fukunaga K, Tsuda M, et al. Roles for the protein tyrosine phosphatase SHP-2 in cytoskeletal organization, cell adhesion and cell migration revealed by overexpression of a dominant negative mutant. Oncogene. 2000;19(1):75–84.
[36] Schoenwaelder SM, Petch LA, Williamson D, Shen R, Feng GS, Burridge K. The protein tyrosine phosphatase Shp-2 regulates RhoA activity. Curr Biol. 2000;10(23):1523–6.
[37] Tsuda M, Matozaki T, Fukunaga K, Fujioka Y, Imamoto A, Noguchi T, et al. Integrin-mediated tyrosine phosphorylation of SHPS-1 and its association with SHP-2. Roles of Fak and Src family kinases. J Biol Chem. 1998;273(21):13223–9.
[38] Miao H, Burnett E, Kinch M, Simon E, Wang B. Activation of EphA2 kinase suppresses integrin function and causes focal-adhesion-kinase dephosphorylation. Nat Cell Biol. 2000;2(2):62–9.

[39] Manes S, Mira E, Gomez-Mouton C, Zhao ZJ, Lacalle RA, Martinez AC. Concerted activity of tyrosine phosphatase SHP-2 and focal adhesion kinase in regulation of cell motility. Mol Cell Biol. 1999;19(4):3125–35.
[40] Inagaki K, Yamao T, Noguchi T, Matozaki T, Fukunaga K, Takada T, et al. SHPS-1 regulates integrin-mediated cytoskeletal reorganization and cell motility. EMBO J. 2000;19 (24):6721–31.
[41] Oh ES, Gu H, Saxton TM, Timms JF, Hausdorff S, Frevert EU, et al. Regulation of early events in integrin signaling by protein tyrosine phosphatase SHP-2. Mol Cell Biol. 1999;19(4):3205–15.
[42] Feng GS. Shp-2 tyrosine phosphatase: signaling one cell or many. Exp Cell Res. 1999;253 (1):47–54.
[43] Noguchi T, Matozaki T, Horita K, Fujioka Y, Kasuga M. Role of SH-PTP2, a protein-tyrosine phosphatase with Src homology 2 domains, in insulin-stimulated Ras activation. Mol Cell Biol. 1994;14(10):6674–82.
[44] Shi ZQ, Yu DH, Park M, Marshall M, Feng GS. Molecular mechanism for the Shp-2 tyrosine phosphatase function in promoting growth factor stimulation of Erk activity. Mol Cell Biol. 2000;20(5):1526–36.
[45] Klinghoffer RA, Kazlauskas A. Identification of a putative Syp substrate, the PDGF beta receptor. J Biol Chem. 1995;270(38):22208–17.
[46] Pawson T, Scott JD. Signaling through scaffold, anchoring, and adaptor proteins. Science. 1997;278(5346):2075–80.
[47] Kawabuchi M, Satomi Y, Takao T, Shimonishi Y, Nada S, Nagai K, et al. Transmembrane phosphoprotein Cbp regulates the activities of Src-family tyrosine kinases. Nature. 2000;404 (6781):999–1003.
[48] Brdicka T, Pavlistova D, Leo A, Bruyns E, Korinek V, Angelisova P, et al. Phosphoprotein associated with glycosphingolipid-enriched microdomains (PAG), a novel ubiquitously expressed transmembrane adaptor protein, binds the protein tyrosine kinase csk and is involved in regulation of T cell activation. J Exp Med. 2000;191(9):1591–604.
[49] Su J, Muranjan M, Sap J. Receptor protein tyrosine phosphatase alpha activates Src-family kinases and controls integrin-mediated responses in fibroblasts. Curr Biol. 1999;9(10):505–11.
[50] Gu M, Warshawsky I, Majerus PW. Cloning and expression of a cytosolic megakaryocyte protein-tyrosine-phosphatase with sequence homology to retinaldehyde-binding protein and yeast SEC14p. Proc Natl Acad Sci U S A. 1992;89(7):2980–4.
[51] Huynh H, Wang X, Li W, Bottini N, Williams S, Nika K, et al. Homotypic secretory vesicle fusion induced by the protein tyrosine phosphatase MEG2 depends on polyphosphoinositides in T cells. J Immunol. 2003;171(12):6661–71.
[52] Kruger JM, Fukushima T, Cherepanov V, Borregaard N, Loeve C, Shek C, et al. Protein-tyrosine phosphatase MEG2 is expressed by human neutrophils. Localization to the phagosome and activation by polyphosphoinositides. J Biol Chem. 2002;277(4):2620–8.
[53] Huynh H, Bottini N, Williams S, Cherepanov V, Musumeci L, Saito K, et al. Control of vesicle fusion by a tyrosine phosphatase. Nat Cell Biol. 2004;6(9):831–9.
[54] Cho CY, Koo SH, Wang Y, Callaway S, Hedrick S, Mak PA, et al. Identification of the tyrosine phosphatase PTP-MEG2 as an antagonist of hepatic insulin signaling. Cell Metab. 2006;3 (5):367–78.
[55] Hao Q, Samten B, Ji HL, Zhao ZJ, Tang H. Tyrosine phosphatase PTP-MEG2 negatively regulates vascular endothelial growth factor receptor signaling and function in endothelial cells. Am J Physiol Cell Physiol. 2012;303(5):C548–53.
[56] Laubach JP, Fu P, Jiang X, Salter KH, Potti A, Arcasoy MO. Polycythemia vera erythroid precursors exhibit increased proliferation and apoptosis resistance associated with abnormal RAS and PI3K pathway activation. Exp Hematol. 2009;37(12):1411–22.

[57] Zhao R, Fu X, Li Q, Krantz SB, Zhao ZJ. Specific interaction of protein tyrosine phosphatase-MEG2 with phosphatidylserine. J Biol Chem. 2003;278(25):22609–14.
[58] Wang Y, Vachon E, Zhang J, Cherepanov V, Kruger J, Li J, et al. Tyrosine phosphatase MEG2 modulates murine development and platelet and lymphocyte activation through secretory vesicle function. J Exp Med. 2005;202(11):1587–97.
[59] Wang X, Huynh H, Gjorloff-Wingren A, Monosov E, Stridsberg M, Fukuda M, et al. Enlargement of secretory vesicles by protein tyrosine phosphatase PTP-MEG2 in rat basophilic leukemia mast cells and Jurkat T cells. J Immunol. 2002;168(9):4612–9.
[60] Polgar J, Chung SH, Reed GL. Vesicle-associated membrane protein 3 (VAMP-3) and VAMP-8 are present in human platelets and are required for granule secretion. Blood. 2002;100(3):1081–3.
[61] Hendriks WJ, Elson A, Harroch S, Pulido R, Stoker A, den Hertog J. Protein tyrosine phosphatases in health and disease. FEBS J. 2013;280(2):708–30.
[62] Liu Y, Stanford SM, Jog SP, Fiorillo E, Orrú V, Comai L, et al. Regulation of lymphoid tyrosine phosphatase activity: inhibition of the catalytic domain by the proximal interdomain. Biochemistry. 2009;48(31):7525–32.
[63] Sun T, Aceto N, Meerbrey KL, Kessler JD, Zhou C, Migliaccio I, et al. Activation of multiple proto-oncogenic tyrosine kinases in breast cancer via loss of the PTPN12 phosphatase. Cell. 2011;144(5):703–18.
[64] Zheng Y, Zhang C, Croucher DR, Soliman MA, St-Denis N, Pasculescu A, et al. Temporal regulation of EGF signalling networks by the scaffold protein Shc1. Nature. 2013;499(7457):166–71.
[65] Cote JF, Charest A, Wagner J, Tremblay ML. Combination of gene targeting and substrate trapping to identify substrates of protein tyrosine phosphatases using PTP-PEST as a model. Biochemistry. 1998;37(38):13128–37.
[66] Angers-Loustau A, Cote JF, Charest A, Dowbenko D, Spencer S, Lasky LA, et al. Protein tyrosine phosphatase-PEST regulates focal adhesion disassembly, migration, and cytokinesis in fibroblasts. J Cell Biol. 1999;144(5):1019–31.
[67] Garton AJ, Burnham MR, Bouton AH, Tonks NK. Association of PTP-PEST with the SH3 domain of p130cas; a novel mechanism of protein tyrosine phosphatase substrate recognition. Oncogene. 1997;15(8):877–85.
[68] Shen Y, Lyons P, Cooley M, Davidson D, Veillette A, Salgia R, et al. The noncatalytic domain of protein-tyrosine phosphatase-PEST targets paxillin for dephosphorylation in vivo. J Biol Chem. 2000;275(2):1405–13.
[69] Li H, Yang F, Liu C, Xiao P, Xu Y, Liang Z, et al. Crystal structure and substrate specificity of PTPN12. Cell Rep. 2016;15(6):1345–58.
[70] Charest A, Wagner J, Kwan M, Tremblay ML. Coupling of the murine protein tyrosine phosphatase PEST to the epidermal growth factor (EGF) receptor through a Src homology 3 (SH3) domain-mediated association with Grb2. Oncogene. 1997;14(14):1643–51.
[71] Davidson D, Cloutier JF, Gregorieff A, Veillette A. Inhibitory tyrosine protein kinase p50csk is associated with protein-tyrosine phosphatase PTP-PEST in hemopoietic and non-hemopoietic cells. J Biol Chem. 1997;272(37):23455–62.
[72] Habib T, Herrera R, Decker SJ. Activators of protein kinase C stimulate association of Shc and the PEST tyrosine phosphatase. J Biol Chem. 1994;269(41):25243–6.
[73] Li J, Nishizawa K, An W, Hussey RE, Lialios FE, Salgia R, et al. A cdc15-like adaptor protein (CD2BP1) interacts with the CD2 cytoplasmic domain and regulates CD2-triggered adhesion. EMBO J. 1998;17(24):7320–36.
[74] Spencer S, Dowbenko D, Cheng J, Li W, Brush J, Utzig S, et al. PSTPIP: a tyrosine phosphorylated cleavage furrow-associated protein that is a substrate for a PEST tyrosine phosphatase. J Cell Biol. 1997;138(4):845–60.

[75] Wise CA, Gillum JD, Seidman CE, Lindor NM, Veile R, Bashiardes S, et al. Mutations in CD2BP1 disrupt binding to PTP PEST and are responsible for PAPA syndrome, an autoinflammatory disorder. Hum Mol Genet. 2002;11(8):961–9.
[76] Satsangi J, Parkes M, Louis E, Hashimoto L, Kato N, Welsh K, et al. Two stage genome-wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7 and 12. Nat Genet. 1996;14(2):199–202.
[77] Wu Y, Spencer SD, Lasky LA. Tyrosine phosphorylation regulates the SH3-mediated binding of the Wiskott-Aldrich syndrome protein to PSTPIP, a cytoskeletal-associated protein. J Biol Chem. 1998;273(10):5765–70.
[78] Badour K, Zhang J, Shi F, McGavin MK, Rampersad V, Hardy LA, et al. The Wiskott-Aldrich syndrome protein acts downstream of CD2 and the CD2AP and PSTPIP1 adaptors to promote formation of the immunological synapse. Immunity. 2003;18(1):141–54.
[79] Faisal A, el-Shemerly M, Hess D, Nagamine Y. Serine/threonine phosphorylation of ShcA. Regulation of protein-tyrosine phosphatase-pest binding and involvement in insulin signaling. J Biol Chem. 2002;277(33):30144–52.
[80] Davidson D, Veillette A. PTP-PEST, a scaffold protein tyrosine phosphatase, negatively regulates lymphocyte activation by targeting a unique set of substrates. EMBO J. 2001; 20(13):3414–26.
[81] O'Neill GM, Fashena SJ, Golemis EA. Integrin signalling: a new Cas(t) of characters enters the stage. Trends Cell Biol. 2000;10(3):111–9.
[82] Sastry SK, Rajfur Z, Liu BP, Cote JF, Tremblay ML, Burridge K. PTP-PEST couples membrane protrusion and tail retraction via VAV2 and p190RhoGAP. J Biol Chem. 2006;281(17):11627–36.
[83] Cohen S, Dadi H, Shaoul E, Sharfe N, Roifman CM. Cloning and characterization of a lymphoid-specific, inducible human protein tyrosine phosphatase, Lyp. Blood. 1999;93(6):2013–24.
[84] Wu J, Katrekar A, Honigberg LA, Smith AM, Conn MT, Tang J, et al. Identification of substrates of human protein-tyrosine phosphatase PTPN22. J Biol Chem. 2006;281(16):11002–10.
[85] Orru V, Tsai SJ, Rueda B, Fiorillo E, Stanford SM, Dasgupta J, et al. A loss-of-function variant of PTPN22 is associated with reduced risk of systemic lupus erythematosus. Hum Mol Genet. 2009;18(3):569–79.
[86] Cloutier JF, Veillette A. Association of inhibitory tyrosine protein kinase p50csk with protein tyrosine phosphatase PEP in T cells and other hemopoietic cells. EMBO J. 1996;15 (18):4909–18.
[87] Wang Y, Shaked I, Stanford SM, Zhou W, Curtsinger JM, Mikulski Z, et al. The autoimmunity-associated gene PTPN22 potentiates toll-like receptor-driven, type 1 interferon-dependent immunity. Immunity. 2013;39(1):111–22.
[88] Bottini N, Musumeci L, Alonso A, Rahmouni S, Nika K, Rostamkhani M, et al. A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. Nat Genet. 2004;36(4):337–8.
[89] Yu X, Sun JP, He Y, Guo X, Liu S, Zhou B, et al. Structure, inhibitor, and regulatory mechanism of Lyp, a lymphoid-specific tyrosine phosphatase implicated in autoimmune diseases. Proc Natl Acad Sci U S A. 2007;104(50):19767–72.
[90] Ronninger M, Guo Y, Shchetynsky K, Hill A, Khademi M, Olsson T, et al. The balance of expression of PTPN22 splice forms is significantly different in rheumatoid arthritis patients compared with controls. Genome Med. 2012;4(1):2.
[91] Wang S, Dong H, Han J, Ho WT, Fu X, Zhao ZJ. Identification of a variant form of tyrosine phosphatase LYP. BMC Mol Biol. 2010;11:78.
[92] Chang HH, Tai TS, Lu B, Iannaccone C, Cernadas M, Weinblatt M, et al. PTPN22.6, a dominant negative isoform of PTPN22 and potential biomarker of rheumatoid arthritis. PLoS One. 2012; 7(3):e33067.

[93] Cloutier JF, Veillette A. Cooperative inhibition of T-cell antigen receptor signaling by a complex between a kinase and a phosphatase. J Exp Med. 1999;189(1):111–21.
[94] Hasegawa K, Martin F, Huang G, Tumas D, Diehl L, Chan AC. PEST domain-enriched tyrosine phosphatase (PEP) regulation of effector/memory T cells. Science. 2004;303 (5658):685–9.
[95] Vang T, Liu WH, Delacroix L, Wu S, Vasile S, Dahl R, et al. LYP inhibits T-cell activation when dissociated from CSK. Nat Chem Biol. 2012;8(5):437–46.
[96] Chien W, Tidow N, Williamson EA, Shih LY, Krug U, Kettenbach A, et al. Characterization of a myeloid tyrosine phosphatase, Lyp, and its role in the Bcr-Abl signal transduction pathway. J Biol Chem. 2003;278(30):27413–20.
[97] Yu X, Chen M, Zhang S, Yu ZH, Sun JP, Wang L, et al. Substrate specificity of lymphoid-specific tyrosine phosphatase (Lyp) and identification of Src kinase-associated protein of 55 kDa homolog (SKAP-HOM) as a Lyp substrate. J Biol Chem. 2011;286(35):30526–34.
[98] Schmedt C, Saijo K, Niidome T, Kuhn R, Aizawa S, Tarakhovsky A. Csk controls antigen receptor-mediated development and selection of T-lineage cells. Nature. 1998;394 (6696):901–4.
[99] Gjorloff-Wingren A, Saxena M, Williams S, Hammi D, Mustelin T. Characterization of TCR-induced receptor-proximal signaling events negatively regulated by the protein tyrosine phosphatase PEP. Eur J Immunol. 1999;29(12):3845–54.
[100] Begovich AB, Carlton VE, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC, et al. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. Am J Hum Genet. 2004;75 (2):330–7.
[101] Fiorillo E, Orru V, Stanford SM, Liu Y, Salek M, Rapini N, et al. Autoimmune-associated PTPN22 R620W variation reduces phosphorylation of lymphoid phosphatase on an inhibitory tyrosine residue. J Biol Chem. 2010;285(34):26506–18.
[102] de la Puerta ML, Trinidad AG, Rodriguez Mdel C, de Pereda JM, Sanchez Crespo M, Bayon Y, et al. The autoimmunity risk variant LYP-W620 cooperates with CSK in the regulation of TCR signaling. PLoS One. 2013;8(1):e54569.
[103] Huang K, Sommers CL, Grinberg A, Kozak CA, Love PE. Cloning and characterization of PTP-K1, a novel nonreceptor protein tyrosine phosphatase highly expressed in bone marrow. Oncogene. 1996;13(7):1567–73.
[104] Kim YW, Wang H, Sures I, Lammers R, Martell KJ, Ullrich A. Characterization of the PEST family protein tyrosine phosphatase BDP1. Oncogene. 1996;13(10):2275–9.
[105] Wang B, Lemay S, Tsai S, Veillette A. SH2 domain-mediated interaction of inhibitory protein tyrosine kinase Csk with protein tyrosine phosphatase-HSCF. Mol Cell Biol. 2001;21 (4):1077–88.
[106] Aoki N, Ueno S, Mano H, Yamasaki S, Shiota M, Miyazaki H, et al. Mutual regulation of protein-tyrosine phosphatase 20 and protein-tyrosine kinase Tec activities by tyrosine phosphorylation and dephosphorylation. J Biol Chem. 2004;279(11):10765–75.
[107] Gjorloff-Wingren A, Saxena M, Han S, Wang X, Alonso A, Renedo M, et al. Subcellular localization of intracellular protein tyrosine phosphatases in T cells. Eur J Immunol. 2000;30(8):2412–21.
[108] Gu MX, York JD, Warshawsky I, Majerus PW. Identification, cloning, and expression of a cytosolic megakaryocyte protein-tyrosine-phosphatase with sequence homology to cytoskeletal protein 4.1. Proc Natl Acad Sci U S A. 1991;88(13):5867–71.
[109] Yang Q, Tonks NK. Isolation of a cDNA clone encoding a human protein-tyrosine phosphatase with homology to the cytoskeletal-associated proteins band 4.1, ezrin, and talin. Proc Natl Acad Sci U S A. 1991;88(14):5949–53.

[110] Pilecka I, Patrignani C, Pescini R, Curchod ML, Perrin D, Xue Y, et al. Protein-tyrosine phosphatase H1 controls growth hormone receptor signaling and systemic growth. J Biol Chem. 2007;282(48):35405–15.
[111] Bauler TJ, Hendriks WJ, King PD. The FERM and PDZ domain-containing protein tyrosine phosphatases, PTPN4 and PTPN3, are both dispensable for T cell receptor signal transduction. PLoS One. 2008;3(12):e4014.
[112] Wang Z, Shen D, Parsons DW, Bardelli A, Sager J, Szabo S, et al. Mutational analysis of the tyrosine phosphatome in colorectal cancers. Science. 2004;304(5674):1164–6.
[113] Kina S, Tezuka T, Kusakawa S, Kishimoto Y, Kakizawa S, Hashimoto K, et al. Involvement of protein-tyrosine phosphatase PTPMEG in motor learning and cerebellar long-term depression. Eur J Neurosci. 2007;26(8):2269–78.
[114] Bauler TJ, Hughes ED, Arimura Y, Mustelin T, Saunders TL, King PD. Normal TCR signal transduction in mice that lack catalytically active PTPN3 protein tyrosine phosphatase. J Immunol. 2007;178(6):3680–7.
[115] Patrignani C, Magnone MC, Tavano P, Ardizzone M, Muzio V, Greco B, et al. Knockout mice reveal a role for protein tyrosine phosphatase H1 in cognition. Behav Brain Funct. 2008;4:36.
[116] Patrignani C, Lafont DT, Muzio V, Greco B, Hooft van Huijsduijnen R, Zaratin PF. Characterization of protein tyrosine phosphatase H1 knockout mice in animal models of local and systemic inflammation. J Inflamm (Lond). 2010;7:16.
[117] Young JA, Becker AM, Medeiros JJ, Shapiro VS, Wang A, Farrar JD, et al. The protein tyrosine phosphatase PTPN4/PTP-MEG1, an enzyme capable of dephosphorylating the TCR ITAMs and regulating NF-kappaB, is dispensable for T cell development and/or T cell effector functions. Mol Immunol. 2008;45(14):3756–66.
[118] Sozio MS, Mathis MA, Young JA, Walchli S, Pitcher LA, Wrage PC, et al. PTPH1 is a predominant protein-tyrosine phosphatase capable of interacting with and dephosphorylating the T cell receptor zeta subunit. J Biol Chem. 2004;279(9):7760–9.
[119] Han S, Williams S, Mustelin T. Cytoskeletal protein tyrosine phosphatase PTPH1 reduces T cell antigen receptor signaling. Eur J Immunol. 2000;30(5):1318–25.
[120] Hironaka K, Umemori H, Tezuka T, Mishina M, Yamamoto T. The protein-tyrosine phosphatase PTPMEG interacts with glutamate receptor delta 2 and epsilon subunits. J Biol Chem. 2000;275 (21):16167–73.
[121] Pasquali C, Curchod ML, Walchli S, Espanel X, Guerrier M, Arigoni F, et al. Identification of protein tyrosine phosphatases with specificity for the ligand-activated growth hormone receptor. Mol Endocrinol. 2003;17(11):2228–39.
[122] Jespersen T, Gavillet B, van Bemmelen MX, Cordonier S, Thomas MA, Staub O, et al. Cardiac sodium channel Na(v)1.5 interacts with and is regulated by the protein tyrosine phosphatase PTPH1. Biochem Biophys Res Commun. 2006;348(4):1455–62.
[123] Zhang SH, Kobayashi R, Graves PR, Piwnica-Worms H, Tonks NK. Serine phosphorylation-dependent association of the band 4.1-related protein-tyrosine phosphatase PTPH1 with 14-3-3beta protein. J Biol Chem. 1997;272(43):27281–7.
[124] Zhang SH, Liu J, Kobayashi R, Tonks NK. Identification of the cell cycle regulator VCP (p97/CDC48) as a substrate of the band 4.1-related protein-tyrosine phosphatase PTPH1. J Biol Chem. 1999;274(25):17806–12.
[125] Hou SW, Zhi HY, Pohl N, Loesch M, Qi XM, Li RS, et al. PTPH1 dephosphorylates and cooperates with p38gamma MAPK to increase ras oncogenesis through PDZ-mediated interaction. Cancer Res. 2010;70(7):2901–10.
[126] Moller NP, Moller KB, Lammers R, Kharitonenkov A, Sures I, Ullrich A. Src kinase associates with a member of a distinct subfamily of protein-tyrosine phosphatases containing an ezrin-like domain. Proc Natl Acad Sci U S A. 1994;91(16):7477–81.

[127] Chen KE, Li MY, Chou CC, Ho MR, Chen GC, Meng TC, et al. Substrate specificity and plasticity of FERM-containing protein tyrosine phosphatases. Structure. 2015;23(4):653–64.
[128] Dunican DS, McWilliam P, Tighe O, Parle-McDermott A, Croke DT. Gene expression differences between the microsatellite instability (MIN) and chromosomal instability (CIN) phenotypes in colorectal cancer revealed by high-density cDNA array hybridization. Oncogene. 2002;21(20): 3253–7.
[129] Dorner C, Ciossek T, Muller S, Moller PH, Ullrich A, Lammers R. Characterization of KIF1C, a new kinesin-like protein involved in vesicle transport from the Golgi apparatus to the endoplasmic reticulum. J Biol Chem. 1998;273(32):20267–75.
[130] Carlucci A, Porpora M, Garbi C, Galgani M, Santoriello M, Mascolo M, et al. PTPD1 supports receptor stability and mitogenic signaling in bladder cancer cells. J Biol Chem. 2010;285(50): 39260–70.
[131] Jiang X, Borgesi RA, McKnight NC, Kaur R, Carpenter CL, Balk SP. Activation of nonreceptor tyrosine kinase Bmx/Etk mediated by phosphoinositide 3-kinase, epidermal growth factor receptor, and ErbB3 in prostate cancer cells. J Biol Chem. 2007;282(45):32689–98.
[132] Cardone L, Carlucci A, Affaitati A, Livigni A, DeCristofaro T, Garbi C, et al. Mitochondrial AKAP121 binds and targets protein tyrosine phosphatase D1, a novel positive regulator of src signaling. Mol Cell Biol. 2004;24(11):4613–26.
[133] Feliciello A, Gottesman ME, Avvedimento EV. cAMP-PKA signaling to the mitochondria: protein scaffolds, mRNA and phosphatases. Cell Signal. 2005;17(3):279–87.
[134] Livigni A, Scorziello A, Agnese S, Adornetto A, Carlucci A, Garbi C, et al. Mitochondrial AKAP121 links cAMP and src signaling to oxidative metabolism. Mol Biol Cell. 2006;17(1):263–71.
[135] Parsons JT. Focal adhesion kinase: the first ten years. J Cell Sci. 2003;116(8):1409.
[136] Alonso A, Sasin J, Bottini N, Friedberg I, Friedberg I, Osterman A, et al. Protein tyrosine phosphatases in the human genome. Cell.117(6):699–711.
[137] Yin HL, Janmey PA. Phosphoinositide regulation of the actin cytoskeleton. Annu Rev Physiol. 2003;65:761–89.
[138] Araki N, Egami Y, Watanabe Y, Hatae T. Phosphoinositide metabolism during membrane ruffling and macropinosome formation in EGF-stimulated A431 cells. Exp Cell Res. 2007; 313(7):1496–507.
[139] Carlucci A, Gedressi C, Lignitto L, Nezi L, Villa-Moruzzi E, Avvedimento EV, et al. Protein-tyrosine phosphatase PTPD1 regulates focal adhesion kinase autophosphorylation and cell migration. J Biol Chem. 2008;283(16):10919–29.
[140] Ilić D, Furuta Y, Kanazawa S, Takeda N, Sobue K, Nakatsuji N, et al. Reduced cell motility and enhanced focal adhesion contact formation in cells from FAK-deficient mice. Nature. 1995;377:539.
[141] Iwahara T, Akagi T, Fujitsuka Y, Hanafusa H. CrkII regulates focal adhesion kinase activation by making a complex with Crk-associated substrate, p130<sup>Cas</sup&gt. Proc Natl Acad Sci U S A. 2004;101(51):17693.
[142] Smith AL, Mitchell PJ, Shipley J, Gusterson BA, Rogers MV, Crompton MR. Pez: a novel human cDNA encoding protein tyrosine phosphatase- and ezrin-like domains. Biochem Biophys Res Commun. 1995;209(3):959–65.
[143] Sjoblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, et al. The consensus coding sequences of human breast and colorectal cancers. Science. 2006;314(5797):268–74.
[144] Belle L, Ali N, Lonic A, Li X, Paltridge JL, Roslan S, et al. The tyrosine phosphatase PTPN14 (Pez) inhibits metastasis by altering protein trafficking. Sci Signal. 2015;8(364):ra18.
[145] Ogata M, Takada T, Mori Y, Oh-hora M, Uchida Y, Kosugi A, et al. Effects of overexpression of PTP36, a putative protein tyrosine phosphatase, on cell adhesion, cell growth, and cytoskeletons in HeLa cells. J Biol Chem. 1999;274(18):12905–9.

[146] Zhang P, Guo A, Possemato A, Wang C, Beard L, Carlin C, et al. Identification and functional characterization of p130Cas as a substrate of protein tyrosine phosphatase nonreceptor 14. Oncogene. 2013;32(16):2087–95.
[147] Wadham C, Gamble JR, Vadas MA, Khew-Goodall Y. The protein tyrosine phosphatase Pez is a major phosphatase of adherens junctions and dephosphorylates beta-catenin. Mol Biol Cell. 2003;14(6):2520–9.
[148] Wyatt L, Wadham C, Crocker LA, Lardelli M, Khew-Goodall Y. The protein tyrosine phosphatase Pez regulates TGFbeta, epithelial-mesenchymal transition, and organ development. J Cell Biol. 2007;178(7):1223–35.
[149] Yagi R, Chen LF, Shigesada K, Murakami Y, Ito Y. A WW domain-containing yes-associated protein (YAP) is a novel transcriptional co-activator. EMBO J. 1999;18(9):2551–62.
[150] Huang J, Wu S, Barrera J, Matthews K, Pan D. The Hippo signaling pathway coordinately regulates cell proliferation and apoptosis by inactivating Yorkie, the Drosophila Homolog of YAP. Cell. 2005;122(3):421–34.
[151] Overholtzer M, Zhang J, Smolen GA, Muir B, Li W, Sgroi DC, et al. Transforming properties of YAP, a candidate oncogene on the chromosome 11q22 amplicon. Proc Natl Acad Sci U S A. 2006;103(33):12405–10.
[152] Wilson KE, Li YW, Yang N, Shen H, Orillion AR, Zhang J. PTPN14 forms a complex with Kibra and LATS1 proteins and negatively regulates the YAP oncogenic function. J Biol Chem. 2014; 289(34):23693–700.
[153] Park M, Kim WK, Song M, Park M, Kim H, Nam HJ, et al. Protein kinase C-delta-mediated recycling of active KIT in colon cancer. Clin Cancer Res. 2013;19(18):4961–71.
[154] Hu H, Bliss JM, Wang Y, Colicelli J. RIN1 is an ABL tyrosine kinase activator and a regulator of epithelial-cell adhesion and migration. Curr Biol. 2005;15(9):815–23.
[155] Balaji K, Mooser C, Janson CM, Bliss JM, Hojjat H, Colicelli J. RIN1 orchestrates the activation of RAB5 GTPases and ABL tyrosine kinases to determine the fate of EGFR. J Cell Sci. 2012; 125(Pt 23):5887–96.
[156] Hagemann N, Ackermann N, Christmann J, Brier S, Yu F, Erdmann KS. The serologically defined colon cancer antigen-3 interacts with the protein tyrosine phosphatase PTPN13 and is involved in the regulation of cytokinesis. Oncogene. 2013;32(39):4602–13.
[157] Maekawa K, Imagawa N, Naito A, Harada S, Yoshie O, Takagi S. Association of protein-tyrosine phosphatase PTP-BAS with the transcription-factor-inhibitory protein IkappaBalpha through interaction between the PDZ1 domain and ankyrin repeats. Biochem J. 1999;337 (Pt 2):179–84.
[158] Kimber WA, Deak M, Prescott AR, Alessi DR. Interaction of the protein tyrosine phosphatase PTPL1 with the PtdIns(3,4)P2-binding adaptor protein TAPP1. Biochem J. 2003;376(Pt 2):525–35.
[159] van den Berk LC, van Ham MA, te Lindert MM, Walma T, Aelen J, Vuister GW, et al. The interaction of PTP-BL PDZ domains with RIL: an enigmatic role for the RIL LIM domain. Mol Biol Rep. 2004;31(4):203–15.
[160] Murthy KK, Clark K, Fortin Y, Shen SH, Banville D. ZRP-1, a zyxin-related protein, interacts with the second PDZ domain of the cytosolic protein tyrosine phosphatase hPTP1E. J Biol Chem. 1999;274(29):20679–87.
[161] Erdmann KS, Kuhlmann J, Lessmann V, Herrmann L, Eulenburg V, Muller O, et al. The Adenomatous Polyposis Coli-protein (APC) interacts with the protein tyrosine phosphatase PTP-BL via an alternatively spliced PDZ domain. Oncogene. 2000;19(34):3894–901.
[162] Sotelo NS, Schepens JT, Valiente M, Hendriks WJ, Pulido R. PTEN-PDZ domain interactions: binding of PTEN to PDZ domains of PTPN13. Methods. 2015;77–78:147–56.
[163] Kozlov G, Gehring K, Ekiel I. Solution structure of the PDZ2 domain from human phosphatase hPTP1E and its interactions with C-terminal peptides from the Fas receptor. Biochemistry. 2000;39(10):2572–80.

[164] Yanagisawa J, Takahashi M, Kanki H, Yano-Yanagisawa H, Tazunoki T, Sawa E, et al. The molecular interaction of Fas and FAP-1. A tripeptide blocker of human Fas interaction with FAP-1 promotes Fas-induced apoptosis. J Biol Chem. 1997;272(13):8539–45.
[165] Saras J, Engstrom U, Gonez LJ, Heldin CH. Characterization of the interactions between PDZ domains of the protein-tyrosine phosphatase PTPL1 and the carboxyl-terminal tail of Fas. J Biol Chem. 1997;272(34):20979–81.
[166] Gross C, Heumann R, Erdmann KS. The protein kinase C-related kinase PRK2 interacts with the protein tyrosine phosphatase PTP-BL via a novel PDZ domain binding motif. FEBS Lett. 2001;496(2–3):101–4.
[167] Irie S, Hachiya T, Rabizadeh S, Maruyama W, Mukai J, Li Y, et al. Functional interaction of Fas-associated phosphatase-1 (FAP-1) with p75(NTR) and their effect on NF-kappaB activation. FEBS Lett. 1999;460(2):191–8.
[168] Saras J, Franzen P, Aspenstrom P, Hellman U, Gonez LJ, Heldin CH. A novel GTPase-activating protein for Rho interacts with a PDZ domain of the protein-tyrosine phosphatase PTPL1. J Biol Chem. 1997;272(39):24333–8.
[169] van Ham M, Croes H, Schepens J, Fransen J, Wieringa B, Hendriks W. Cloning and characterization of mCRIP2, a mouse LIM-only protein that interacts with PDZ domain IV of PTP-BL. Genes Cells. 2003;8(7):631–44.
[170] Ghiran I, Glodek AM, Weaver G, Klickstein LB, Nicholson-Weller A. Ligation of erythrocyte CR1 induces its clustering in complex with scaffolding protein FAP-1. Blood. 2008;112(8):3465–73.
[171] Villa F, Deak M, Bloomberg GB, Alessi DR, van Aalten DM. Crystal structure of the PTPL1/FAP-1 human tyrosine phosphatase mutated in colorectal cancer: evidence for a second phosphotyrosine substrate recognition pocket. J Biol Chem. 2005;280(9):8180–7.
[172] van Bergen En Henegouwen PM. Eps15: a multifunctional adaptor protein regulating intracellular trafficking. Cell Commun Signal. 2009;7:24.
[173] Confalonieri S, Salcini AE, Puri C, Tacchetti C, Di Fiore PP. Tyrosine phosphorylation of Eps15 is required for ligand-regulated, but not constitutive, endocytosis. J Cell Biol. 2000;150(4):905.
[174] Li MY, Lai PL, Chou YT, Chi AP, Mi YZ, Khoo KH, et al. Protein tyrosine phosphatase PTPN3 inhibits lung cancer cell proliferation and migration by promoting EGFR endocytic degradation. Oncogene. 2015;34(29):3791–803.
[175] Glondu-Lassis M, Dromard M, Lacroix-Triki M, Nirde P, Puech C, Knani D, et al. PTPL1/PTPN13 regulates breast cancer cell aggressiveness through direct inactivation of Src kinase. Cancer Res. 2010;70(12):5116–26.
[176] Bompard G, Puech C, Prebois C, Vignon F, Freiss G. Protein-tyrosine phosphatase PTPL1/FAP-1 triggers apoptosis in human breast cancer cells. J Biol Chem. 2002;277(49):47861–9.
[177] Han XJ, Xue L, Gong L, Zhu SJ, Yao L, Wang SM, et al. Stat3 inhibits PTPN13 expression in squamous cell lung carcinoma through recruitment of HDAC5. Biomed Res Int. 2013;2013:468963.
[178] Abaan OD, Levenson A, Khan O, Furth PA, Uren A, Toretsky JA. PTPL1 is a direct transcriptional target of EWS-FLI1 and modulates Ewing's Sarcoma tumorigenesis. Oncogene. 2005;24(16):2715-22.
[179] Schickel R, Park SM, Murmann AE, Peter ME. miR-200c regulates induction of apoptosis through CD95 by targeting FAP-1. Mol Cell. 2010;38(6):908–15.
[180] Ying J, Li H, Cui Y, Wong AH, Langford C, Tao Q. Epigenetic disruption of two proapoptotic genes MAPK10/JNK3 and PTPN13/FAP-1 in multiple lymphomas and carcinomas through hypermethylation of a common bidirectional promoter. Leukemia. 2006;20(6):1173–5.
[181] Meinhold-Heerlein I, Stenner-Liewen F, Liewen H, Kitada S, Krajewska M, Krajewski S, et al. Expression and potential role of Fas-associated phosphatase-1 in ovarian cancer. Am J Pathol. 2001;158(4):1335–44.

[182] Zhu JH, Chen R, Yi W, Cantin GT, Fearns C, Yang Y, et al. Protein tyrosine phosphatase PTPN13 negatively regulates Her2/ErbB2 malignant signaling. Oncogene. 2008;27(18):2525–31.
[183] Dromard M, Bompard G, Glondu-Lassis M, Puech C, Chalbos D, Freiss G. The putative tumor suppressor gene PTPN13/PTPL1 induces apoptosis through insulin receptor substrate-1 dephosphorylation. Cancer Res. 2007;67(14):6806–13.
[184] Kuchay S, Duan S, Schenkein E, Peschiaroli A, Saraf A, Florens L, et al. FBXL2- and PTPL1-mediated degradation of p110-free p85beta regulatory subunit controls the PI(3)K signalling cascade. Nat Cell Biol. 2013;15(5):472–80.
[185] Huang W, Bei L, Eklund EA. Fas-associated phosphatase 1 (Fap1) influences betacatenin activity in myeloid progenitor cells expressing the Bcr-abl oncogene. J Biol Chem. 2013;288(18):12766–76.
[186] Hoover AC, Strand GL, Nowicki PN, Anderson ME, Vermeer PD, Klingelhutz AJ, et al. Impaired PTPN13 phosphatase activity in spontaneous or HPV-induced squamous cell carcinomas potentiates oncogene signaling through the MAP kinase pathway. Oncogene. 2009;28(45): 3960–70.
[187] Lopez-Ruano G, Prieto-Bermejo R, Ramos TL, San-Segundo L, Sanchez-Abarca LI, Sanchez-Guijo F, et al. PTPN13 and beta-catenin regulate the quiescence of hematopoietic stem cells and their interaction with the bone marrow niche. Stem Cell Reports. 2015;5(4):516–31.
[188] Ali N, Zhang L, Taylor S, Mironov A, Urbe S, Woodman P. Recruitment of UBPY and ESCRT exchange drive HD-PTP-dependent sorting of EGFR to the MVB. Curr Biol. 2013;23(6):453–61.
[189] Tanase CA. Histidine domain-protein tyrosine phosphatase interacts with Grb2 and GrpL. PLoS One. 2010;5(12):e14339.
[190] Gingras MC, Zhang YL, Kharitidi D, Barr AJ, Knapp S, Tremblay ML, et al. HD-PTP is a catalytically inactive tyrosine phosphatase due to a conserved divergence in its phosphatase domain. PLoS One. 2009;4(4):e5105.
[191] Lin G, Aranda V, Muthuswamy SK, Tonks NK. Identification of PTPN23 as a novel regulator of cell invasion in mammary epithelial cells from a loss-of-function screen of the “PTP-ome”. Genes Dev. 2011;25(13):1412–25.
[192] Mariotti M, Castiglioni S, Garcia-Manteiga JM, Beguinot L, Maier JA. HD-PTP inhibits endothelial migration through its interaction with Src. Int J Biochem Cell Biol. 2009;41(3):687–93.
[193] Chen MJ, Dixon JE, Manning G. Genomics and evolution of protein phosphatases. Sci Signal. 2017;10(474).
[194] Ichioka F, Takaya E, Suzuki H, Kajigaya S, Buchman VL, Shibata H, et al. HD-PTP and Alix share some membrane-traffic related proteins that interact with their Bro1 domains or proline-rich regions. Arch Biochem Biophys. 2007;457(2):142–9.
[195] Chen DY, Li MY, Wu SY, Lin YL, Tsai SP, Lai PL, et al. The Bro1-domain-containing protein Myopic/HDPTP coordinates with Rab4 to regulate cell adhesion and migration. J Cell Sci. 2012;125(Pt 20):4841–52.
[196] Castiglioni S, Maier JA, Mariotti M. The tyrosine phosphatase HD-PTP: a novel player in endothelial migration. Biochem Biophys Res Commun. 2007;364(3):534–9.
[197] Tanaka K, Kondo K, Kitajima K, Muraoka M, Nozawa A, Hara T. Tumor-suppressive function of protein-tyrosine phosphatase non-receptor type 23 in testicular germ cell tumors is lost upon overexpression of miR142-3p microRNA. J Biol Chem. 2013;288(33):23990–9.
[198] Mariotti M, Castiglioni S, Beguinot L, Maier JA. The tyrosine phosphatase HD-PTP is regulated by FGF-2 through proteasome degradation. Front Biosci. 2006;11:2138–43.
[199] Castiglioni S, Maier JA. The tyrosine phosphatase HD-PTP (PTPN23) is degraded by calpains in a calcium-dependent manner. Biochem Biophys Res Commun. 2012;421(2):380–3.
[200] Ohsugi M, Kuramochi S, Matsuda S, Yamamoto T. Molecular cloning and characterization of a novel cytoplasmic protein-tyrosine phosphatase that is specifically expressed in spermatocytes. J Biol Chem. 1997;272(52):33092–9.

[201] Fodero-Tavoletti MT, Hardy MP, Cornell B, Katsis F, Sadek CM, Mitchell CA, et al. Protein tyrosine phosphatase hPTPN20a is targeted to sites of actin polymerization. Biochem J. 2005;389(Pt 2):343–54.
[202] Krueger NX, Saito H. A human transmembrane protein-tyrosine-phosphatase, PTP zeta, is expressed in brain and has an N-terminal receptor domain homologous to carbonic anhydrases. Proc Natl Acad Sci U S A. 1992;89(16):7417–21.
[203] Maurel P, Meyer-Puttlitz B, Flad M, Margolis RU, Margolis RK. Nucleotide sequence and molecular variants of rat receptor-type protein tyrosine phosphatase-zeta/beta. DNA Seq. 1995;5(5):323–8.
[204] Matteoni R, Kreis TE. Translocation and clustering of endosomes and lysosomes depends on microtubules. J Cell Biol. 1987;105(3):1253–65.
[205] Du Y, Ferro-Novick S, Novick P. Dynamics and inheritance of the endoplasmic reticulum. J Cell Sci. 2004;117(Pt 14):2871–8.
[206] Allan VJ, Thompson HM, McNiven MA. Motoring around the Golgi. Nat Cell Biol. 2002;4(10): E236–42.
[207] Giannakakou P, Sackett DL, Ward Y, Webster KR, Blagosklonny MV, Fojo T. p53 is associated with cellular microtubules and is transported to the nucleus by dynein. Nat Cell Biol. 2000; 2(10):709–17.
[208] Lam MH, Thomas RJ, Loveland KL, Schilders S, Gu M, Martin TJ, et al. Nuclear transport of parathyroid hormone (PTH)-related protein is dependent on microtubules. Mol Endocrinol. 2002;16(2):390–401.
[209] Andersen JN, Mortensen OH, Peters GH, Drake PG, Iversen LF, Olsen OH, et al. Structural and evolutionary relationships among protein tyrosine phosphatase domains. Mol Cell Biol. 2001;21(21):7117–36.
[210] Bardwell L, Thorner J. A conserved motif at the amino termini of MEKs might mediate high-affinity interaction with the cognate MAPKs. Trends Biochem Sci. 1996;21(10):373–4.
[211] Pulido R, Zuniga A, Ullrich A. PTP-SL and STEP protein tyrosine phosphatases regulate the activation of the extracellular signal-regulated kinases ERK1 and ERK2 by association through a kinase interaction motif. EMBO J. 1998;17(24):7337–50.
[212] Eswaran J, von Kries JP, Marsden B, Longman E, Debreczeni JE, Ugochukwu E, et al. Crystal structures and inhibitor identification for PTPN5, PTPRR and PTPN7: a family of human MAPK-specific protein tyrosine phosphatases. Biochem J. 2006;395(3):483–91.
[213] Mustelin T, Tautz L, Page R. Structure of the hematopoietic tyrosine phosphatase (HePTP) catalytic domain: structure of a KIM phosphatase with phosphate bound at the active site. J Mol Biol. 2005;354(1):150–63.
[214] Lombroso PJ, Murdoch G, Lerner M. Molecular characterization of a protein-tyrosine-phosphatase enriched in striatum. Proc Natl Acad Sci U S A. 1991;88(16):7242–6.
[215] Olausson P, Venkitaramani DV, Moran TD, Salter MW, Taylor JR, Lombroso PJ. The tyrosine phosphatase STEP constrains amygdala-dependent memory formation and neuroplasticity. Neuroscience. 2012;225:1–8.
[216] Yang CH, Huang CC, Hsu KS. A critical role for protein tyrosine phosphatase nonreceptor type 5 in determining individual susceptibility to develop stress-related cognitive and morphological changes. J Neurosci. 2012;32(22):7550–62.
[217] Kurup P, Zhang Y, Xu J, Venkitaramani DV, Haroutunian V, Greengard P, et al. Abeta-mediated NMDA receptor endocytosis in Alzheimer's disease involves ubiquitination of the tyrosine phosphatase STEP61. J Neurosci. 2010;30(17):5948–57.
[218] Zhang Y, Kurup P, Xu J, Anderson GM, Greengard P, Nairn AC, et al. Reduced levels of the tyrosine phosphatase STEP block beta amyloid-mediated GluA1/GluA2 receptor internalization. J Neurochem. 2011;119(3):664–72.

[219] Pelov I, Teltsh O, Greenbaum L, Rigbi A, Kanyas-Sarner K, Lerer B, et al. Involvement of PTPN5, the gene encoding the striatal-enriched protein tyrosine phosphatase, in schizophrenia and cognition. Psychiatr Genet. 2012;22(4):168–76.
[220] Nguyen TH, Liu J, Lombroso PJ. Striatal enriched phosphatase 61 dephosphorylates Fyn at phosphotyrosine 420. J Biol Chem. 2002;277(27):24274–9.
[221] Xu J, Kurup P, Foscue E, Lombroso PJ. Striatal-enriched protein tyrosine phosphatase regulates the PTPalpha/Fyn signaling pathway. J Neurochem. 2015;134(4):629–41.
[222] Adachi M, Sekiya M, Isobe M, Kumura Y, Ogita Z, Hinoda Y, et al. Molecular cloning and chromosomal mapping of a human protein-tyrosine phosphatase LC-PTP. Biochem Biophys Res Commun. 1992;186(3):1607–15.
[223] Zanke B, Suzuki H, Kishihara K, Mizzen L, Minden M, Pawson A, et al. Cloning and expression of an inducible lymphoid-specific, protein tyrosine phosphatase (HePTPase). Eur J Immunol. 1992;22(1):235–9.
[224] Swieter M, Berenstein EH, Swaim WD, Siraganian RP. Aggregation of IgE receptors in rat basophilic leukemia 2H3 cells induces tyrosine phosphorylation of the cytosolic protein-tyrosine phosphatase HePTP. J Biol Chem. 1995;270(37):21902–6.
[225] Zanke B, Squire J, Griesser H, Henry M, Suzuki H, Patterson B, et al. A hematopoietic protein tyrosine phosphatase (HePTP) gene that is amplified and overexpressed in myeloid malignancies maps to chromosome 1q32.1. Leukemia. 1994;8(2):236–44.
[226] Oh-hora M, Ogata M, Mori Y, Adachi M, Imai K, Kosugi A, et al. Direct suppression of TCR-mediated activation of extracellular signal-regulated kinase by leukocyte protein tyrosine phosphatase, a tyrosine-specific phosphatase. J Immunol. 1999;163(3):1282–8.
[227] Gronda M, Arab S, Iafrate B, Suzuki H, Zanke BW. Hematopoietic protein tyrosine phosphatase suppresses extracellular stimulus-regulated kinase activation. Mol Cell Biol. 2001;21(20):6851–8.
[228] Saxena M, Williams S, Gilman J, Mustelin T. Negative regulation of T cell antigen receptor signal transduction by hematopoietic tyrosine phosphatase (HePTP). J Biol Chem. 1998;273(25):15340–4.
[229] Munoz JJ, Tarrega C, Blanco-Aparicio C, Pulido R. Differential interaction of the tyrosine phosphatases PTP-SL, STEP and HePTP with the mitogen-activated protein kinases ERK1/2 and p38alpha is determined by a kinase specificity sequence and influenced by reducing agents. Biochem J. 2003;372(Pt 1):193–201.
[230] Nika K, Hyunh H, Williams S, Paul S, Bottini N, Tasken K, et al. Haematopoietic protein tyrosine phosphatase (HePTP) phosphorylation by cAMP-dependent protein kinase in T-cells: dynamics and subcellular location. Biochem J. 2004;378(Pt 2):335–42.

8 Protein tyrosine phosphatases: strategies for drug development

Protein phosphorylation regulation is undoubtedly a fundamental process that mediates key biological processes of growth, cell division, cell motility and cell physiology [1]. The first protein tyrosine phosphatase was identified and cloned a decade after the discovery of the first protein tyrosine kinase [2, 3], and for the longest time, it was widely believed that these enzymes work as housekeeping genes that maintain a basal level of protein phosphorylation in the cell. However, perturbations in the protein tyrosine phosphatome got increasingly linked to numerous diseases, including metabolic disorders, cardiovascular and neurological diseases, autoimmune disorders and also cancer [4, 5]. Dysregulation of these phosphatases was linked to variation in their expression levels and also amino acid polymorphisms in their protein chains [6]. For example, reduced expression of SHP1 is associated with the onset of cancer while activating mutations in SHP2 gene are linked to Noonan Syndrome and leukemia [7, 8]. Polymorphisms in the PTPσ gene are linked to ulcerative colitis [9]. Similarly, polymorphism in *PTPN22* gene is associated with the development of type-I diabetes, rheumatoid arthritis and systemic lupus erythematosus [10, 11]. Protein tyrosine phosphatases have now, long been the keen interest of pharmaceutical companies for being promising drug targets for various diseases. PTP1B has been of particular interest in drug designing endeavors for diabetes and obesity [12, 13]. Animal model studies using transgenic mice show PTP1B to function in concert with Erb2 tyrosine kinases implicated in breast cancers, hence providing an exciting opportunity for protein kinase–phosphatase combination drug discovery [14, 15]. Viability of protein phosphatase targeting drugs is exemplified by the immuno-suppressant drugs cyclosporine and FK-506 that target calcineurin and oligonucleotides for downregulation of PTP1B to improve insulin sensitivity [16, 17].

Drug discovery for protein tyrosine phosphatases has mainly faced two critical challenges: [1] enzyme selectivity and [2] membrane permeability. The highly conserved protein tyrosine phosphatase active site poses a major challenge for achieving selectivity. Similarity between the phosphatases from the same subtype is even closer. For example, PTP1B and TCPTP are 72% identical in sequence and have a 92% identical active site. In addition, their catalytic domains superimpose with a root-mean-square-deviation of less than 1.0Å [18]. Given this high similarity in these two phosphatases, cross-reaction between their inhibitors would be very likely and of disastrous consequences. Inhibiting PTP1B enhances insulin sensitivity and provides resistance to diet-induced obesity. However, TCPTP inhibition is lethal, and TCPTP knockout mice die within weeks of birth [12, 19]. The second challenge to protein tyrosine phosphatase targeted drug design efforts is posed by the polar nature of their conserved active site. The large polar active site of the protein tyrosine

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phosphatase catalytic domain requires highly charged anionic phosphate mimetics to serve as potent inhibitors. However, these charged molecules have poor membrane permeability and are transported poorly inside the cells and tissues. The important role of protein tyrosine phosphatases in cellular physiology have provided impetus to find approaches that would help overcome these key challenges.

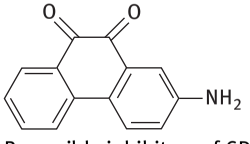
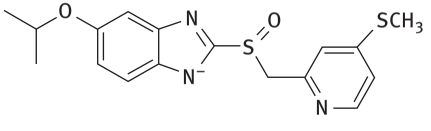
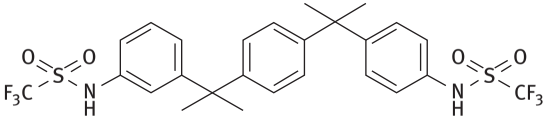
Key efforts and strategies for these approaches are covered in this chapter. These efforts include standard molecular designing of orthosteric inhibitors that bind to the active site of these enzymes and also the discovery of natural compounds that could selectively inhibit these proteins (Table 8.1) [20]. More novel approaches include the designing of bidentate inhibitors that target a specific pocket of a given protein tyrosine phosphatase, alongside its active site (Figure 8.1). Certain compounds have been designed to bind and prevent the opening-and-closing of the WPD-loop. Allosteric inhibitor designs rely on protein-specific pockets, including the unique inter-domain interface of the double domain receptor protein tyrosine phosphatases. Biologics-based approaches include designing of antibodies that binds to induce dimerization of the receptor forms to promote their inhibition. Novel methods also include use of anti-sense RNA aptamers to switch off the production of these enzymes in cells without compromising with any other elements of the signaling machinery.

8.1 Natural Products as protein tyrosine phosphatase inhibitors

Various natural compounds have been identified as potent inhibitors of protein tyrosine phosphatases. Some of the most potent natural inhibitors are listed in Table 8.1 and Figure 8.2. The first natural compound to be discovered as a protein tyrosine inhibitor was dephostatin in 1993 [62]. Isolated as a nitrosamino-containing hydroquinone, it was isolated from *Streptomyces* and is reported to competitively inhibit the activity of CD45. It is also reported to inhibit the activities of SHP-1, PTPε and PTP1B but is reported to be ineffective against the Ser/Thr-specific phosphatases PP2A and PP2B [63]. *Streptomyces* have also proved to be helpful for discovery of related quinone molecules called as phosphatoquinone A and phosphatoquinone B [64]. These are reported to inhibit protein tyrosine phosphatase activity of extracts of human Ball-1 cells with IC_{50} value of 28.0 μM and 2.9 μM, respectively. Non-competitive inhibitors of PTP1B include amentoflavone isolated from spikemoss *Selaginella* [65]. Flavonoid-based inhibitors of PTP1B include five compounds isolated from the roots of *Broussonetia papyrifera* [66]. Quercetin, uralenol and broussochalcone A inhibit PTP1B with IC_{50} value of 23.3 μM, 21.5 μM and 36.8μM, respectively. The more decorated flavonoid rings of 3′-(3-methylbut-2-enyl)-3′,4′,7-trihydroxyflavane and 8-(1,1-dimethylallyl)-5′-(3-methylbut-2-enyl)-3′,4′,5,7-tetrahydroxyflanvone are reported to inhibit PTP1B with IC_{50} value of 41.5 μM and 4.3 μM, respectively [66, 67].

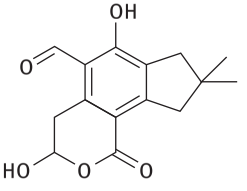
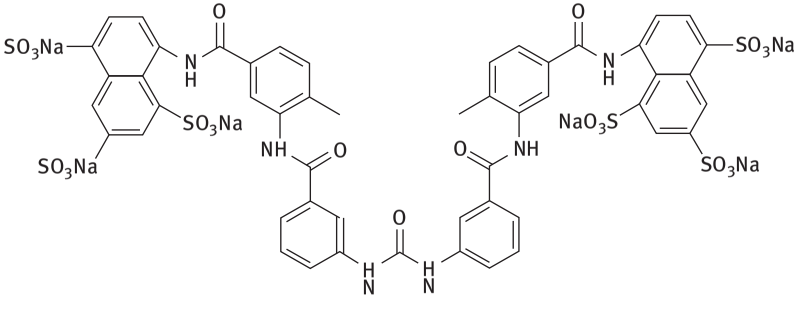
Two compounds derived from the Indian beech tree *Pongamia pinnata* called karajin and kanjone are reported to be anti-hyperglycemic, and karajin has been

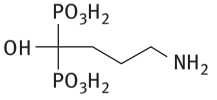
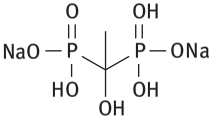
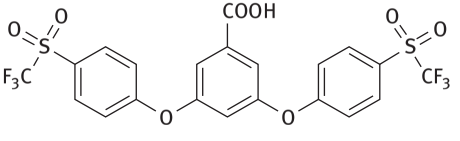
Table 8.1: Natural compounds as inhibitors of protein tyrosine phosphatases.

Protein Tyrosine Phosphatase	Inhibitor(s)	Reference
CD45	– Best drug target for graft rejection, autoimmunity and inflammatory disorders	[21, 22]
	– 9,10-phenanthrenedione derivatives	[23]
		
	Reversible inhibitors of CD45 with IC_{50} = 0.2μM. It is suggested to use the active site cysteine of CD45 for attacking the carbonyl of the dione	
	– 2-[4-(methylthiopyridin-2-yl)methylsulfinyl] benzimidazole	
		
	Inhibitor TU-572 inhibits CD45 with IC_{50} = 0.25μM, shows specificity for CD45 over PTP1B, LAR, VHR and Ser/Thr phosphatases	
PTPμ	– Nonselective; *bis*(4-trifluoro-methylsulfonamidophenyl)-1,4-diisopropylbenzene, IC_{50} = 6.7μM	[26]
		

(*continued*)

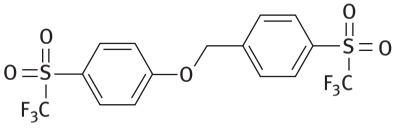
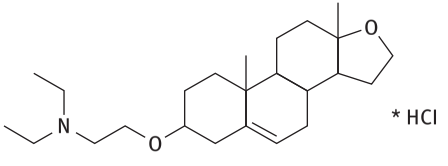
Table 8.1: (*continued*)

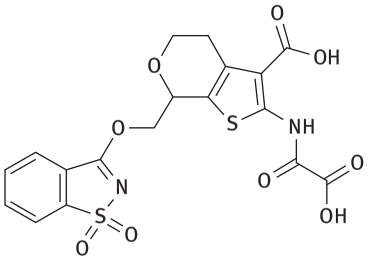
Protein Tyrosine Phosphatase	Inhibitor(s)	Reference
LAR	– Illudalic acid  Competitive inhibitor, IC_{50} = 1.1 µM. K_I = 330 nM, selectivity for LAR and PTPσ	[27]
	– Wedge-domain peptides: Homophilic binding to the full length protein by peptides derived from the N-terminal α1′-α2′ helix-turn-helix region of the catalytic domain, membrane penetrant Tat-derived peptide: *NH_2-DLADNIERLKANDGLKFSQEYESI-GRKKRRQRRRC-NH_2*	[28] [29]
	– **Sumarin and its derivatives: potentiate LAR activity* 	

PTPσ	– Illudalic acid, competitive inhibitor	[27]
	– Alendronate; Nonspecific inhibitor 	[6]
	– Nonselective; *bis*(4-trifluoro-methylsulfonamidophenyl)-1,4-diisopropylbenzene, IC_{50} = 20.0 μM	[26]
PTPδ	– Nonspecific inhibition by vanadate ≥ 10μM	[30]
	– Etidronate; (1-hydroxy-1-phosphonoethyl) phosphonic acid  (Selectivity unexplored)	[31]
PTPα	– Inhibition by reactive oxygen species H_2O_2; dose of 50–250μM, rotational coupling of receptor dimers of PTPα due to oxidation of the active site	[32, 33]
	– Si–RNA-mediated inhibition	[34]
	– * *Sumarin and its derivatives: potentiate PTPα activity*	[29]
PTPε	– Bisphosphonates, including alendronate	[35]
	– Trifluoromethyl sulfonyl-based compounds having 2.5–5-fold selectivity for PTPε over PTPβ, SHPs, PTP1B, PTP–MEG,PTPμ 	[26]
PTPγ	– Expression is downregulated by keratinocyte growth factor (at 20ng/mL)	[36]
	– Expression is downregulated by nonsteroidal growth promoters 17α-estradiol and zeranol	[37]

(*continued*)

Table 8.1: (*continued*)

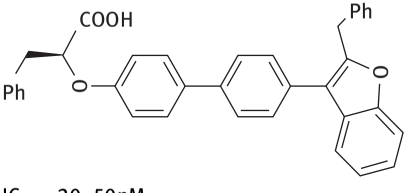
Protein Tyrosine Phosphatase	Inhibitor(s)	Reference
PTPβ	– 4-trifluoromethylsulfonylbenzyl 4-trifluoromethylsulonylphenyl ether, IC_{50} = 3.5 μM  Twofold selective over PTPε >tenfold selective over PTP1B, SHPs, PTP-MEG, PTPμ – Thiophosphotyrosylated peptides derived from PLCγ, insulin receptor and Src phosphotyrosine containing sequences Peptide based on sequence TAEPDpYGALYE (K_M = 1.0 μM), is selective for PTPβ, K_I = 3.0 μM 110-fold over CD45 and 75-fold over LAR	[26] [38]
DEP1	– Reported to be downregulated by U18666A, apoptosis-promoting compound that mediates phosphorylation of Tau protein 	[39]
IA-2	– Derivatives of 2-(oxalylamino)benzoic acid 7-(1,1-dioxo-1H-benzo[d]isothiazol-3-yloxymethyl)-2-(oxalylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid, K_I = 310.0 μM	[40]



Cross-reacts with IA-2β (K_I = 8.0 μM) and PTP1B (K_I < 1.0 μM)

PTP1B

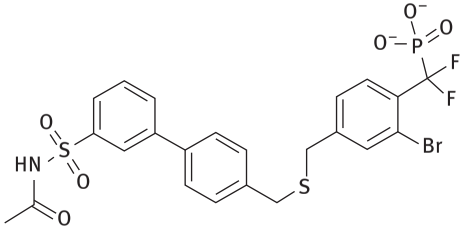
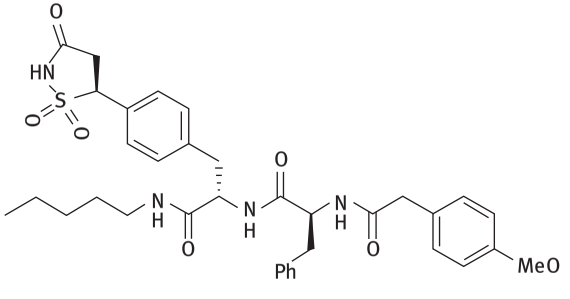
– Anti-sense oligonucleotide ISIS-113715 [41]
  ISIS-113715 has passed class II clinical trials
  20nt 2′-O-methylethylribose-modified phosphorothioate oligonucleotide that binds the coding region of the PTP1B sequence
  Binds at an exon–exon junction (nucleotide 861–880) near the putative splicing site for intron 7
– Phosphotyrosine mimetics [42, 43]
  Replacement of labile phosphate group to generate sulfotyrosyl, thiophosphoryltyrosyl, O-dithiophosphoryltyrosyl, fluoro O-malonyltyrosyl, difluoromethylenesulfonic acid or phosphonodifluoromethyl phenylalanine (F_2PmP)
– Natural pentacyclic triterpenoid ursolic acid and its derivative UA0713; promotes glucose uptake in L6 myotubes [44]
– Benzofuran and Benzothiophene biphenyls [45]



IC_{50} =20–50nM
Bioactive when administered orally

(*continued*)

Table 8.1: (*continued*)

Protein Tyrosine Phosphatase	Inhibitor(s)	Reference
	– Difluoromethyl phosphonates, $IC_{50} \geq 2.2$ nM	[46]
		
	– Izothiazolidinones, $IC_{50} \geq 40.0$ nM	[47]
		

– Cinnamic acid peptide conjugate, IC_{50} = 44 nM, K_I = 79 nM

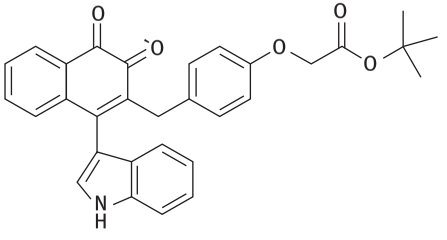
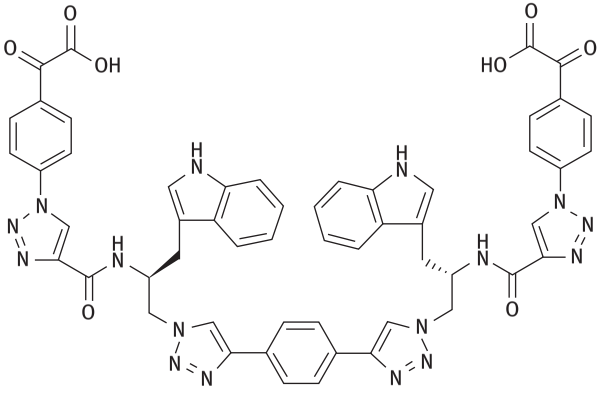
[48]

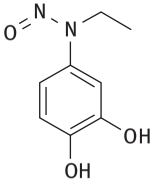
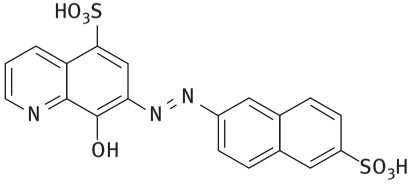
– Ethyl-3,4-dephostatin, IC_{50} = 0.58 µg/mL
Methoxime-3,4-dephostatin, IC_{50} = 2.9 µg/mL

[49]

(*continued*)

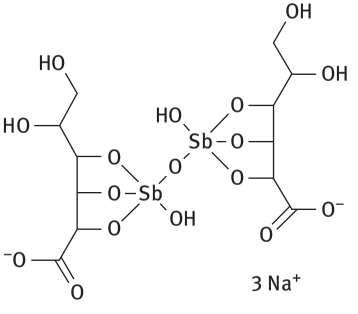
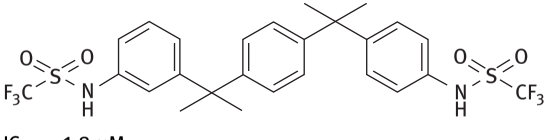
Table 8.1: (*continued*)

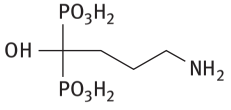
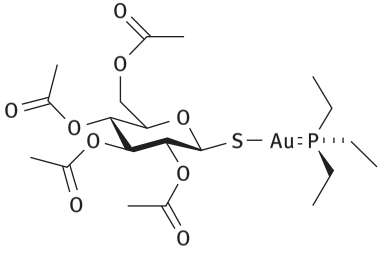
Protein Tyrosine Phosphatase	Inhibitor(s)	Reference
	– KR61639 (1,2-naphthoquinone compound), IC_{50} = 650 nM, K_I = 390 nM 	[50]
TCPTP	– RNA-interference to suppress expression	[51]
	– α-ketoacid-based protein tyrosine phosphatase inhibitor, IC_{50} ≥ 710 nM, not very specific 	[52]

SHP1	– Peptidyl aldehydes, 4-carboxy cinnamaldehyde attached to NH_2–EEG–COOH tripeptide, K_I = 10.7 μM, reversible, slow inhibitors, cross-react with PTP1B and VHR	[48]
	– Ethyl-3,4-dephostatin 	[53]
	– NSC-87877, commercial name for 8-hydroxy-7-(6-sulfonaphthalen-2-yl)diazenyl-quinoline-5-sulfonic acid Membrane permeable inhibitor of SHPs, IC_{50} = 355 nM 	[54]

(*continued*)

Table 8.1: (*continued*)

Protein Tyrosine Phosphatase	Inhibitor(s)	Reference
	– Sodium stibogluconate, anti-*Leishmania* drug, decreases melanoma growth rate Glucantime is the methylglucamine form of the compound sold commercially  3 Na^+	[55]
SHP2	– Anti-sense si-RNA, nt 199–219 of SHP2 sequence 5′ CTC CGC GAT GTC ATG TTC CT 3′ – NSC-87877, inhibitor of SHPs, IC_{50} = 318 nM – PtpI IV inhibitor, another name for *bis*(4-trifluoromethylsulfonamidophenyl)-1,4-diisopropylbenzene  IC_{50} = 1.8 µM	[54] [26]

PTP-MEG1	– Alendronate, a type of bisphosphonate, IC_{50} = 25 μM 	[57]
PTP-PEST	– Auranofin, IC_{50} = 9.0 μM (specific for PEST subfamily of PTPs, cross-reacts with PTP–PEP, IC_{50} = 200 μM)  – Auranofin-derived gold(I) N-heterocyclic carbene complex IC_{50} = 7.0 μM (specific for PEST subfamily of PTPs, cross-reacts with PTP–PEP, IC_{50} = 11 μM)	[58]
PTPD1	– Small hairpin RNA (shRNA) for downregulation of gene expression, shRNA made against the human nt 184–204 and nt 335–355	[59]
HD–PTP	– siRNA for downregulation of expression	[60]
PTP–Typ	– siRNA for downregulation of expression, siRNA for exon 6	[61]

*Chemical structures adapted from Ref [20]

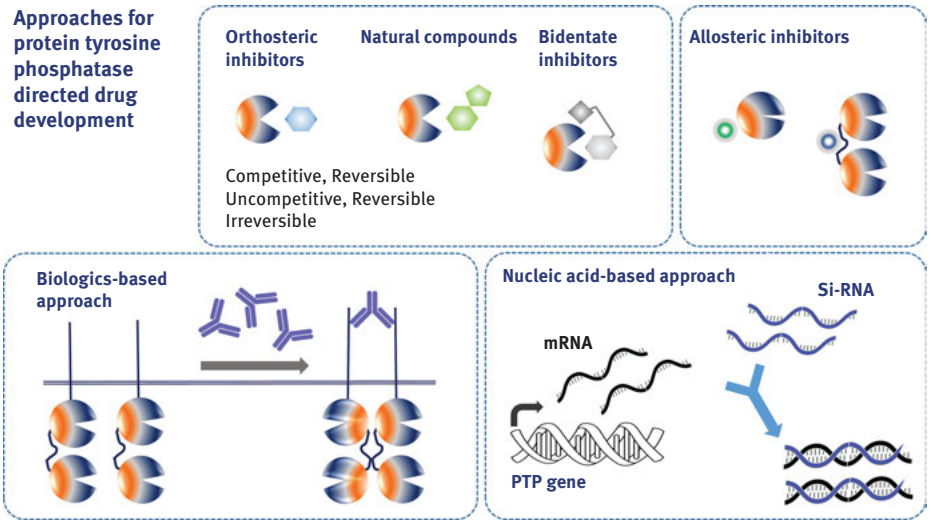


Figure 8.1: Schematic for various strategies used for modulation of protein tyrosine phosphatases.

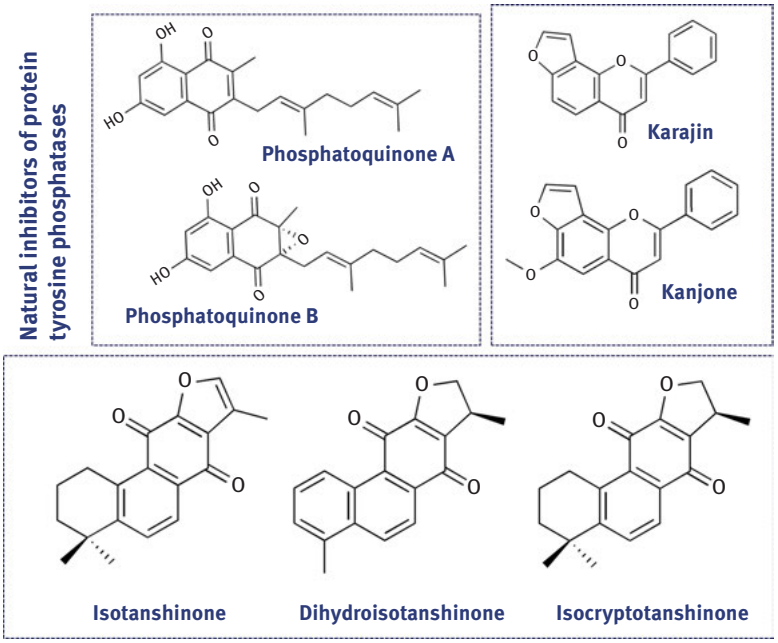
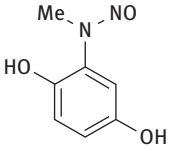
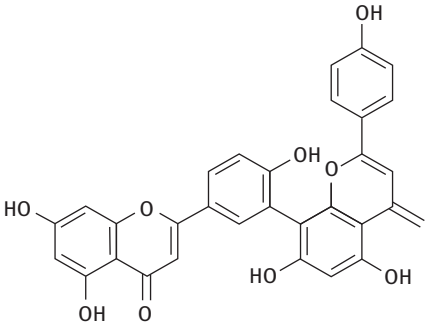
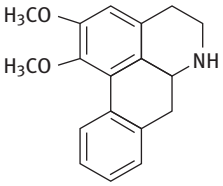
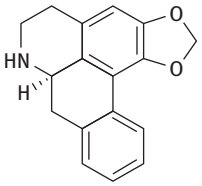
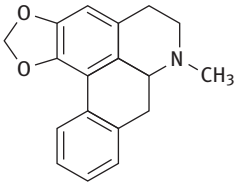


Figure 8.2: Chemical structures of a few natural compounds that are protein tyrosine phosphatase inhibitors. Structures have been adapted from Ref. [20].

used to develop a series of benzofuran isoxazolines pharmacores as potent protein tyrosine phosphatase inhibitors [68]. Aporphine alkaloids have also been reported to be specific inhibitors of protein tyrosine phosphatases and show specificity toward

inhibiting them over Ser/Thr phosphatases. Anonaine, roemerine and N-nornuciferine are the most potent alkaloids (Table 8.2) [69–71]. Specific inhibitors of CD45 include α,β unsaturated lactone-containing inhibitors dihydrocarolic acid and penitricin D. These

Table 8.2: Natural inhibitors of protein tyrosine phosphatases.

Inhibitor	Structure	IC_{50} (μM)
Dephostatin		7.70 (CD45)
Amentoflavone		7.3 (PTP1B)
Nornuciferine		5.3 (CD45)
Anonaine		17.0 (CD45)
Roemerine		107 (CD45)

(*continued*)

Table 8.2: (*continued*)

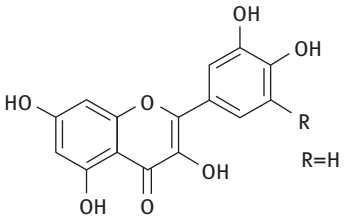
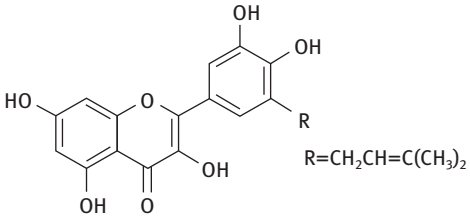
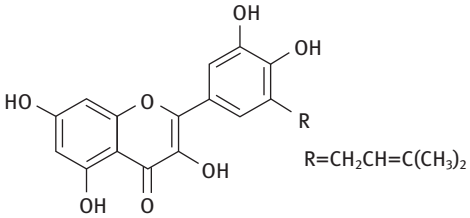
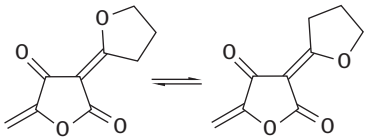
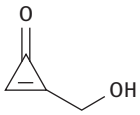
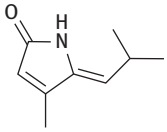
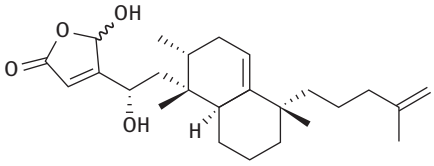
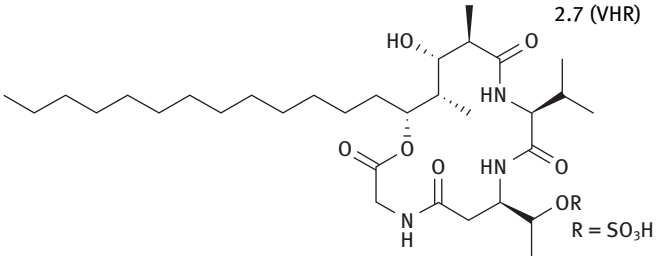
Inhibitor	Structure	IC_{50} (μM)
Quercetin		23.3 (PTP1B)
Uralenol		21.5 (PTP1B)
Broussochalcone A		36.8 (PTP1B)
Dihydrocarolic acid		1.2 (CD45) 38.0 (PTP1B)
Penitricin D		2.3 (CD45) 15.8 (PTP1B)
Pulchellalactam		124 (CD45)
Dysidiolide		9.4 (Cdc25A)

Table 8.2: (*continued*)

Inhibitor	Structure	IC_{50} (μM)
Stevasteline A		2.7 (VHR)

[a]Chemical structures adapted from Ref [20]

show specificity for CD45 with lower IC_{50} value of 1.2 μM and 2.3 μM respectively, as opposed to PTP1B, who they inhibit with IC_{50} value of 38.0 μM and 15.8 μM, respectively [72]. Inhibitors selective for CD45 over PTP1B also include the lactam-based inhibitor pulchellalactam [73, 74].

Dysidiolide is a compound extracted from the marine sponge *Dysidea etheria* that is reported to be specific for the Class III Cdc25 protein tyrosine phosphatases [75]. Dysidiolide is reported to be effective in reducing the growth of lung carcinoma A549 cell lines and the murine P388 leukemia cell lines. Stevastelines are a kind of depsipeptides that are known to be inhibitors of the dual-specificity phosphatases, including VHR. Stevastelines are reported to be immuno-suppressants that modulate human lymphocyte blastogenesis by serving as VHR inhibitors with IC_{50} value of 2.7 μM *in vitro* [76, 77]. Tanshinones are diterpene pigments found in the roots of mint-family perennial plants. Three types of tanshinones, namely isotanshinone, dihydroisotanshinone and isocryptotanshinone are reported to be PTP1B inhibitors and are selective for PTP1B over VHR [78, 79].

8.2 Natural Product analogues as protein tyrosine phosphatase inhibitors

Generation of analogues of natural products is a popular theme for inhibitor design and drug discovery. Among these analogues are multi-cyclic aryl compounds 1,2-naphthalenediones and 9,10-phenanthrenediones that reversibly inhibit CD45 [23], indolyldihydroxyquinone derivatives that inhibit Cdc25 phosphatases [80] and benzbromarone that inhibits PTP1B with an IC_{50} value of 23.0 μM [81]. α-ketocarboxylic acids (such as phenylglyoxylic acid) are reported to be inhibitors of the *Yersinia* protein tyrosine phosphatase but owing to their small size are weak inhibitors with IC_{50} value of 2.7 mM. These are used for designing peptide-based ketocarboxylic acid

derivatives that can achieve higher protein tyrosine phosphatase inhibition reaching IC_{50} value of 15.0 μM [82]. Oxaloaminobenzoic acid is a potent protein tyrosine phosphatase inhibitor and is the minimal phenyl phosphate memetic used to make further analogues [83]. Oxamic acid-based derivatives of oxalyaminobenzoic acid have been used to generate inhibitors selective for PTP1B over TCPTP and also CD45, SHPs, LAR and Cdc25 phosphatases (Figure 8.3) [83].

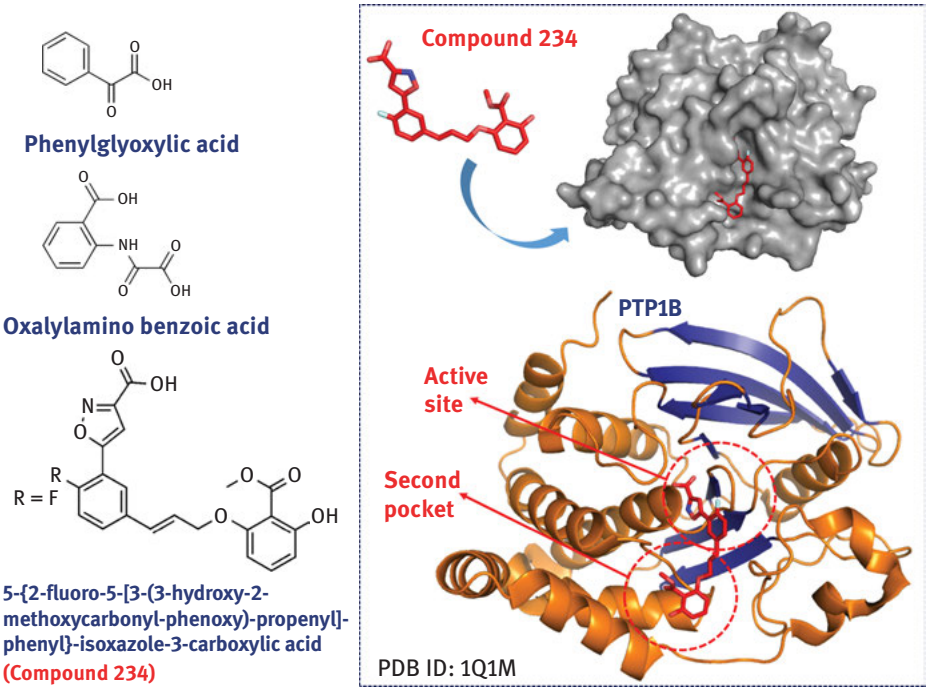


Figure 8.3: Mode of protein tyrosine phosphatase inhibition by an analogue (Compound 234) derived from natural inhibitor precursor phenylglyoxylic acid.

TU-572 is another natural product analogue that is reported to be a potent protein tyrosine phosphatase inhibitor [25]. TU-572 is reported to impede the release of histamine from rat peritoneal mast cells and inhibit hypersensitivity. TU-572 is a derivative of benzimidazole of microbial origin and is the short form for 2-[(4-methylthiopyridin-2-yl)methylsulfinyl]-5-isopropoxybenzimidazole. TU-572 is specific for CD45 inhibition and is reported to select CD45 over LAR, PTP1B and VHR [84]. Although its mode of interaction with CD45 protein is unclear, it is suggested that it does not involve irreversible covalent bond formation with the active site cysteine.

8.3 Reversible orthosteric inhibitors of protein tyrosine phosphatases

Reversible inhibitor use non-covalent interactions to bind to enzymes and typically have fast association and dissociation rates. Reversible inhibitors are either competitive, the ones that bind to occlude the substrate from the active site or non-competitive or inhibitors that bind the enzyme:substrate complex [85]. Reversible competitive inhibitors of protein tyrosine phosphatases target their charged active sites and are usually aryl derivatives that use their ring structures to navigate through the active site cleft of these proteins. For example, 2-(benzothiazol-2-ylamino)-2-oxo-1-phenylethanesulfonic acid has been identified from α-sulfophenylacetic amide (SPAA) derivative-based library screen has been identified as a potent, highly selective inhibitor of Class II low molecular weight phosphatases [86]. The high selectivity of the compound for LMPTP over the Class I protein tyrosine phosphatases is attributed to the distinct electrostatic potential of the active site surface and differences in the signature motif. More importantly, LMPTP is seen to accommodate the inhibitor α-phenyl ring using an induced-fit mechanism to generate a hydrophobic pocket near the active site (Figure 8.4). Low micromolar concentrations of 2-(benzothiazol-2-ylamino)-2-oxo-1-phenylethanesulfonic acid is reported to increase the phosphorylation of Protein Kinase B in response to insulin signaling in the human HepG2 hepatocytes [86, 87]. Fragment-

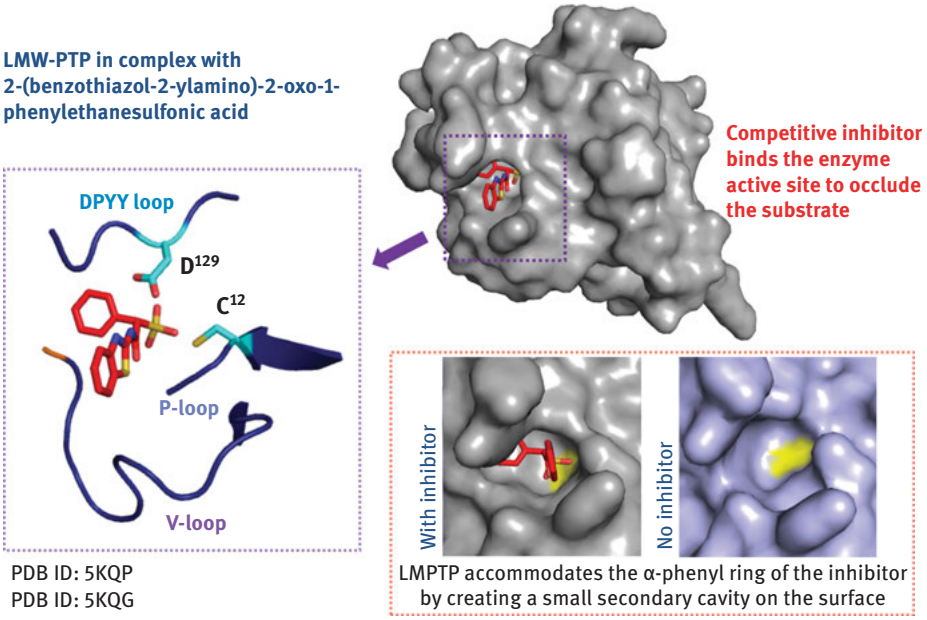


Figure 8.4: Competitive mode of enzyme inhibition: Class II LMPTP inhibited by 2-(benzothiazol-2-ylamino)-2-oxo-1-phenylethanesulfonic acid.

based inhibitor design using the SPAA library has also identified an inhibitor that is specific to the *Mycobacterium tuberculosis* LMPTP [88]. This inhibitor known as L335-M34 is selective for the bacterial LMPTP over the human isoform and only targets to inhibit signaling by the parasitic phosphatase. L335-M34 is reported to be bioavailable and effective in lower lung inflammation when administered in combination with other antituberculosis agents in guinea pig models [89].

Orthosteric uncompetitive inhibitors of LMPTP have been obtained from quinolone-based series of inhibitors using screens that use V_{max} levels of the LMPTP substrate 3-O-methylfluorescein phosphate (OMFP) [90]. These inhibitors and their optimized version have been shown to be specific for class II LMPTP over the classic class I protein tyrosine phosphatases and are reported to enhance insulin receptor-mediated tyrosine phosphorylation in the liver. Biophysical experiments using isothermal titration calorimetry (ITC), NMR spectroscopy and also X-ray crystallography have provided evidence for the uncompetitive inhibition mode of these inhibitors. Binding of these inhibitors to the apo LMPTP is weak and entropy driven, which is switched to a stronger binding using an enthalpic drive under conditions saturating the phosphatase with orthovanadate ions. 2-(4-([3-(piperidin-1-yl)propyl]amino)quinolin-2-yl) benzonitrile essentially binds the enzyme:substrate complex in the phosphocysteine intermediate form to block the hydrolysis of the reaction intermediate and locks the phosphatase in an inactive form (Figure 8.5). These inhibitors have shown remarkable specificity for LMPTP, oral bioavailability and are reported to reverse high-fat diet-induced diabetes in animal models.

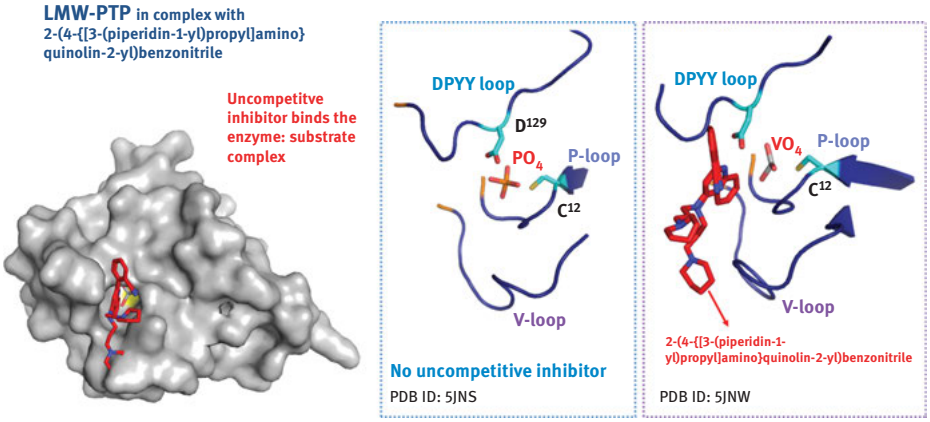


Figure 8.5: Uncompetitive mode of enzyme inhibition: Class II LM-PTP inhibited by 2-(4-{[3-(piperidin-1-yl)propyl]amino} quinolin-2-yl) benzonitrile.

A reversible competitive orthosteric inhibitor of protein tyrosine phosphatases, AKB-9778 has recently been under clinical trials for therapeutics for diabetic macular edema

(DME). AKB-9778 was originally identified as a phosphotyrosine mimetic from a phenylsulfamic acid core of Proctor & Gamble Pharmaceuticals [91]. Improvement in drug design has allowed for the development of this inhibitor that targets the vascular endothelial protein tyrosine phosphatase (VE-PTP or PTPβ) with picomolar efficiency (IC_{50} = 17.0 pM) [92]. The specificity of AKB-9778 for its target protein tyrosine phosphatase is substantiated by three biological evidences: (1) Treatment of adult mice with AKB-9778 increases the phosphorylation status of angiopoietin receptor 2 (Tie2) and results in decreased vascular leakage and neovascularization [93, 94], thereby mimicking the phenotype of inducible VE-PTP deletion in mice models; (2) AKB-9778 administration mimics the effect of use of VE-PTP-specific antibodies in mice models; and (3) AKB-9778 is reported to inhibit vascular leakage equally in healthy mice and mice knockouts of DEP-1 protein tyrosine phosphatase, hence emphasizing the selectivity.

CPT-157633 is reported to be a PTP1B inhibitor and has been used to explore relief from Rett Syndrome [95]. PTP1B is reported to be overexpressed in patients with Rett Syndrome due to the mutations in its transcription regulator MECP2 [96]. CPT-157633 is a competitive inhibitor that targets the active site of PTP1B and occludes the binding of its substrate proteins. Administration of CPT-157633 is reported to increase the phosphorylation levels of tropomyosin receptor kinase B (TRKB) in wild type and *MECP2*$^{-/+}$ mice models. PTP1B substrate-trapping mutants have identified TRKB as a physiological tyrosine phosphatase substrate in the brain and augmentation of this pathway via PTP1B is fast emerging as an efficient therapeutic strategy [97].

LTV-1 has been identified as an efficient inhibitor for the lymphoid tyrosine phosphatase Lyp (or PTPN22) in a screen using small molecule substrates [98]. LTV-1 interacts with P-loop active site cysteine and the nearby hydrophobic pocket of Lyp in the WPD-loop open conformation. LTV-1 shows moderate selectivity for Lyp over PTP1B and TCPTP but is effective in binding to Lyp over other PEST-PTPs. The role of LTV-1 has been explored in resetting impaired immune cell tolerance in mice models of autoimmunity that utilize human hematopoietic stem cells grafted on NSG-knockout mice [99].

8.4 Irreversible inhibitors of protein tyrosine phosphatases

The design of irreversible inhibitor relies on the chemical fragility of the active site of cysteine and its susceptibility to oxidation. Selenic acid-based inhibitors have been shown to be successful in covalently binding and inactivating the active site cysteines of protein tyrosine phosphatases. The crystal structure of PTP1B with (4-((2S)-2-[(tert-butoxycarbonyl)amino]-3-methoxy-3-oxopropyl)phenyl)methaneseleninic acid can be used to visualize the seleno-sulfide bond between the irreversible inhibitor and Cys215 of PTP1B [100]. The WPD-loop is seen in the open conformation and the inhibitor is seen to make additional interactions with the main-chain atoms of Gly220 and Arg221 of the P-loop and also the phenyl ring of Tyr46 of the phosphotyrosine-recognition loop (Figure 8.6).

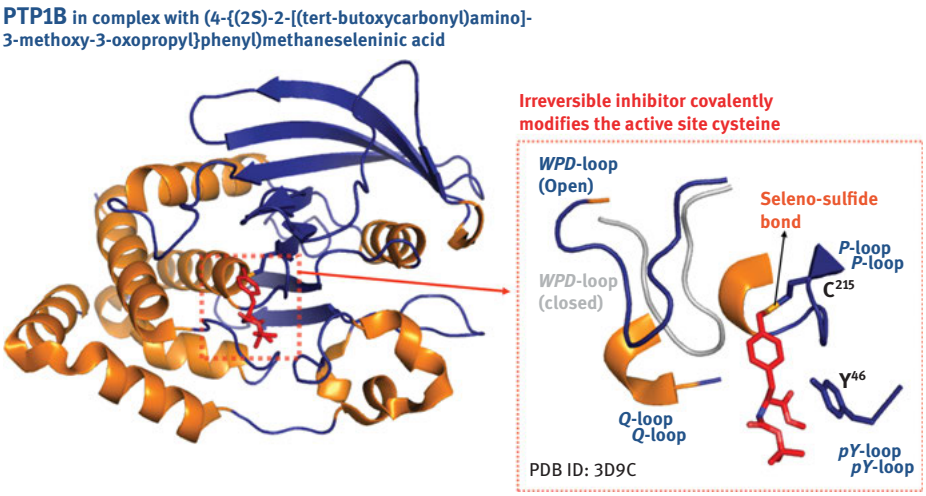


Figure 8.6: Irreversible inhibition of protein tyrosine phosphatases: PTP1B in a dead-end complex with (4-{(2S)-2-[(tert-butoxycarbonyl)amino]-3-methoxy-3-oxopropyl}phenyl)methaneseleninic acid.

STEP protein tyrosine phosphatase is a promising target for Alzheimer's disease and its deletion in mice models of the diseases is reported to improve cognition in these animals [101, 102]. An irreversible inhibitor, TC-2153 was serendipitously discovered from a sulfur-contaminated library of STEP inhibitors and is suggested to bind irreversibly with active site Cys472 of STEP to lock it in the inactive state [103]. TC-2153 has been reported to be a potent antianxiolytic and anticonvulsant in mice and also improves memory and object recognition in mice models of Alzheimer's disease. Administration of TC-2153 increases the phosphorylated levels of Erk 1/2 and PTK 2β (Pyk2) protein kinases and also the glutamate NMDA receptor subunit 2B [101].

Irreversible inhibitors have also been explored for the class III Cdc25 protein tyrosine phosphatases. Collective inhibition of Cdc25A/B/C is reported to be promising strategy for cancer therapy [104]. Discovery of quinone-based BN82685 compound has paved the way for designing of a more efficient IRC-083864 heterocyclic *bis*-quinone inhibitor of the Cdc25 phosphatases [105]. Both BN82685 and IRC-083864 are suggested to deactivate Cdc25 phosphatases by modulating and/or oxidizing their active site cysteine residue. Success of these inhibitors has been reported in inhibiting the growth of primary acute myeloid leukemia and prostrate carcinomas [104, 106].

8.5 Bidentate inhibitors of protein tyrosine phosphatases

The bidentate inhibitor employs a strategy of targeting the conserved protein tyrosine phosphatase active site in concert with an adjacent less conserved and hence protein-specific pocket. This strategy is rooted in the specificity of protein tyrosine phosphatases

for sequences that flank the incoming substrate phosphotyrosine for an efficient turnover [107]. The most crucial push for this approach has been provided by the uncovering of second aryl-binding site in PTP1B that allows the protein to bind two phosphotyrosine residues that are present in tandem in a given substrate sequence [108]. This pocket was reported to be specific to PTP1B and promoted the idea of design of bivalent inhibitors that could simultaneously target the adjacent pockets of the PTP1B surface [42]. The active site is targeted using non-hydrolysable phosphotyrosine mimics such as phosphonodifluoromethyl phenylalanine (F_2Pmp) (Figure 8.7) [109]. F_2Pmp is a small molecule that has low affinity for the active site of protein tyrosine phosphatases (0.55mM for PTP1B and 11.2mM for PTP-MEG2). Using a lysine linker to fuse F_2Pmp to a 3-bromo-4-methylbenzoic amide group generates a compound with high selectivity for PTP1B and an IC_{50} of 120nM [110] (Inhibitor 1, Figure 8.7). In addition, this compound is ineffective in inhibiting PTP-MEG2 even at high concentrations of 20μM. Accordingly, using an ornithine linker to attach a 3-iodobenzoic amide group to F_2Pmp creates an inhibitor that is specific for PTP-MEG2 (IC_{50} = 0.9 μM) (Inhibitor 2, Figure 8.7) [111]. Similarly, this approach of using chemically modified F_2PMP as a core for development of bivalent inhibitors has been used for TCPTP (Inhibitor 3, Figure 8.7) [112]. Improved bidentate inhibitors of TCPTP and PTP-MEG2 (Inhibitors 3 and 4, Figure 8.7) have also shown biological efficacy. Intracere-broventricular administration of the TCPTP inhibitor is reported to enhance leptin phosphorylation levels at Tyr705 in mice models [110, 113]. PTP-MEG2 inhibitor has been reported to improve insulin sensitivity in diet-induced obese mice [111].

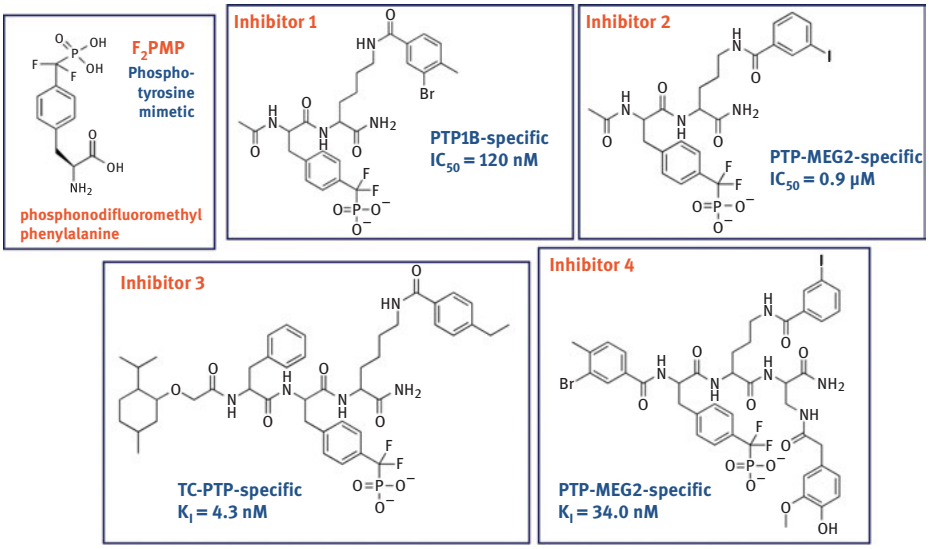


Figure 8.7: Phosphonodifluoromethyl phenylalanine (F_2PMP) is a phosphotyrosine mimetic that serves as precursor for generation of protein tyrosine phosphatase-specific inhibitors. Chemical structures adapted from Ref. [20].

The exploration of phosphotyrosine mimetics as active site inhibitors allowed for the discovery of natural agents that could serve as protein tyrosine phosphatase inhibitors. Salicylic acid was found to be a potent inhibitor of protein tyrosine phosphatases and its napthyl and polyaryl derivatives were successful in achieving protein selectivity [114, 115]. Bicyclic salicylic acid-based inhibitors have been shown to bind and block various protein tyrosine phosphatases, including Lyp [116]. The 6-hydroxy-benzofuran-5-carboxylic acid moiety of inhibitor is seen to bind into the active site of the protein and the inhibitor is reported to show a ninefold selectivity for Lyp over other protein tyrosine phosphatases. 3-[(3-chlorophenyl)ethynyl]-2-(4-[2-(cyclopropylamino)-2-oxoethoxy]phenyl)-6-hydroxy-1-benzofuran-5-carboxylic acid binds Lyp with a K_i = 110nM and uses its entire surface to access the peripheral pockets of the protein (Figure 8.8). Salicylic acid-based bidentate II-B08 was identified as a potent inhibitor of SHP2 with an IC_{50}=5.5 μM [117]. The compound has proven to be effective in SHP2 inhibition in mast cell leukemia and xenograft models of lung cancer [118, 119]. II-B08 has been developed into a second-generation inhibitor that inhibits SHP2 with an IC_{50}=220nM and shows antitumor activity [120, 121].

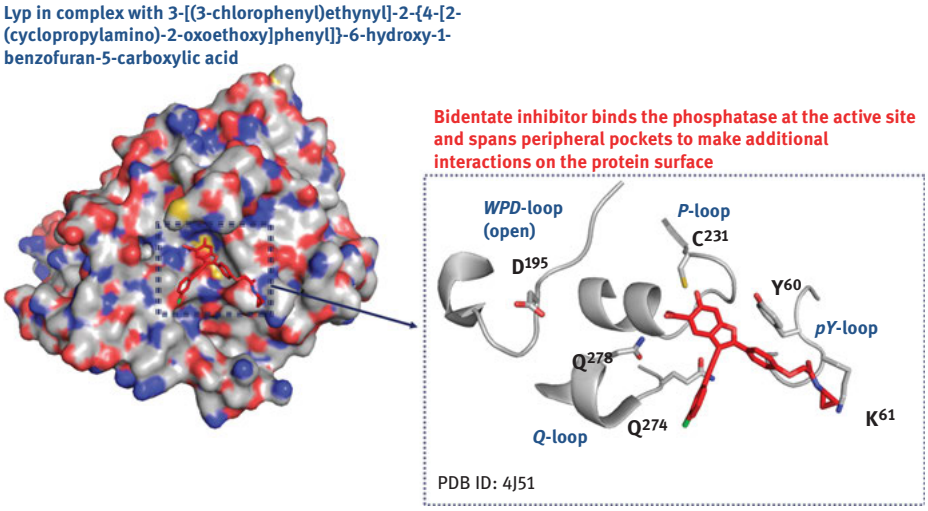


Figure 8.8: Bidentate inhibitors block the active site in conjunction with a peripheral site of the surface of protein tyrosine phosphatases.

8.6 Allosteric inhibitors of protein tyrosine phosphatases

Allosteric inhibitors target specific pockets on the protein tyrosine phosphatase to bind and lock the protein into a catalytically incompetent conformation. One of the earliest reports of using an allosteric inhibitor for controlling protein tyrosine phosphatases involves the benzofuran sulfonamide derivative of benzbromarone that allowed

exploring the possibility of blocking the closing of the WPD-loop of PTP1B [81, 122]. Elaborate efforts by the scientific community to harness the allosteric pocket of PTP1B has now led to the design of inhibitor MSI-1436 that is presently in phase I clinical trial as a potent therapeutic for metastatic breast cancer. MSI-1436 was initially discovered as an appetite suppressant in mice and was later known to target PTP1B at its disordered C-terminal region [123, 124]. MSI-1436 binds PTP1B in a reversible noncompetitive manner near the C-terminal α9′ to induce a conformational change in the protein leading to inactivation. This conformational change has been studied by biophysical tools, including fluorescence energy transfer, NMR spectroscopy, isothermal calorimetry and also protease sensitivity measurements. MSI-1436 shows an IC_{50} = 600nM for PTP1B and is tenfold selective for PTP1B over its most similar TCPTP. It is also about thirtyfold selective for PTP1B over CD45, PTP-PEST and LAR proteins. Potential of MSI-1436 is also being explored for the cure of Duchene muscular dystrophy where PTP1B inhibition could allow for skeletal muscle regeneration [125].

The SHP2 allosteric inhibitor SHP099 has shown potential in slowing growth of hematopoietic cancer cells dependent on JAK signaling [126, 127]. SHP099 has been improved from the initial hit of SHP836 that binds a novel allosteric pocket formed at the interface of its C-terminal SH2 and the protein tyrosine phosphatase domain and stabilizes the enzyme in an autoinhibited closed inactive conformation (Figure 8.9). The inhibitor is specific for SHP2 with an IC_{50} = 71nM and is reported to not cross-reacting with about 66 other protein tyrosine phosphatases. The inhibitor is also shown to be bioavailable upon oral administration and a dosage of 75–100 mg/kg p.o. mice for 10 days is shown to decrease tumor cell growth in xenograft models.

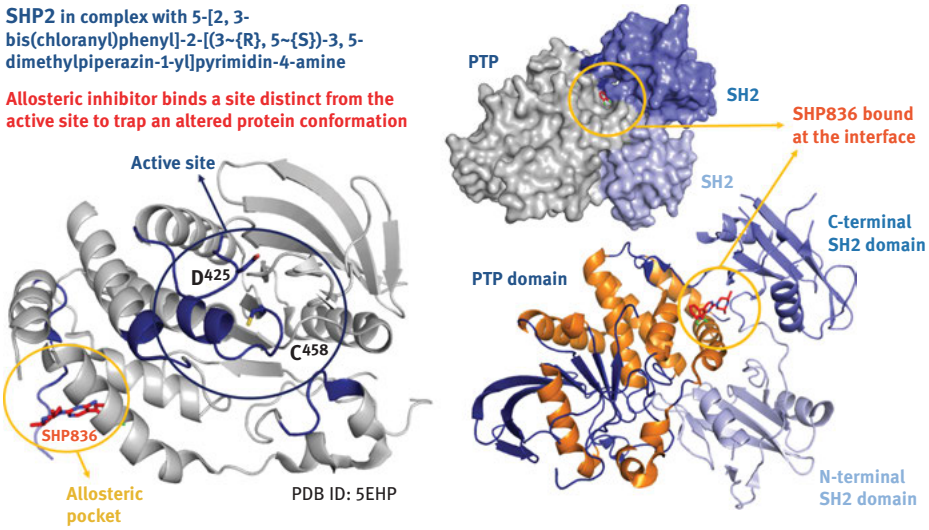


Figure 8.9: Allosteric inhibitors target pockets specific to a certain protein tyrosine phosphatase to stabilize a distinct protein conformation.

A CD45 allosteric inhibitor is reported to use the interface of its D1 and D2 protein tyrosine phosphatase domains in the double domain protein [128]. The inhibitor known as compound 211 was discovered from a 120,000 compound screen using various structure analysis tools, including circular dichroism. The compound is suggested to bind irreversibly in a noncompetitive way in the grove created by the interface of the two domains of CD45. Conformational change is seen in CD45 but not in LAR in the presence of this allosteric inhibitor. Administration of the compound is reported to reduce inflammation in hypersensitive mouse models and increase in the phosphorylation levels of LCK in Jurkat cells.

An allosteric inhibitor of dual-specificity phosphatase DUSP6 has been identified from 5,000 diverse compounds using zebrafish as the model [129]. The compound BCI inhibits DUSP6 by predictive binding to a crevice near helix α7 to prevent the positioning of the general acid/base Asp262 required for its catalytic cycle. This inhibitor-binding mode to DUSP6 has been predicted by docking studies and requires further structural validation [130]. The compound is reported to be efficacious in inducing death of cancer cells (IC_{50} = 2.1 μM) and promotes growth factor signaling in zebrafish embryos [131].

A unique inhibitor of the phosphatase of the regenerating liver (PRLs) is reported to work by interfering with their oligomerization [132]. Compound 43 inhibits the trimerization of PRLs but does not directly inhibit its catalytic activity. This inhibition of PRL trimerization is enough for reducing the migration and viability of cancer cells and affects their metastasis [133, 134]. Administration of this compound to mice melanoma xenograft models blocked tumor growth and was well tolerated by these mice after even 3 weeks of daily dosage.

8.7 Biologics-based therapeutic intervention of protein tyrosine phosphatases

Two biologic-based approaches have been especially useful for targeting PTPσ that plays a crucial role in recovery after spinal cord injury and rheumatoid arthritis [135, 136]. PTPσ uses its extracellular domains to interact with the components of the extracellular matrix, including chondroitin sulfate proteoglycans (CSPG) and heparin sulfate proteoglycans (HSPG) in a "proteoglycan switch" [137]. In this switching mechanism, the transmembrane HSPG bind the receptor PTPσ to sequester them away from sites of axonal growth into oligomeric clusters that occupy certain microdomains on the cell surface. This uneven distribution of the receptor protein tyrosine phosphatase allows for enhanced tyrosine phosphorylation levels at axon growth sites that require a signal to promote neuronal extension (Figure 8.10). Alternatively, binding of the secreted CSPG competes with HSPG to bind PTPσ and release their oligomerization, thereby inhibiting neuronal growth by evenly distributing their phosphotyrosine phosphatase activity.

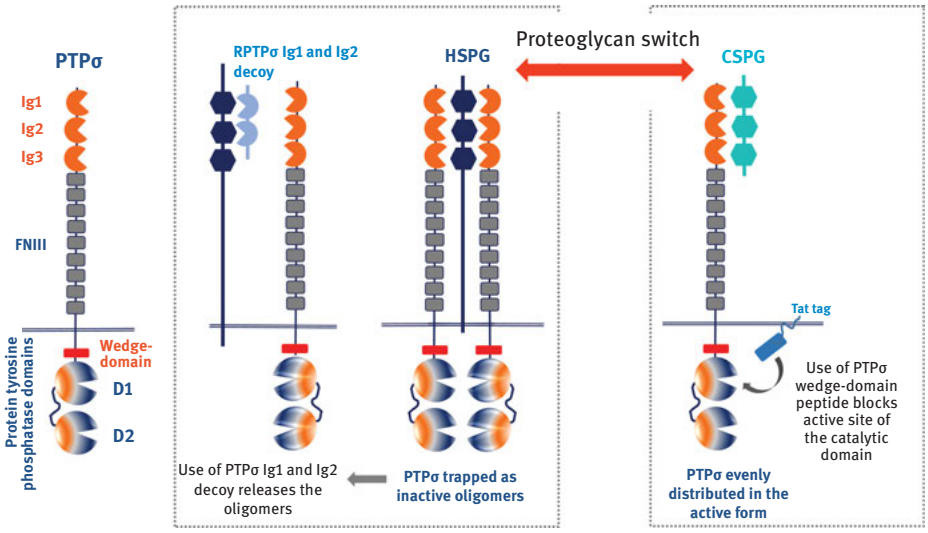


Figure 8.10: Harnessing the "proteoglycan switch" of PTPσ for biologics-based therapeutics.

PTPσ is expressed constitutively in the joint-lining cells called the fibroblasts-like synoviocytes (FLS) where it is a target for therapeutics directed toward rheumatoid arthritis [136]. FLS cells are reported to be hyper activated in rheumatoid arthritis where they signal destruction of the joints by mediating inflammation. On the surface of these synoviocytes, PTPσ interacts with the proteoglycans present in the extracellular matrix. The most prominent is its interaction with HSPG syndecan-4. Constitutive interaction between syndecan-4 and PTPσ maintains PTPσ in inactive oligomers that are sequestered away from its substrate protein ezrin. As ezrin is maintained in its phosphorylated state (tyrosine phosphorylation by the PDGF-receptor tyrosine kinase), it interacts with the cytoskeleton to promote cell invasion. Targeting this pathway with as low as 20nM of RPTPσ-Ig1 and Ig2 decoy protein has allowed for potential therapeutic intervention. The RPTPσ-Ig1 and Ig2 decoy protein competes with the functional PTPσ from the syndecan-4:PTPσ complex and allows its displacement to reach ezrin and dephosphorylate it. Dephosphorylation of ezrin is sufficient to stall cell invasiveness and migration. Treatment using RPTPσ-Ig1and Ig2 decoy protein has been shown to be effective in reversing arthritis symptoms in the K/BxN serum-transfer model. In addition, studies on mouse xenograft models have shown the potential of RPTPσ-Ig1 and Ig2 decoy protein in blocking FLS invasion in the cartilage in reversing symptoms of rheumatoid arthritis. Very encouragingly, RPTPσ-Ig1 and Ig2 decoy protein did not show any effect on PTPσ-null mouse models of rheumatoid arthritis or PTPσ-null FLS cells, thus indicating its high target specificity.

CSPG are reported to be more abundant in scar tissue, and their binding to PTPσ induces an inhibitory signal that stops axon regeneration. Manipulation of PTPσ activity in this scenario forms the basis of therapeutic intervention for recovery from

spinal cord injury. In animal models of spinal cord injury, introducing a cell permeable wedge-domain peptide has allowed recuperation of these animals [135, 138]. Animals exhibit restoration of innervation to the spinal cord and also recovery of locomotor and urinary functions. Wedge-domain peptide treatment of cultured sensory neurons has allowed reduced CSPG-mediated inhibition of axon regeneration by direct inhibition of PTPσ. Peptide treatment is seen to mimic the PTPσ knockout phenotype showing axons penetrating the chondroitin sulfate-rich glial scars [139]. Inspiration for wedge-domain peptides stems from structural studies on PTPα and LAR that exhibited a potential for inhibition via a homophilic interaction with peptides designed around the N-terminal "wedge" of their membrane proximal D1 domains (Figure 8.10) [28]. The interaction between the wedge peptide and protein target is reported to be highly specific, wherein LAR is only inhibited by the wedge of LAR itself and not that of PTPμ. The mechanistic working of the peptide is attributed to the presence of a D1 domain-specific cleft in the double domain receptor protein tyrosine phosphatases [140]. Recently, the therapeutic potential of these peptides has also been explored in animal models of myocardial infarction [141]. The administration of PTPσ-based wedge peptides (called intracellular sigma peptide or ISP) beginning 3 days after myocardial injury showed a marked decrease in arrhythmia susceptibility and promoted innervation of the damaged cardiac tissue.

8.8 Concluding remarks

Protein tyrosine phosphatases remain a lucrative and challenging drug target for modulation of protein phosphorylation-based signaling pathways. The past few years have seen substantial progress in the scientific community overcoming the challenges posed by the conserved nature of the active site of protein tyrosine phosphatase to achieve protein selectivity. Successful strategies have employed protein-specific determinants to target distinctive exploratory methods in inhibitor design. Molecular knowledge of the working of each protein tyrosine phosphatase has hence emerged to be of utmost importance. The more challenging field of achieving protein tyrosine phosphatase activation by small molecules remains unconquered for use for therapeutic benefit. As more studies find their way to understanding the working of these enzymes, more avenues will open up for their molecular manipulation and strategic interplay.

References

[1] Soulsby M, Bennett AM. Physiological signaling specificity by protein tyrosine phosphatases. Physiology (Bethesda). 2009;24:281–9.

[2] Czernilofsky AP, Levinson AD, Varmus HE, Bishop JM, Tischer E, Goodman HM. Nucleotide sequence of an avian sarcoma virus oncogene (src) and proposed amino acid sequence for gene product. Nature. 1980;287(5779):198–203.

[3] Guan KL, Haun RS, Watson SJ, Geahlen RL, Dixon JE. Cloning and expression of a protein-tyrosine-phosphatase. Proc Natl Acad Sci. 1990;87(4):1501–5.
[4] Wellcome Trust Case Control C. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007;447(7145):661–78.
[5] Tautz L, Pellecchia M, Mustelin T. Targeting the PTPome in human disease. Expert Opin Ther Targets. 2006;10(1):157–77.
[6] Easty D, Gallagher W, Bennett DC. Protein tyrosine phosphatases, new targets for cancer therapy. Curr Cancer Drug Targets. 2006;6(6):519–32.
[7] Ostman A, Hellberg C, Bohmer FD. Protein-tyrosine phosphatases and cancer. Nat Rev Cancer. 2006;6(4):307–20.
[8] Wang Z, Shen D, Parsons DW, Bardelli A, Sager J, Szabo S, et al. Mutational analysis of the tyrosine phosphatome in colorectal cancers. Science. 2004;304(5674):1164–6.
[9] Muise AM, Walters T, Wine E, Griffiths AM, Turner D, Duerr RH, et al. Protein-tyrosine phosphatase sigma is associated with ulcerative colitis. Curr Biol. 2007;17(14):1212–8.
[10] Stanford SM, Mustelin TM, Bottini N. Lymphoid tyrosine phosphatase and autoimmunity: human genetics rediscovers tyrosine phosphatases. Semin Immunopathol. 2010;32(2):127–36.
[11] Bottini N, Musumeci L, Alonso A, Rahmouni S, Nika K, Rostamkhani M, et al. A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. Nat Genet. 2004;36(4):337–8.
[12] Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL, et al. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. Science. 1999;283(5407):1544–8.
[13] Blaskovich MA. Drug discovery and protein tyrosine phosphatases. Curr Med Chem. 2009;16 (17):2095–176.
[14] Julien SG, Dube N, Read M, Penney J, Paquet M, Han Y, et al. Protein tyrosine phosphatase 1B deficiency or inhibition delays ErbB2-induced mammary tumorigenesis and protects from lung metastasis. Nat Genet. 2007;39(3):338–46.
[15] Bentires-Alj M, Neel BG. Protein-tyrosine phosphatase 1B is required for HER2/Neu-induced breast cancer. Cancer Res. 2007;67(6):2420–4.
[16] Liu G. Technology evaluation: ISIS-113715, Isis. Curr Opin Mol Ther. 2004;6(3):331–6.
[17] Zinker BA, Rondinone CM, Trevillyan JM, Gum RJ, Clampit JE, Waring JF, et al. PTP1B antisense oligonucleotide lowers PTP1B protein, normalizes blood glucose, and improves insulin sensitivity in diabetic mice. Proc Natl Acad Sci U S A. 2002;99(17):11357–62.
[18] Iversen LF, Moller KB, Pedersen AK, Peters GH, Petersen AS, Andersen HS, et al. Structure determination of T cell protein-tyrosine phosphatase. J Biol Chem. 2002;277(22):19982–90.
[19] You-Ten KE, Muise ES, Itie A, Michaliszyn E, Wagner J, Jothy S, et al. Impaired bone marrow microenvironment and immune function in T cell protein tyrosine phosphatase-deficient mice. J Exp Med. 1997;186(5):683–93.
[20] Heneberg P. Use of protein tyrosine phosphatase inhibitors as promising targeted therapeutic drugs. Curr Med Chem. 2009;16(6):706–33.
[21] McNeill L, Salmond RJ, Cooper JC, Carret CK, Cassady-Cain RL, Roche-Molina M, et al. The differential regulation of Lck kinase phosphorylation sites by CD45 is critical for T cell receptor signaling responses. Immunity. 2007;27(3):425–37.
[22] Lazarovits AI, Poppema S, Zhang Z, Khandaker M, Le Feuvre CE, Singhal SK, et al. Prevention and reversal of renal allograft rejection by antibody against CD45RB. Nature. 1996;380 (6576):717–20.
[23] Urbanek RA, Suchard SJ, Steelman GB, Knappenberger KS, Sygowski LA, Veale CA, et al. Potent reversible inhibitors of the protein tyrosine phosphatase CD45. J Med Chem. 2001;44(11): 1777–93.

[24] Hamaguchi T, Takahashi A, Kagamizono T, Manaka A, Sato M, Osada H. Synthesis and characterization of a potent and selective protein tyrosine phosphatase inhibitor, 2-[(4-Methylthiopyridin-2-yl)methylsulfinyl]benzimidazole 2000. 2657–60 p.
[25] Hamaguchi T, Takahashi A, Manaka A, Sato M, Osada H. TU-572, a potent and selective CD45 inhibitor, suppresses IgE-mediated anaphylaxis and murine contact hypersensitivity reactions. Int Arch Allergy Immunol. 2001;126(4):318–24.
[26] Huang P, Ramphal J, Wei J, Liang C, Jallal B, McMahon G, et al. Structure-based design and discovery of novel inhibitors of protein tyrosine phosphatases. Bioorg Med Chem. 2003;11 (8):1835–49.
[27] Yang X, Li J, Zhou Y, Shen Q, Chen J, Li J. Discovery of novel inhibitor of human leukocyte common antigen-related phosphatase. Biochim Biophys Acta. 2005;1726(1):34–41.
[28] Xie Y, Massa SM, Ensslen-Craig SE, Major DL, Yang T, Tisi MA, et al. Protein-tyrosine phosphatase (PTP) wedge domain peptides: a novel approach for inhibition of PTP function and augmentation of protein-tyrosine kinase function. J Biol Chem. 2006;281(24):16482–92.
[29] McCain DF, Wu L, Nickel P, Kassack MU, Kreimeyer A, Gagliardi A, et al. Suramin derivatives as inhibitors and activators of protein-tyrosine phosphatases. J Biol Chem. 2004;279 (15):14713–25.
[30] Nugent AA, Kolpak AL, Engle EC. Human disorders of axon guidance. Curr Opin Neurobiol. 2012;22(5):837–43.
[31] Schmidt A, Rutledge SJ, Endo N, Opas EE, Tanaka H, Wesolowski G, et al. Protein-tyrosine phosphatase activity regulates osteoclast formation and function: inhibition by alendronate. Proc Natl Acad Sci U S A. 1996;93(7):3068–73.
[32] Hao Q, Rutherford SA, Low B, Tang H. Suppression of the phosphorylation of receptor tyrosine phosphatase-alpha on the Src-independent site tyrosine 789 by reactive oxygen species. Mol Pharmacol. 2006;69(6):1938–44.
[33] van der Wijk T, Blanchetot C, Overvoorde J, den Hertog J. Redox-regulated rotational coupling of receptor protein-tyrosine phosphatase alpha dimers. J Biol Chem. 2003;278(16):13968–74.
[34] MacKeigan JP, Murphy LO, Blenis J. Sensitized RNAi screen of human kinases and phosphatases identifies new regulators of apoptosis and chemoresistance. Nat Cell Biol. 2005;7 (6):591–600.
[35] Burke TR, Jr., Ye B, Akamatsu M, Ford H, Jr., Yan X, Kole HK, et al. 4′-O-[2-(2-fluoromalonyl)]-L-tyrosine: a phosphotyrosyl mimic for the preparation of signal transduction inhibitory peptides. J Med Chem. 1996;39(5):1021–7.
[36] Chang HL, Sugimoto Y, Liu S, Ye W, Wang LS, Huang YW, et al. Keratinocyte growth factor (KGF) induces tamoxifen (Tam) resistance in human breast cancer MCF-7 cells. Anticancer Res. 2006;26(3A):1773–84.
[37] Liu S, Sugimoto Y, Kulp SK, Jiang J, Chang HL, Park KY, et al. Estrogenic down-regulation of protein tyrosine phosphatase gamma (PTP gamma) in human breast is associated with estrogen receptor alpha. Anticancer Res. 2002;22(6C):3917–23.
[38] Cho H, Krishnaraj R, Itoh M, Kitas E, Bannwarth W, Saito H, et al. Substrate specificities of catalytic fragments of protein tyrosine phosphatases (HPTP beta, LAR, and CD45) toward phosphotyrosylpeptide substrates and thiophosphotyrosylated peptides as inhibitors. Protein Sci. 1993;2(6):977–84.
[39] Koh CH, Qi RZ, Qu D, Melendez A, Manikandan J, Bay BH, et al. U18666A-mediated apoptosis in cultured murine cortical neurons: role of caspases, calpains and kinases. Cell Signal. 2006;18 (10):1572–83.
[40] Drake PG, Peters GH, Andersen HS, Hendriks W, Moller NP. A novel strategy for the development of selective active-site inhibitors of the protein tyrosine phosphatase-like proteins islet-cell antigen 512 (IA-2) and phogrin (IA-2beta). Biochem J. 2003;373(Pt 2):393–401.

[41] Kennewell P. Technology evaluation: ISIS-104838, OraSense. Curr Opin Mol Ther. 2003;5 (1):76–80.
[42] Shen K, Keng YF, Wu L, Guo XL, Lawrence DS, Zhang ZY. Acquisition of a specific and potent PTP1B inhibitor from a novel combinatorial library and screening procedure. J Biol Chem. 2001;276(50):47311–9.
[43] Leung C, Grzyb J, Lee J, Meyer N, Hum G, Jia C, et al. The difluoromethylenesulfonic acid group as a monoanionic phosphate surrogate for obtaining PTP1B inhibitors. Bioorg Med Chem. 2002;10(7):2309–23.
[44] Zhang W, Hong D, Zhou Y, Zhang Y, Shen Q, Li JY, et al. Ursolic acid and its derivative inhibit protein tyrosine phosphatase 1B, enhancing insulin receptor phosphorylation and stimulating glucose uptake. Biochim Biophys Acta. 2006;1760(10):1505–12.
[45] Malamas MS, Sredy J, Moxham C, Katz A, Xu W, McDevitt R, et al. Novel benzofuran and benzothiophene biphenyls as inhibitors of protein tyrosine phosphatase 1B with antihyperglycemic properties. J Med Chem. 2000;43(7):1293–310.
[46] Montalibet J, Skorey K, McKay D, Scapin G, Asante-Appiah E, Kennedy BP. Residues distant from the active site influence protein-tyrosine phosphatase 1B inhibitor binding. J Biol Chem. 2006;281(8):5258–66.
[47] Yue EW, Wayland B, Douty B, Crawley ML, McLaughlin E, Takvorian A, et al. Isothiazolidinone heterocycles as inhibitors of protein tyrosine phosphatases: synthesis and structure-activity relationships of a peptide scaffold. Bioorg Med Chem. 2006;14(17):5833–49.
[48] Fu H, Park J, Pei D. Peptidyl aldehydes as reversible covalent inhibitors of protein tyrosine phosphatases. Biochemistry. 2002;41(34):10700–9.
[49] Umezawa K, Kawakami M, Watanabe T. Molecular design and biological activities of protein-tyrosine phosphatase inhibitors. Pharmacol Ther. 2003;99(1):15–24.
[50] Cheon HG, Kim SM, Yang SD, Ha JD, Choi JK. Discovery of a novel protein tyrosine phosphatase-1B inhibitor, KR61639: potential development as an antihyperglycemic agent. Eur J Pharmacol. 2004;485(1–3):333–9.
[51] Xu J, Li L, Hong J, Huang W. Effects of small interference RNA against PTP1B and TCPTP on insulin signaling pathway in mouse liver: evidence for non-synergetic cooperation. Cell Biol Int. 2007;31(1):88–91.
[52] Xie J, Seto CT. A two stage click-based library of protein tyrosine phosphatase inhibitors. Bioorg Med Chem. 2007;15(1):458–73.
[53] Paling NR, Welham MJ. Role of the protein tyrosine phosphatase SHP-1 (Src homology phosphatase-1) in the regulation of interleukin-3-induced survival, proliferation and signalling. Biochem J. 2002;368(Pt 3):885–94.
[54] Chen L, Sung SS, Yip ML, Lawrence HR, Ren Y, Guida WC, et al. Discovery of a novel shp2 protein tyrosine phosphatase inhibitor. Mol Pharmacol. 2006;70(2):562–70.
[55] Yi T, Pathak MK, Lindner DJ, Ketterer ME, Farver C, Borden EC. Anticancer activity of sodium stibogluconate in synergy with IFNs. J Immunol. 2002;169(10):5978–85.
[56] Pazdrak K, Adachi T, Alam R. Src homology 2 protein tyrosine phosphatase (SHPTP2)/Src homology 2 phosphatase 2 (SHP2) tyrosine phosphatase is a positive regulator of the interleukin 5 receptor signal transduction pathways leading to the prolongation of eosinophil survival. J Exp Med. 1997;186(4):561–8.
[57] Opas EE, Rutledge SJ, Golub E, Stern A, Zimolo Z, Rodan GA, et al. Alendronate inhibition of protein-tyrosine-phosphatase-meg1. Biochem Pharmacol. 1997;54(6):721–7.
[58] Krishnamurthy D, Karver MR, Fiorillo E, Orru V, Stanford SM, Bottini N, et al. Gold(I)-mediated inhibition of protein tyrosine phosphatases: a detailed in vitro and cellular study. J Med Chem. 2008;51(15):4790–5.

[59] Carlucci A, Gedressi C, Lignitto L, Nezi L, Villa-Moruzzi E, Avvedimento EV, et al. Protein-tyrosine phosphatase PTPD1 regulates focal adhesion kinase autophosphorylation and cell migration. J Biol Chem. 2008;283(16):10919–29.
[60] Castiglioni S, Maier JA, Mariotti M. The tyrosine phosphatase HD-PTP: A novel player in endothelial migration. Biochem Biophys Res Commun. 2007;364(3):534–9.
[61] Fodero-Tavoletti MT, Hardy MP, Cornell B, Katsis F, Sadek CM, Mitchell CA, et al. Protein tyrosine phosphatase hPTPN20a is targeted to sites of actin polymerization. Biochem J. 2005;389(Pt 2):343–54.
[62] Imoto M, Kakeya H, Sawa T, Hayashi C, Hamada M, Takeuchi T, et al. Dephostatin, a novel protein tyrosine phosphatase inhibitor produced by Streptomyces. I. Taxonomy, isolation, and characterization. J Antibiot (Tokyo). 1993;46(9):1342–6.
[63] Peretz A, Gil-Henn H, Sobko A, Shinder V, Attali B, Elson A. Hypomyelination and increased activity of voltage-gated K(+) channels in mice lacking protein tyrosine phosphatase epsilon. EMBO J. 2000;19(15):4036–45.
[64] Kagamizono T, Hamaguchi T, Ando T, Sugawara K, Adachi T, Osada H. Phosphatoquinones A and B, novel tyrosine phosphatase inhibitors produced by Streptomyces sp. J Antibiot (Tokyo). 1999;52(2):75–80.
[65] Na M, Kim KA, Oh H, Kim BY, Oh WK, Ahn JS. Protein tyrosine phosphatase 1B inhibitory activity of amentoflavone and its cellular effect on tyrosine phosphorylation of insulin receptors. Biol Pharm Bull. 2007;30(2):379–81.
[66] Chen RM, Hu LH, An TY, Li J, Shen Q. Natural PTP1B inhibitors from Broussonetia papyrifera. Bioorg Med Chem Lett. 2002;12(23):3387–90.
[67] Carr G, Berrue F, Klaiklay S, Pelletier I, Landry M, Kerr RG. Natural products with protein tyrosine phosphatase inhibitory activity. Methods. 2014;65(2):229–38.
[68] Ahmad G, Mishra PK, Gupta P, Yadav PP, Tiwari P, Tamrakar AK, et al. Synthesis of novel benzofuran isoxazolines as protein tyrosine phosphatase 1B inhibitors. Bioorg Med Chem Lett. 2006;16(8):2139–43.
[69] Kuo RY, Chang FR, Chen CY, Teng CM, Yen HF, Wu YC. Antiplatelet activity of N-methoxycarbonyl aporphines from Rollinia mucosa. Phytochemistry. 2001;57(3):421–5.
[70] Montenegro H, Gutierrez M, Romero LI, Ortega-Barria E, Capson TL, Rios LC. Aporphine alkaloids from Guatteria spp. with leishmanicidal activity. Planta Med. 2003;69(7):677–9.
[71] Miski M, Xing S, Cooper R, Gillum AM, Fisher DK, Miller RW, et al. Aporphine alkaloids, CD45 protein tyrosine phosphatase inhibitors, from Rollinia ulei. Bioorg Med Chem Lett. 1995;5 (14):1519–22.
[72] Alvi KA, Nair BG, Rabenstein J, Davis G, Baker DD. CD45 tyrosine phosphatase inhibitory components from Aspergillus niger. J Antibiot (Tokyo). 2000;53(2):110–3.
[73] Alvi KA, Casey A, Nair BG. Pulchellalactam: a CD45 protein tyrosine phosphatase inhibitor from the marine fungus Corollospora pulchella. J Antibiot (Tokyo). 1998;51(5):515–7.
[74] Li WR, Lin ST, Hsu NM, Chern MS. Efficient total synthesis of pulchellalactam, a CD45 protein tyrosine phosphatase inhibitor. J Org Chem. 2002;67(14):4702–6.
[75] Gunasekera SP, McCarthy PJ, Kelly-Borges M, Lobkovsky E, Clardy J. Dysidiolide: novel protein phosphatase inhibitor from the caribbean sponge Dysidea etheria de Laubenfels. J Am Chem Soc. 1996;118(36):8759–60.
[76] Morino T, Masuda A, Yamada M, Nishimoto M, Nishikiori T, Saito S, et al. Stevastelins, novel immunosuppressants produced by Penicillium. J Antibiot (Tokyo). 1994;47(11):1341–3.
[77] Hamaguchi T, Masuda A, Morino T, Osada H. Stevastelins, a novel group of immunosuppressants, inhibit dual-specificity protein phosphatases. Chem Biol. 1997;4(4):279–86.
[78] Han YM, Oh H, Na M, Kim BS, Oh WK, Kim BY, et al. PTP1B inhibitory effect of abietane diterpenes isolated from Salvia miltiorrhiza. Biol Pharm Bull. 2005;28(9):1795–7.

[79] Lee S, Wang Q. Recent development of small molecular specific inhibitor of protein tyrosine phosphatase 1B. Med Res Rev. 2007;27(4):553–73.
[80] Sohn J, Kiburz B, Li Z, Deng L, Safi A, Pirrung MC, et al. Inhibition of Cdc25 phosphatases by indolyldihydroxyquinones. J Med Chem. 2003;46(13):2580–8.
[81] Wrobel J, Sredy J, Moxham C, Dietrich A, Li Z, Sawicki DR, et al. PTP1B inhibition and anti-hyperglycemic activity in the ob/ob mouse model of novel 11-arylbenzo[b]naphtho[2,3-d] furans and 11-arylbenzo[b]naphtho[2,3-d]thiophenes. J Med Chem. 1999;42(17):3199–202.
[82] Chen YT, Xie J, Seto CT. Peptidic alpha-ketocarboxylic acids and sulfonamides as inhibitors of protein tyrosine phosphatases. J Org Chem. 2003;68(10):4123–5.
[83] Liu G, Xin Z, Pei Z, Hajduk PJ, Abad-Zapatero C, Hutchins CW, et al. Fragment screening and assembly: a highly efficient approach to a selective and cell active protein tyrosine phosphatase 1B inhibitor. J Med Chem. 2003;46(20):4232–5.
[84] Hamaguchi T, Takahashi A, Kagamizono T, Manaka A, Sato M, Osada H. Synthesis and characterization of a potent and selective protein tyrosine phosphatase inhibitor, 2. Bioorg Med Chem Lett. 2000;10(23):2657–60.
[85] Ring B, Wrighton SA, Mohutsky M. Reversible mechanisms of enzyme inhibition and resulting clinical significance. Methods Mol Biol. 2014;1113:37–56.
[86] He R, Wang J, Yu ZH, Zhang RY, Liu S, Wu L, et al. Inhibition of low molecular weight protein tyrosine phosphatase by an induced-fit mechanism. J Med Chem. 2016.
[87] Caselli A, Paoli P, Santi A, Mugnaioni C, Toti A, Camici G, et al. Low molecular weight protein tyrosine phosphatase: Multifaceted functions of an evolutionarily conserved enzyme. Biochim Biophys Acta. 2016;1864(10):1339–55.
[88] Dutta NK, He R, Pinn ML, He Y, Burrows F, Zhang ZY, et al. Mycobacterial protein tyrosine phosphatases A and B inhibitors augment the bactericidal activity of the standard anti-tuberculosis regimen. ACS Infect Dis. 2016;2(3):231–9.
[89] He Y, Xu J, Yu ZH, Gunawan AM, Wu L, Wang L, et al. Discovery and evaluation of novel inhibitors of mycobacterium protein tyrosine phosphatase B from the 6-Hydroxy-benzofuran-5-carboxylic acid scaffold. J Med Chem. 2013;56(3):832–42.
[90] Stanford SM, Aleshin AE, Zhang V, Ardecky RJ, Hedrick MP, Zou J, et al. Diabetes reversal by inhibition of the low-molecular-weight tyrosine phosphatase. Nat Chem Biol. 2017;13(6):624–32.
[91] Klopfenstein SR, Evdokimov AG, Colson AO, Fairweather NT, Neuman JJ, Maier MB, et al. 1,2,3,4-Tetrahydroisoquinolinyl sulfamic acids as phosphatase PTP1B inhibitors. Bioorg Med Chem Lett. 2006;16(6):1574–8.
[92] Goel S, Gupta N, Walcott BP, Snuderl M, Kesler CT, Kirkpatrick ND, et al. Effects of vascular-endothelial protein tyrosine phosphatase inhibition on breast cancer vasculature and metastatic progression. J Natl Cancer Inst. 2013;105(16):1188–201.
[93] Shen J, Frye M, Lee BL, Reinardy JL, McClung JM, Ding K, et al. Targeting VE-PTP activates TIE2 and stabilizes the ocular vasculature. J Clin Invest. 2014;124(10):4564–76.
[94] Frye M, Dierkes M, Küppers V, Vockel M, Tomm J, Zeuschner D, et al. Interfering with VE-PTP stabilizes endothelial junctions in vivo via Tie-2 in the absence of VE-cadherin. J Exp Med. 2015;212(13):2267–87.
[95] Krishnan N, Krishnan K, Connors CR, Choy MS, Page R, Peti W, et al. PTP1B inhibition suggests a therapeutic strategy for Rett syndrome. J Clin Invest. 2015;125(8):3163–77.
[96] Lombardi LM, Baker SA, Zoghbi HY. MECP2 disorders: from the clinic to mice and back. J Clin Invest. 2015;125(8):2914–23.
[97] Ozek C, Kanoski SE, Zhang ZY, Grill HJ, Bence KK. Protein-tyrosine phosphatase 1B (PTP1B) is a novel regulator of central brain-derived neurotrophic factor and tropomyosin receptor kinase B (TrkB) signaling. J Biol Chem. 2014;289(46):31682–92.

[98] Vang T, Liu WH, Delacroix L, Wu S, Vasile S, Dahl R, et al. LYP inhibits T-cell activation when dissociated from CSK. Nat Chem Biol. 2012;8(5):437–46.
[99] Schickel JN, Kuhny M, Baldo A, Bannock JM, Massad C, Wang H, et al. PTPN22 inhibition resets defective human central B cell tolerance. Sci Immunol. 2016;1(1).
[100] Abdo M, Liu S, Zhou B, Walls CD, Wu L, Knapp S, et al. Seleninate in place of phosphate: irreversible inhibition of protein tyrosine phosphatases. J Am Chem Soc. 2008;130(40):13196–7.
[101] Xu J, Hartley BJ, Kurup P, Phillips A, Topol A, Xu M, et al. Inhibition of STEP61 ameliorates deficits in mouse and hiPSC-based schizophrenia models. Mol Psychiatry. 2018;23(2):271–81.
[102] Zhang Y, Kurup P, Xu J, Carty N, Fernandez SM, Nygaard HB, et al. Genetic reduction of striatal-enriched tyrosine phosphatase (STEP) reverses cognitive and cellular deficits in an Alzheimer's disease mouse model. Proc Natl Acad Sci U S A. 2010;107(44):19014–9.
[103] Xu J, Chatterjee M, Baguley TD, Brouillette J, Kurup P, Ghosh D, et al. Inhibitor of the tyrosine phosphatase STEP reverses cognitive deficits in a mouse model of Alzheimer's disease. PLoS Biol. 2014;12(8):e1001923.
[104] Brenner AK, Reikvam H, Lavecchia A, Bruserud O. Therapeutic targeting the cell division cycle 25 (CDC25) phosphatases in human acute myeloid leukemia – the possibility to target several kinases through inhibition of the various CDC25 isoforms. Molecules. 2014;19(11):18414–47.
[105] Brezak MC, Valette A, Quaranta M, Contour-Galcera MO, Jullien D, Lavergne O, et al. IRC-083864, a novel bis quinone inhibitor of CDC25 phosphatases active against human cancer cells. Int J Cancer. 2009;124(6):1449–56.
[106] Bertoli S, Boutzen H, David L, Larrue C, Vergez F, Fernandez-Vidal A, et al. CDC25A governs proliferation and differentiation of FLT3-ITD acute myeloid leukemia. Oncotarget. 2015;6 (35):38061–78.
[107] Zhang ZY. Protein tyrosine phosphatases: structure and function, substrate specificity, and inhibitor development. Annu Rev Pharmacol Toxicol. 2002;42:209–34.
[108] Puius YA, Zhao Y, Sullivan M, Lawrence DS, Almo SC, Zhang ZY. Identification of a second aryl phosphate-binding site in protein-tyrosine phosphatase 1B: a paradigm for inhibitor design. Proc Natl Acad Sci U S A. 1997;94(25):13420–5.
[109] Chen L, Wu L, Otaka A, Smyth MS, Roller PP, Burke TR, Jr., et al. Why is phosphonodifluoromethyl phenylalanine a more potent inhibitory moiety than phosphonomethyl phenylalanine toward protein-tyrosine phosphatases? Biochem Biophys Res Commun. 1995;216(3):976–84.
[110] Zhang ZY. Drugging the undruggable: therapeutic potential of targeting protein tyrosine phosphatases. Acc Chem Res. 2017;50(1):122–9.
[111] Zhang S, Liu S, Tao R, Wei D, Chen L, Shen W, et al. A highly selective and potent PTP-MEG2 inhibitor with therapeutic potential for type 2 diabetes. J Am Chem Soc. 2012;134(43):18116–24.
[112] Zhang S, Chen L, Luo Y, Gunawan A, Lawrence DS, Zhang ZY. Acquisition of a potent and selective TC-PTP inhibitor via a stepwise fluorophore-tagged combinatorial synthesis and screening strategy. J Am Chem Soc. 2009;131(36):13072–9.
[113] Loh K, Fukushima A, Zhang X, Galic S, Briggs D, Enriori PJ, et al. Elevated hypothalamic TCPTP in obesity contributes to cellular leptin resistance. Cell Metab. 2011;14(5):684–99.
[114] Sarmiento M, Wu L, Keng YF, Song L, Luo Z, Huang Z, et al. Structure-based discovery of small molecule inhibitors targeted to protein tyrosine phosphatase 1B. J Med Chem. 2000;43 (2):146–55.
[115] Liang F, Huang Z, Lee SY, Liang J, Ivanov MI, Alonso A, et al. Aurintricarboxylic acid blocks in vitro and in vivo activity of YopH, an essential virulent factor of Yersinia pestis, the agent of plague. J Biol Chem. 2003;278(43):41734–41.
[116] He Y, Liu S, Menon A, Stanford S, Oppong E, Gunawan AM, et al. A potent and selective small-molecule inhibitor for the lymphoid-specific tyrosine phosphatase (LYP), a target associated with autoimmune diseases. J Med Chem. 2013;56(12):4990–5008.

[117] Zhang X, He Y, Liu S, Yu Z, Jiang Z-X, Yang Z, et al. Salicylic acid based small molecule inhibitor for the oncogenic Src Homology-2 domain containing protein tyrosine phosphatase-2 (SHP2). J Med Chem. 2010;53(6):2482–93.
[118] Xu J, Zeng LF, Shen W, Turchi JJ, Zhang ZY. Targeting SHP2 for EGFR inhibitor resistant non-small cell lung carcinoma. Biochem Biophys Res Commun. 2013;439(4):586–90.
[119] Bunda S, Burrell K, Heir P, Zeng L, Alamsahebpour A, Kano Y, et al. Inhibition of SHP2-mediated dephosphorylation of Ras suppresses oncogenesis. Nat Commun. 2015;6:8859.
[120] Zeng LF, Zhang RY, Yu ZH, Li S, Wu L, Gunawan AM, et al. Therapeutic potential of targeting the oncogenic SHP2 phosphatase. J Med Chem. 2014;57(15):6594–609.
[121] Zhang RY, Yu ZH, Zeng L, Zhang S, Bai Y, Miao J, et al. SHP2 phosphatase as a novel therapeutic target for melanoma treatment. Oncotarget. 2016;7(45):73817–29.
[122] Wiesmann C, Barr KJ, Kung J, Zhu J, Erlanson DA, Shen W, et al. Allosteric inhibition of protein tyrosine phosphatase 1B. Nat Struct Mol Biol. 2004;11(8):730–7.
[123] Zasloff M, Williams JI, Chen Q, Anderson M, Maeder T, Holroyd K, et al. A spermine-coupled cholesterol metabolite from the shark with potent appetite suppressant and antidiabetic properties. Int J Obes Relat Metab Disord. 2001;25(5):689–97.
[124] Krishnan N, Koveal D, Miller DH, Xue B, Akshinthala SD, Kragelj J, et al. Targeting the disordered C terminus of PTP1B with an allosteric inhibitor. Nat Chem Biol. 2014;10(7):558–66.
[125] Smith AM, Maguire-Nguyen KK, Rando TA, Zasloff MA, Strange KB, Yin VP. The protein tyrosine phosphatase 1B inhibitor MSI-1436 stimulates regeneration of heart and multiple other tissues. npj Regenerative Medicine. 2017;2(1):4.
[126] Chen YN, LaMarche MJ, Chan HM, Fekkes P, Garcia-Fortanet J, Acker MG, et al. Allosteric inhibition of SHP2 phosphatase inhibits cancers driven by receptor tyrosine kinases. Nature. 2016;535(7610):148–52.
[127] Garcia Fortanet J, Chen CH, Chen YN, Chen Z, Deng Z, Firestone B, et al. Allosteric inhibition of SHP2: Identification of a potent, selective, and orally efficacious phosphatase inhibitor. J Med Chem. 2016;59(17):7773–82.
[128] Perron MD, Chowdhury S, Aubry I, Purisima E, Tremblay ML, Saragovi HU. Allosteric noncompetitive small molecule selective inhibitors of CD45 tyrosine phosphatase suppress T-cell receptor signals and inflammation in vivo. Mol Pharmacol. 2014;85(4):553–63.
[129] Molina G, Vogt A, Bakan A, Dai W, Queiroz de Oliveira P, Znosko W, et al. Zebrafish chemical screening reveals an inhibitor of Dusp6 that expands cardiac cell lineages. Nat Chem Biol. 2009;5(9):680–7.
[130] Korotchenko VN, Saydmohammed M, Vollmer LL, Bakan A, Sheetz K, Debiec KT, et al. In vivo structure-activity relationship studies support allosteric targeting of a dual specificity phosphatase. Chembiochem. 2014;15(10):1436–45.
[131] Owens DM, Keyse SM. Differential regulation of MAP kinase signalling by dual-specificity protein phosphatases. Oncogene. 2007;26(22):3203–13.
[132] Bai Y, Yu ZH, Liu S, Zhang L, Zhang RY, Zeng LF, et al. Novel anticancer agents based on targeting the trimer interface of the PRL phosphatase. Cancer Res. 2016;76(16):4805–15.
[133] Campbell AM, Zhang ZY. Phosphatase of regenerating liver: a novel target for cancer therapy. Expert Opin Ther Targets. 2014;18(5):555–69.
[134] Kobayashi M, Chen S, Gao R, Bai Y, Zhang ZY, Liu Y. Phosphatase of regenerating liver in hematopoietic stem cells and hematological malignancies. Cell Cycle. 2014;13(18):2827–35.
[135] Lang BT, Cregg JM, DePaul MA, Tran AP, Xu K, Dyck SM, et al. Modulation of the proteoglycan receptor PTPsigma promotes recovery after spinal cord injury. Nature. 2015;518(7539):404–8.
[136] Doody KM, Stanford SM, Sacchetti C, Svensson MN, Coles CH, Mitakidis N, et al. Targeting phosphatase-dependent proteoglycan switch for rheumatoid arthritis therapy. Sci Transl Med. 2015;7(288):288ra76.

[137] Coles CH, Shen Y, Tenney AP, Siebold C, Sutton GC, Lu W, et al. Proteoglycan-specific molecular switch for RPTPsigma clustering and neuronal extension. Science. 2011;332(6028):484–8.
[138] Li H, Wong C, Li W, Ruven C, He L, Wu X, et al. Enhanced regeneration and functional recovery after spinal root avulsion by manipulation of the proteoglycan receptor PTPsigma. Sci Rep. 2015;5:14923.
[139] Shen Y, Tenney AP, Busch SA, Horn KP, Cuascut FX, Liu K, et al. PTPsigma is a receptor for chondroitin sulfate proteoglycan, an inhibitor of neural regeneration. Science. 2009;326 (5952):592–6.
[140] Majeti R, Weiss A. Regulatory mechanisms for receptor protein tyrosine phosphatases. Chem Rev. 2001;101(8):2441–8.
[141] Gardner RT, Wang L, Lang BT, Cregg JM, Dunbar CL, Woodward WR, et al. Targeting protein tyrosine phosphatase sigma after myocardial infarction restores cardiac sympathetic innervation and prevents arrhythmias. Nat Commun. 2015;6:6235.

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