Protein tyrosine phosphatases (PTPs), the enzymes that dephosphorylate tyrosyl phosphoproteins, were initially believed to be few in number and serve a ‘housekeeping’ role in signal transduction. Recent work indicates that this is totally incorrect. Instead, PTPs comprise a large superfamily whose members play critical roles in a wide variety of cellular processes. Moreover, PTPs exhibit exquisite substrate specificity in vivo. Recent evidence has led us to propose that members of the PTP family achieve selectivity through different combinations of specific targeting strategies and intrinsic catalytic domain specificity.

Introduction

The regulated phosphorylation of proteins on specific tyrosyl residues is a major control mechanism for processes as diverse as cell growth and proliferation, metabolism, differentiation and locomotion. Accordingly, the activities of the enzymes that regulate reversible tyrosyl phosphorylation, the protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs), must be integrated and exquisitely regulated. Abnormal regulation of tyrosyl phosphorylation can lead to a variety of diseases, and therefore, it is important to understand how specificity and function are regulated for members of both enzyme families.

Until recently, most studies of tyrosyl phosphorylation focused on the PTKs. Characterization of PTPs proceeded at a slower pace for technical and philosophical reasons. The requirement for a suitable purified phosphorylated substrate for measurement of PTP activity represented a significant technical hurdle. Furthermore, PTPs were initially believed to be few in number and consigned to boring ‘housekeeping’ roles. Indeed, many investigators believed (and, unfortunately, some still believe) that PTPs have little specificity. However, results from the past ten years wholly belie such notions. It is now clear that PTPs comprise a large superfamily of related enzymes (Figure 1) that are subject to sophisticated modes of regulation and play critical roles in the control of a wide array of signaling pathways. Moreover, PTPs are highly specific, not only for particular phosphorylated protein and even non-protein (i.e. phospholipid) substrates, but also, in some cases, for specific phosphorylation sites within those substrates.

Structural, biochemical, reverse genetic and genetic approaches have begun to clarify the mechanisms underlying PTP specificity. Initially, the prevailing view was that PTP catalytic domains possessed little intrinsic substrate specificity; instead, specificity was thought to be conferred largely, if not entirely, by intracellular targeting (the ‘zip code’ model; see [1]). More recent studies have revealed several examples in which PTP catalytic domains display exquisite specificity in their recognition of substrates. The emerging theme is that overall PTP specificity is governed by targeting and catalytic domains acting in a combinatorial fashion. This review focuses on a few of the clearest examples of this theme.

Structural features of the PTP superfamily

A number of reviews cover the basics of PTP structure and enzymology [2,3]; only those features essential for understanding PTP specificity will be summarized here. Members of the PTP superfamily are characterized by the presence of a signature motif, H-C-X-X-G-X-X-R; they can be further categorized as classical PTPs or dual-specificity phosphatases (DSPs). Classical PTPs (of which the prototypic members are PTP1B and CD45) contain one or two ‘PTP domains’, conserved stretches of 240–250 amino acids that surround the signature motif. The structure of the active site of a classical PTP, in particular the depth of the active site cleft, renders it specific for pTyr residues, compared with pSer or pThr, ([4,5]; reviewed in [6]). Classical enzymes are further subdivided into transmembrane, receptor-like (RPTPs) and non-transmembrane (non-TM) forms, on the basis of the sequences that flank their PTP domains. Flanking domains regulate catalytic activity, either directly or indirectly, by providing sites of interaction for other regulatory or targeting proteins. For example, the extracellular segment of RPTPs offers a potential binding site for other regulatory or targeting proteins.
Combinatorial control of the specificity of protein tyrosine phosphatases

Tonks and Neel

site for regulatory ligands, thus permitting regulation of RPTP activity in response to specific stimuli. Most RPTPs have two intracellular PTP domains; however, the reason for this remains unclear. In nearly all cases, the more carboxy-terminal PTP domain has little or no catalytic activity but may function as a site of interaction with regulatory/targeting proteins.

Examples of DSPs include the MAP kinase phosphatases (MKPs), cdc25 family members and VH1 of vaccinia virus. Similarity between classical PTPs and DSPs is largely restricted to the signature motif, although there is conservation in the fold of the catalytic domain [7,8]. As their name implies, DSPs typically dephosphorylate pTyr and pSer/pThr residues. Analyses of the physiological function of these enzymes provided some of the first indications that members of the PTP family can exhibit profound selectivity in their effects on signal transduction pathways. For example, MKPs dephosphorylate particular members of the MAP kinase (MAPK) family (reviewed in [9**–11**]), whereas the different isoforms of cdc25 dephosphorylate Thr14 and Tyr15 of cyclin-dependent kinases (Cdks), thereby stimulating Cdk activity (reviewed in [12]). On the other hand, some ‘DSPs’ dephosphorylate primarily one type of phosphoamino acid residue in vivo. For example, KAP dephosphorylates Thr160 from the activation loop of Cdks [13]. Likewise, VHR displays a preference for the tyrosyl residue in the activation loop of Erk MAP kinases [14]. VH1 dephosphorylates at least three substrates in Vaccinia virions, including the transmembrane protein A14 [15,16**] and the soluble DNA-binding protein F18 [15], both of which are phosphorylated on seryl residues. However, another virion protein, A17, is phosphorylated on seryl, threonyl and tyrosyl residues, but VH1 primarily targets the tyrosyl phosphorylation site [17**].

The physiological substrates of some ‘DSPs’ are not even proteins, but rather phospholipids. The best characterized
example is PTEN, the product of a tumor suppressor gene on 10q23 [18,19], which is absolutely specific for phosphate on the 3 position in the sugar ring of phosphatidylinositol(3,4)P_2, phosphatidylinositol(3,4,5)P_3 (PIP_3) [20–22], and myotubularin (NTM), the product of the gene mutated in X-linked myotubular myopathy [23], which dephosphorylates the 3 phosphate of phosphatidylinositol-3P (PI3P) [24••,25••]. Once again, these are striking examples of specificity, not only for particular substrates but also for defined sites with that substrate. For further discussion of the structural basis of specificity of PTEN and other phosphoinositide phosphatases see the review in this issue by Wishart et al. (pp 172–181).

**Features of the PTP active site**

All members of the PTP superfamily utilize the same basic catalytic mechanism, in which the cysteinyl residue in the signature motif executes a nucleophilic attack on the phosphate group in the substrate (reviewed in [7]). In addition, an invariant Asp residue (Asp181 in PTP1B or the cognate residue in other family members) plays two critical roles in catalysis. First, it acts as a general acid, protonating the phenolate leaving group in the substrate. Second, it serves as a general base, activating a water molecule to promote hydrolysis of the cysteinyl–phosphate intermediate [26].

Crystal structures have been solved for several classical PTPs, including RPTPs and non-TM PTPs, and for some DSPs, including PTEN. These structural studies provide insight into how classical PTPs and DSPs retain the same general catalytic mechanism, yet target different phosphate esters. The PTP signature motif, containing the catalytic cysteinyl residue, forms a continuous loop located at the base of the catalytic cleft. In classical PTPs, a tyrosyl residue (Tyr46 in PTP1B) forms one side of the cleft, determining the depth of the pocket and contributing to the absolute specificity of classical PTPs for tyrosyl phosphoproteins. Only the side chain of a pTyr residue in a target substrate is of sufficient length to be accessible to the nucleophilic cysteinyl residue when inserted into the catalytic site; pSer and pThr residues are too short to be dephosphorylated. Another important feature is the dramatic change in conformation that occurs upon substrate binding. In the absence of substrate, the active site adopts an ‘open’ conformation in which the general acid Asp is pointed away from the active site, precluding it from serving as a proton donor. Upon substrate binding, the active site closes around the side chain of the pTyr residue. In this ‘closed’ conformation the loop containing the general acid residue (the so-called ‘WPD loop’) has moved, repositioning the Asp for its catalytic function. This also juxtaposes the Asp residue to the negatively charged phosphate group, which limits the stability of the enzyme–substrate complex.

These structural features inspired the development of ‘substrate-trapping’ mutant forms of PTPs, which retain the ability to bind substrates with high affinity but display impaired catalytic activity [27,28]. Mutants in which the general acid aspartyl residue is converted to Ala (Asp→Ala mutants) display such properties. In addition, mutation of the catalytic cysteinyl residue from the signature motif abrogates enzyme activity completely, often without diminishing substrate binding. Substrate-trapping mutants can recognize and form stable complexes with their target substrates, either from cell lysates or in the intact cell. Such complexes can be purified and identified by immunoblotting or direct sequencing. In several cases, Asp→Ala mutants have proven superior to Cys→Ser mutants for such studies [27]. This is probably because substitution of Asp with Ala enhances the hydrophobic properties of the active site cleft and removes the potential for electrostatic repulsion between the general acid and the phosphate moiety of the substrate, thereby stabilizing the interaction between the mutant PTP and its substrate.

Substrate-trapping mutants have helped to identify candidate physiological targets of PTPs. For example, the non-TM enzyme PTP-PEST displays a remarkable degree of selectivity for p130Cas as a substrate both in vitro and in intact cells [27]. Interestingly, the results of an unrelated strategy, that of gene disruption in mice, led to the same conclusion [29,30••]. The highly specific interaction between PTP-PEST and p130Cas results from a combination of two distinct substrate recognition mechanisms: the catalytic domain of PTP-PEST has intrinsic specificity for p130Cas [27]; and interaction between the SH3 domain of p130Cas and a polyPro motif in the noncatalytic segment of PTP-PEST dramatically increases the efficiency of the interaction [31]. Therefore, a specific targeting mechanism (the SH3–polyPro interaction) combines with intrinsic specificity within the catalytic domain to determine the specificity of PTP-PEST for p130Cas. Very recently, Goff and colleagues [32••] reported a novel twist to the combined use of targeting and PTP domain specificity by PTP-PEST. They found that PSTPIP1, a PTP-PEST-binding protein, may function to recruit the PTK Abl to PTP-PEST. Thus, proper targeting to a PTP may, at least in some cases, be accomplished by means of an intervening adapter protein. The end result is that by dephosphorylating p130Cas, and possibly Abl, PTP-PEST controls signaling events that regulate cell migration [30••,33••]. As discussed below, the substrate-trapping approach has revealed that many other PTPs also display remarkable substrate specificity.

**Substrate specificity of PTP1B and TC-PTP**

One of the mostly clearly defined examples of PTP specificity involves the non-TM PTPs, PTP1B and TC-PTP. These PTPs share a similar topology and a high degree of sequence similarity (~75% sequence identity in their catalytic domains [34–37]). Both consist of an amino-terminal, classical PTP domain and a carboxy-terminal regulatory sequence. The extreme carboxyl terminus of PTP1B has comparable hydrophobicity to typical transmembrane domains and targets the PTP to the cytoplasmic face of ER.
membranes [38,39]. Between the PTP and carboxy-terminal targeting domain is a stretch of 100 amino acids that contains several phosphorylation sites, which are modified in a signal [40] and cell-cycle-dependent manner [41,42], and a prolyl-rich sequence with the potential to bind SH3 domains [43]. TC-PTP exists in two alternatively spliced forms that share the same catalytic domain but differ at their carboxyl termini: TC48 has a similar carboxy-terminal hydrophobic domain to PTP1B and localizes to the same intracellular compartment, whereas in TC45 the carboxy-terminal hydrophobic domain is replaced by the six residue sequence Pro-Arg-Leu-Thr-Asp-Thr. In the case of TC45, this unmasks a carboxy-terminal bipartite nuclear localization signal, and, in the basal state, the enzyme is localized to the nucleus (reviewed in [44*]). Despite their marked sequence similarity, PTP1B and TC-PTP recognize different targets and have distinct biological functions. Again, this difference in specificity derives from both the catalytic and targeting domains of these enzymes. Initial evidence was provided by substrate-trapping mutants. When transfected into 293T cells, the Asp→Ala mutant of PTP1B recognize only a small subset of the pTyr proteins in the cell, binding to the EGF receptor (EGFR) and an unidentified 68 kDa protein. Moreover, transfection of wild-type PTP1B leads to decreased EGFR phosphorylation, even though the EGFR is not the most highly tyrosyl phosphorylated protein in such cells [28]. These results suggest that the EGFR is dephosphorylated preferentially by PTP1B. Analogous experiments, using cells that overexpress the insulin receptor (IR), suggest that the IR may also be a PTP1B substrate [45]. Subcellular targeting is also important in the selection of PTP1B targets, as expression of PTP1B lacking its carboxy-terminal targeting sequence results in global dephosphorylation of cellular phosphotyrosyl proteins [27].

The Asp→Ala mutant of ER-localized TC48 also ‘traps’ the EGFR. However, unlike PTP1B, TC48 does not recognize a 68 kDa p1yr protein [46]. Therefore, even when targeted to a comparable intracellular location, and despite their sequence similarity, PTP1B and TC-PTP display clear differences in substrate specificity in vivo. Differential targeting is also important for determining the specificity of TC-PTP isoforms. Following EGF stimulation, TC45, which has the same catalytic domain as TC48, exits the nucleus and interacts with EGFR-containing signaling complexes at the plasma membrane. However, in addition to the EGFR, the Asp→Ala mutant of TC45 (but not TC48) traps p52Shc, as well as another unidentified p1yr protein. TC45 not only targets a specific isoform of Shc (p52Shc, but not p46Shc or p66Shc) but also appears to select a particular tyrosyl phosphorylation site within Shc (Y239 but not Y240 or Y317) [46]. Subsequent studies suggest that TC45 regulates PI-3-kinase-dependent signals emanating from the EGFR, but not the Erk MAP kinase pathway [47**], providing a vivid illustration of the ability of a PTP to modulate signal transduction events selectively. As TC-PTP isoforms are generated by alternative splicing and recognize distinct targets, differential regulation of TC-PTP gene splicing may represent a novel mechanism for altering responses within tyrosyl phosphorylation pathways.

Perhaps the strongest evidence that PTP1B and TC-PTP display distinct specificities in vivo is provided by genetic analyses. Each gene has been disrupted by standard homologous recombination approaches, and the resulting homozygotic mutant mice display dramatically different phenotypes. TC-PTP–/– mice are born at normal Mendelian ratios, but by two weeks of age they begin to display runting and appear unwell. Within three to five weeks, all TC-PTP–/– mice die as a consequence of a complex hematopoietic disorder due largely to abnormal marrow stromal cells [48]. The detailed mechanism underlying the stromal cell abnormality, and the key target(s) that are hyperphosphorylated, remain to be identified (reviewed in [44*]). In contrast, PTP1B–/– mice are born at normal Mendelian ratios, appear generally healthy and live a normal lifespan. However, further analysis reveals that PTP1B–/– mice are insulin-sensitive and resistant to obesity induced by a high fat diet [49**,50**]. Consistent with their insulin sensitivity and the earlier experiments suggesting that the IR is a substrate of PTP1B, IR tyrosyl phosphorylation is enhanced and/or sustained in liver and muscle following insulin administration. Nevertheless, IR tyrosyl phosphorylation is unaltered in adipose tissue from PTP-1B–/– mice [50••]. Consistent with these observations, insulin-stimulated glucose transport is enhanced substantially in the muscle, but not the fat, of PTP1B–/– mice [50**]. Thus, although PTP1B appears to be a physiologically relevant IR PTP, its actions are tissue-specific. PTP1B may have additional targets. For example, as predicted by the trapping-mutant studies described above, tyrosyl phosphorylation of the EGFR is enhanced and sustained in the absence of PTP1B. The PDGFR is also hyperphosphorylated in PTP1B–/– fibroblasts. Despite the hyperphosphorylation of these growth factor receptors, there is little or no hyperactivation of the downstream Erk and Akt pathways, suggesting that there are compensatory mechanisms within the cell to accommodate the increased receptor activity (F Haj, L Klaman, BG Neel, unpublished data). Such compensatory mechanisms may explain why PTP1B–/– mice do not exhibit signs of increased EGFR and/or PDGFR activity (e.g., tumors). Moreover, both the EGFR and the PDGFR are eventually dephosphorylated (as is the IR in insulin-responsive tissues of PTP1B–/– mice). These findings illustrate two important principles of PTP action in vivo: that individual PTPs act against a limited number of cellular targets and that a given cellular target is probably regulated by more than one PTP.

The distinct phenotypes of PTP1B- and TC-PTP-deficient mice, together with earlier biochemical studies, strongly suggest that even these two highly related PTPs...
the activation loop of the IR was examined, it was found that the phosphatase displays the lowest Km for peptides in which both Tyr1162 and Tyr1163 are phosphorylated. Furthermore, PTP1B substrate-trapping mutants preferentially recognize forms of the peptide that are doubly phosphorylated on these adjacent sites [51**].

Crystal structures of complexes between the PTP1B Cys→Ala mutant and phosphorylated forms of the IR activation loop peptide reveal how selectivity is achieved ([51**]; Figure 2). The pTyr1162 residue binds to the active site of PTP1B in a manner identical to that described previously for the pTyr residue in the PTP1B–EGF-receptor peptide complex [53]. Specificity for pTyr1162, the critical autophosphorylation site for regulation of the activity of the IR, is determined by extensive and specific interactions between residues on the surface of PTP1B and on both the amino- and carboxy-terminal side of this phosphorylation site in the IR activation loop. Strikingly, the adjacent phosphotyrosyl residue, pTyr1163, is located within a shallow groove on the surface of PTP1B, which is connected to the catalytic cleft by a channel. The interactions of pTyr1163 in this ‘second pocket’ are dominated by salt bridges between its phosphate group and the side chains of Arg24 and Arg254 in PTP1B. The positioning of these arginyl residues also confers specificity for pTyr to the pocket. Interestingly, amongst PTP superfamily members, Arg24 is unique to PTP1B and TC-PTP. Moreover, access to the second pocket is possible because of the presence of a Gly at position 259 in PTP1B. Nearly all the other PTPs, including LAR, which also has been suggested to be an IR phosphate [54,55], have a bulky hydrophobic residue at the cognate position. Indeed, enzymological studies suggest that LAR prefers pTyr1158 [56,57], whereas experiments with transgenic mice overexpressing LAR in muscle suggest that it may target IRS proteins, not the IR, in vivo [58**].

In addition to tandem pTyr residues, there are further interactions between PTP1B and the IR activation loop. The guanidinium side chain of Arg47 of PTP1B, a residue that is poorly conserved within the PTP family, hydrogen bonds to the carboxylic acid of Asp1161 on the amino-terminal side (P-1) of the pTyr1162 substrate site. Similar interactions were noted previously with other substrates and explain the preference of PTP1B for acidic residues at the P-1 and P-2 positions. The side chain of Arg47 is flexible and can accommodate various combinations of acidic residues amino-terminal to the substrate pTyr site. There are further interactions between PTP1B and residues carboxy-terminal to the phosphorylation site. In particular, the guanidinium side chain of Arg1164 at the P+2 position in the substrate forms a classical π-cation interaction with Phe182 of the WPD loop in PTP1B, another residue that is poorly conserved within the PTP family. This interaction only occurs upon substrate binding and closure of the WPD loop, highlighting the concerted nature of the PTP–substrate interaction [51**].

**Structural basis for specific recognition of the IR by PTP1B**

The phenotype of PTP1B−/− mice indicates that the IR is an important substrate in vivo. Recent crystallographic, kinetic and binding analyses have revealed the mechanism underlying the specificity of this interaction at the molecular level [51**]. The activation loop of the IR contains three sites of autophosphorylation: pTyr1158, 1162 and 1163. In the inactive state of the IR, the activation loop impedes access to the catalytic center, with pTyr1162 occupying the position of the tyrosyl side chain of a potential substrate. Following hormone binding and autophosphorylation, the activation loop moves out of the active site and is then accessible for dephosphorylation [52]. When the activity of PTP1B towards synthetic peptides modeled on the activation loop of the IR was examined, it was found that the phosphatase displays exquisite specificity *in vivo*. However, a few caveats to this interpretation remain. Although both PTP1B and TC-PTP are widely expressed, their expression in various tissues has not been carefully quantified. Therefore, it is conceivable that at least part of the reason for the difference in phenotype of mice lacking these PTPs reflects differences in their pattern of expression throughout the animal. Studies in which TC-PTP is knocked into the PTP1B locus or vice-versa will be required to exclude this possibility. Also, it is possible that the dire consequences of TC-PTP deficiency are due to the absence of the nuclear isoform of TC-PTP, TC45, rather than the ER-targeted isoform of TC-PTP, TC48. It will be important to study mice in which only one of these two isoforms is expressed.
These observations provide definition at the molecular level of the recognition of substrate and determination of specificity for PTP1B. Interestingly, the third pTyr residue in the IR activation loop, pTyr 1158, is not recognized by PTP1B in any of the structures determined to date. Presumably, other PTPs dephosphorylate this site. The critical role of the Asp/Glu-pTyr-pTyr-Arg/Lys motif for optimal substrate recognition by PTP1B may suggest additional potential physiological substrates. Several interesting candidates contain this motif, including the receptor PTKs Tnk, FGFR and Axl. This motif is also found in the JAK family of PTKs, which play critical roles in transmitting signals from cytokine receptors. This raises the intriguing possibility that the effects of PTP1B on obesity may be mediated via effects on JAK2, which is associated with the receptor for the satiety hormone leptin.

**Differences in specificity between the mammalian SH2-containing PTPs**

The mammalian SH2-domain-containing PTPs (Shps), Shp-1 and Shp-2, are highly related (~60% sequence identity) non-TM PTPs. As with PTP1B and TC-PTP, Shps have dramatically different biological functions. Several recent studies provide insight into how Shp specificity is achieved.

Both Shp-1 and Shp-2 share the same overall topography, consisting of two amino-terminal SH2 domains, a single PTP domain and a carboxyl terminus with two tyrosyl phosphorylation sites that, in the case of Shp-2, but not Shp-1, flank a prolyl rich domain. The SH2 domains of Shps have two important roles in Shp regulation. First, as is the case for most SH2-domain-containing proteins, they recruit Shps to appropriate binding proteins within cells (reviewed in [2,59–62]). However, the N-SH2 domains of Shps also regulate catalytic activity directly. In the absence of an appropriate pTyr ligand, the N-SH2 binds to and inactivates the PTP domain; ligand binding disrupts this interaction and activates the enzyme (reviewed in [63]). This restricts Shp activation to particular locations within the cell, which helps to regulate their specificity.

The optimal phosphotyrosyl peptide-binding sequences for binding to the Shp SH2 domains are not completely understood. However, the binding specificity of the SH2 domains of the Shps is partially overlapping. Both prefer pTyr peptides that have hydrophobic residues (Ile/Val/Leu) at the −2 (i.e., amino-terminal to the pTyr) and +3 position (i.e., carboxy-terminal of the pTyr) (reviewed in [59,60,64]). There may be some preference for β-branched aliphatic residues at the +1 position and for acidic residues at the +3 and +5 positions for the N-SH2 domain of Shp-2 [65], whereas many Shp-1-binding pTyr peptides and proteins have small aliphatic residues at the +1 position [66,67]. Nevertheless, some tyrosyl phosphoproteins (e.g. Shps-1/Sirpt1, Pir-B/p91A) bind to both Shps, whereas others (e.g. Gab1, Gab2) bind to only one or the other. Thus, differential SH2 domain binding alone is unlikely to explain differences in the physiological functions of the Shps.

The strongest evidence for distinct roles for the Shps comes from genetic analyses. There are two naturally occurring mutations of murine Shp-1, the so-called *motheaten* (me) and *motheaten viable* (mev) mutations. Mice heterozygotic for either of these alleles have no apparent phenotype. However, *me/me* or *me/mev* mice have a panoply of hematopoietic abnormalities, resulting in their early demise (at 2–3 weeks for *me/me*, 9–12 weeks for *me/mev*). These abnormalities are the subject of several reviews [60,61,68–70], so we only summarize them here. In general, Shp-1 is a negative regulator of multiple hematopoietic signaling pathways, including those emanating from cytokine, growth factor, adhesion and antigen receptors. In these pathways, Shp-1 is recruited, most often by means of its SH2 domain, to the relevant receptor signaling complex. This may occur by direct recruitment of Shp-1 to receptors (e.g. the erythropoietin receptor [71,72]), and the receptor tyrosine kinase, Ros (H Keilhack, F Bohmer, personal communication) that contain appropriate binding sites. Alternatively, to regulate antigen and, most likely, integrin receptor signaling, Shp-1 is recruited to one of several so-called ‘inhibitory receptors,’ which are transmembrane glycoproteins that contain one or more binding sites for Shp-1, Shp-2 or the 5′ phosphoinositide phosphatases, Shp-1 or Shp-2 (reviewed in [73••]). Examples of inhibitory receptors that bind to Shp-1 include CD22, Pir-B/p91A, Shps-1/Sirpt1 and the killer inhibitory receptors of NK cells. Although Shp-1 is a negative (i.e. signal-diminishing) signaling molecule most of the time, there have been reports of some positive functions [74].

In contrast, Shp-2 most often plays a positive signaling role. Again, strong evidence is provided by genetic studies. Two targeted mutations in Shp-2 have been generated. These delete exon (Ex) 2 or 3 (Ex3). Although heterozygotes bearing either Ex2 or Ex3 mutations are phenotypically normal, Ex2+/− [75] and Ex3+/− mice [76] exhibit early embryonic lethality (see [65,68] for fuller description of the phenotype). Notably, fibroblasts derived from Ex3+/− mice have impaired responses to a variety of growth factors (e.g. EGF, FGF, IGFl-1 and possibly PDGF [76,77]), as well as defective integrin signaling [78–80]. Furthermore, recent analyses of compound mutations of the EGFR (so-called waved-2) mice and Shp-2 [81,82] provide genetic confirmation that Shp-2 is a positive component in EGFR signal transduction. A potential caveat is that neither the Ex3 [78,83] nor the Ex 2 (L Klaman, B Chen, BG Neel, unpublished data) mutation is protein-null; instead, both mutations give rise to truncations of the Shp-2 protein that lack an intact N-SH2 domain. This is potentially problematic because truncated proteins might have increased basal (i.e. in the absence of SH2 domain engagement) activity; indeed, this has been shown for the Ex3 mutation [83]. However, the decreased growth factor and integrin responses in Ex2+/− or Ex3+/− mice, and that these mutations are genetic hypomorphs.
(i.e. decreased function mutations) not neomorphs (i.e. gratuitous gain-of-function mutations). Consistent with this notion, recent studies of Shp-2 true-null mice (W Yang, L Klaman, B Chen, E George, BG Neel, unpublished data) reveal that they are embryonic lethal at an even earlier stage (day 6.5). The published data) reveal that they are embryonic lethal at an even earlier stage (day 6.5). The

The Drosophila and Caenorhabditis elegans homologs of Shp-2 also are essential positive components of growth factor receptor signaling pathways (reviewed in [59]). However, it should be noted that Shp-2 also acts as a negative regulator in other signaling pathways (reviewed in [59,62]).

These distinct signaling properties are intrinsic to the Shps. Initial evidence was provided by reverse genetic studies in tissue culture cells and early Xenopus embryos. Expression of either a Cys→Ser mutant or an internal deletion mutant within the Shp-2 PTP domain blocks growth-factor- and cytokine-induced activation of the Erk MAP kinase pathway. The same mutants block FGF-induced activation of the Erk pathway in Xenopus embryos, thereby disrupting mesoderm induction both in ex vivo 'animal cap' assays and in vivo (reviewed in [2,59,62]). Indeed, the latter studies anticipated the effects of Shp-2 mutations in mice, providing further evidence that the murine mutants are hypomorphic not neomorphic (see above). In both of these systems, coexpression of wild-type Shp-2, but not similar amounts of Shp-1, rescues the effects of the Shp-2 mutants. In the Xenopus studies, in particular, the levels of expression of the two Shps in the rescue experiments were quantified carefully, showing that they were unable to serve the same function in vivo. Transient transfection experiments also show that Shp-1, but not Shp-2, promotes dephosphorylation of the EGFR receptor [84].

Shp-1/Shp-2 chimeras have been used to probe the determinants of Shp-2 specificity in vivo. They clearly indicate that the PTP domain contains the critical specificity determinants. Chimeras containing the PTP domain of Shp-1 dephosphorylate the EGFR in transient transfection experiments; in contrast, chimeras containing the Shp-2 PTP domain do not promote EGFR dephosphorylation, regardless of which Shp contributes the SH2 and carboxy-terminal domains [84]. Likewise, chimeras containing the Shp-2 PTP domain rescue impaired Xenopus development and FGF-induced Erk activation caused by Shp-2 PTP domain mutants, even if the rest of the chimera is composed of Shp-1-derived sequences. Chimeras containing the Shp-1 PTP domain (and the rest of Shp-2) are completely inactive in such assays. However, the PTP domain is not the only determinant of Shp specificity: although chimeras containing the SH2 domains of Shp-1 and the PTP domain of Shp-2 rescue impaired development, they are less potent than wild-type Shp-2 in the Xenopus bioassay [85].

These findings support a combinatorial specificity model in which the SH2 domains of the Shps direct the PTP domain to appropriate intracellular locations, where it then surveys the local pTyr proteins and selects the correct target. Solely on the basis of the above studies, an alternative possibility is that the SH2 domains play no role in targeting per se; instead they may simply control Shp activation; in this case, all of the specificity for substrate selection would derive from the PTP domain. However, recent studies using the Xenopus system argue strongly against this model. O'Reilly et al. [86] generated mutants of the N-SH2 domain that retain the ability to bind pTyr peptide ligands, yet lose basal repression of the PTP domain. Such 'activated' mutants are able to evoke elongation of animal caps in the absence of added FGF, indicating that they are biologically active. However, mutation of the FLVRES sequences necessary for binding pTyr peptides in the SH2 domains of the activated mutants results in loss of bioactivity [86]. Thus, it appears that activated mutants, and by inference, endogenous Shp-2, must be both localized correctly and activated by means of their SH2 domains to allow the PTP domain specificity to select the ultimate target(s).

Although the PTP domain of Shps plays a vital role in determining substrate specificity, how this occurs at the molecular level remains unclear. Structural analyses of Shp-substrate complexes are needed. Unfortunately, the biologically relevant target(s) of both Shps remain subjects of controversy. Crystal structures of both Shp apoenzymes have been reported [87,88], as have complexes between two pTyr peptides derived from Shps-1–Sirpt1 and the Shp-1 PTP domain [89••]. However, in the latter structures the WPD loop remained partially open and the two phosphopeptides were bound in quite different orientations, raising concerns as to whether they reflect bona fide catalytic intermediates.

Questions also remain about the Shp carboxyl terminus. There is no compelling evidence for a role for this domain, even though it is conserved across vertebrates and some of its structural features (one phosphorylation site, a prolyl rich sequence) are found in its Drosophila (but not its C. elegans) ortholog (reviewed in [59]). Some reports suggest that the carboxyl terminus regulates PTP activity; other studies, at least for Shp-2, appear to refute this possibility (reviewed in [63]). Another possibility is that the carboxyl terminus helps target Shps to some substrate(s); indeed, some evidence exists to support this idea [90]. In such cases, it is unclear how basal repression by the N-SH2 would be overcome. Experiments in which the effects of carboxy-terminal truncations are explored would help shed more light on the role of the carboxyl terminus.

**MAP kinase phosphatases**

The MAPKs are critical constituents of signal transduction pathways activated in response to a wide range of stimuli (reviewed in [91,92]). MAPKs are activated following phosphorylation of a pThr-X-pTyr motif in their activation loops by a dual specificity MAPK kinase. The major MAPK isoforms include the Erks (where X is a Glu), JNKs (X is Pro) and p38 (X is Gly). Phosphorylation of both the threonyl and the tyrosyl residue within this motif is required for activity.
Therefore, inactivation of MAPKs may be achieved either through the action of two distinct single specificity enzymes acting individually on the phosphorylated residues or through the action of a single DSP that recognizes both residues. Not surprisingly, Nature takes advantage of both strategies, and, thus, specific PTPs and DSPs exist that target different MAPKs.

The first mammalian DSP to be identified was the product of the 3CH134 immediate-early gene, which is expressed in response to a variety of growth, differentiation and stress stimuli [93]. Subsequently, the 3CH134 gene product was renamed MKP-1 (MAP kinase phosphatase-1) following demonstration of its ability to dephosphorylate and inactivate Erk MAPKs both in vitro and in vivo [94]. Nevertheless, questions remain as to whether the Erks, Jnks or p38 are its primary physiological targets. To date, a total of ten DSPs with the ability to inactivate MAPKs have been identified, including MKP-1–5, VHR, hVH3/B23, hVH5/M3/6, PAC1 and Pyst2 (reviewed in [9••,10••]). These enzymes display differences in relative specificity for MAPK family members, tissue distribution and subcellular localization. Moreover, some DSPs are expressed constitutively, whereas others are induced only in response to some type of cell stimulation. Therefore, the MKPs constitute a complex response network that mediates the attenuation of specific MAPK-dependent signaling pathways in particular tissues and subcellular compartments following defined stimuli.

One key element of this specificity is the recognition of particular MAPKs by individual DSPs. Recently, how this is accomplished has become apparent (Figure 3). For several DSPs, the non-catalytic, amino-terminal segment of the protein interacts directly and specifically with a particular MAPK. For example, MKP-3/Pyst1 forms a stable complex with the Erks, but not the Jnks or p38. The specificity of this interaction mirrors the selectivity of MKP-3 for inactivation of Erks [95]. Interaction between the two proteins also stimulates the catalytic activity of the DSP. If the amino-terminal segment of the DSP is deleted, MKP-3 displays a low level of activity for all MAPKs and specificity for Erk is lost. One of the sites critical for the interaction of Erk2 with MKP-3 is the kinase interaction motif (KIM) (at residues 61–75), in which Arg65 plays a particularly important role, interacting with Asp319 in Erk2. It will be interesting to investigate the signaling effects of chimeric DSPs, in which distinct targeting and catalytic domains have been fused, to define more precisely the relative contributions of these segments to specificity.

In mammalian cells, PTP-SL (which exists in both receptor-like and non-TM forms) and STEP both regulate the phosphorylation status of Erk. Selectivity was ascribed to a 16-residue KIM in the non-catalytic segment of the PTP that interacts directly with MAPK [96]. Interestingly, the KIM in MKP-3 was recognized, in part, through its similarity to this sequence in these PTPs. Similar results have been obtained with HePTP/LC-PTP, a PTP that is preferentially expressed in hematopoietic cells. This enzyme displays selectivity for the Erks over Jnk; specificity is conferred by interaction of the MAPK with the KIM sequence [97–99]. This interaction not only regulates MAPK function but also sequesters MAPKs to defined subcellular locations. However, there is controversy over whether HePTP can target p38, with two groups arguing that it does [97,98] and another reporting that only Erks are HePTP targets [99].

Interestingly, the KIM–MAPK interaction is also a target for other regulatory mechanisms [100••]. HePTP is phosphorylated by protein kinase A (PKA) on Ser23 within the KIM, which antagonizes its association with Erk. Thus, PKA-dependent phosphorylation of HePTP results in release of Erk from the complex and activation of MAPK. Therefore, these PTPs are not only specific regulators of MAPKs but also underlie a mechanism of crosstalk between PKA and MAPK-dependent signaling systems.

**Specificity of RPTP function**

As their name implies, RPTPs are believed to be receptors, and so at least part of their biological specificity may be derived from their ligands. However, the ligands for most RPTPs remain poorly defined. Certain RPTPs (e.g. RPTPα, RPTPκ) participate in homophilic binding interactions, recognizing another molecule of the same enzyme on an adjacent cell (reviewed in [101]). Such observations, together with the known ability of PTPs to counter the growth promoting function of PTKs, generated excitement regarding the potential role of PTPs in the control of cell-contact-based phenomena, such as contact inhibition of cell growth. Indeed, many RPTPs display features of cell adhesion molecules and are implicated in the regulation of cell contact, such as in neuronal migration. For
example, RPTPζ/β, which is expressed on the surface of glial cells, recognizes contactin, a GPI-anchored protein on the surface of neurons [102]. This interaction triggers the assembly of signaling complexes at locations defined by points of contact between glial and neuronal cells, initiating either unidirectional or bidirectional signaling responses between the cells that underlie neurite outgrowth. Interestingly, the same RPTP binds the soluble growth factor pleiotrophin [103**].

Even for those RPTPs for which candidate ligands exist the consequences of ligand binding often are unclear. In nearly all cases reported, there was no detectable effect on RPTP activity. The single exception involves RPTPζ/β. Although contactin does not appear to affect its activity [102], binding of pleiotrophin was reported to inhibit RPTPζ/β activity and cause enhanced tyrosyl phosphorylation of β-catenin in treated cells [103**]. Structural analyses of RPTPζ led to the proposal that (presumably ligand-controlled) dimerization regulates RPTP activity [104]. Studies of effects of mutant forms of RPTPζ [105**,106**] and EGFR-CD45 chimeras [107], as well as very recent analyses of mutant CD45 knockin mice [108**], support this idea, at least for RPTPζ and CD45. However, it is unclear whether this model applies to all the PTP family and whether it operates under normal physiological conditions. Notably, crystal structural studies of RPTPα [109] and LAR [110**] are inconsistent with the type of dimer-mediated inhibition observed in RPTPα crystals [104].

The presence of catalytically inactive (or low activity) carboxy-terminal PTP domains in two-domain RPTPs hints that these domains play a role in substrate selection by binding to regulatory proteins. Some such binding proteins (liprins for the LAR family [111], fodrin for CD45 [112]) may contribute to targeting of RPTPζ to particular subcellular locations, whereas others (Tri for the LAR family [113,114]) have defined signaling functions. Modulation of RPTPζ activity and target selection by protein–protein interactions, such as those described above, may represent an exciting potential mechanism for maintaining specificity in RPTPζ function.

Currently, the best understood RPTP is CD45 (reviewed in [115**]). Its only clearly defined targets are members of the Src family of PTKs (SFKs). SFKs are regulated by tyrosyl phosphorylation on two sites: an inhibitory carboxy-terminal site (Y527 of c-Src) and an activating autophosphorylation site (Y416 of c-Src). Until recently, it was believed that CD45 specifically targeted the carboxy-terminal negative regulatory pTyr of SFKs. However, CD45 also dephosphorylates the activating (auto) phosphorylation sites in vivo. The mechanisms that govern this specificity for SFKs remain largely undefined. Possibilities include interaction with associated proteins such as CD45AP or fodrin, together with control of cell surface distribution, including movement into and out of lipid rafts.

RPTPζ also targets SFKs. In contrast to CD45, RPTPζ appears to be selective for the inhibitory tyrosyl residue of SFKs, thereby leading to their activation. This was initially suggested by experiments in which overexpression of RPTPζ led to transformation of fibroblasts, accompanied by dephosphorylation of Tyr527 of c-Src [116]. Recently, this was dramatically confirmed by gene knockout studies, which revealed that deletion of RPTPζ results in an 80% decrease in SFK activity, both basally and in response to growth factor and integrin activation [117**,118**]. The determinants of the selectivity of RPTPζ for SFKs are not well established. SFKs present a particular problem for PTPs: the carboxy-terminal inhibitory tyrosyl residue is bound in a ‘closed’ structure by the SH2 domain of the SFK. This closed structure is buttressed by intramolecular interactions between the SH3 domain and a prolyl-rich region in the linker domain of the SFK (reviewed in [119]). Recent data suggest that RPTPζ may utilize a novel mechanism to circumvent this problem. Unlike most RPTPs, RPTPζ is constitutively tyrosyl phosphorylated (to ~20% stoichiometry) on Tyr789, a site that binds to the adapter protein, Grb2 [120,121]. Shalloway and coworkers [122**] propose that pTyr789 helps to ‘pry open’ the closed form of Src by displacing the intramolecular SH2-pTyr527 interaction, thereby exposing the carboxy-terminal phosphotyrosyl residue in Src for dephosphorylation by the RPTPζ catalytic domain. Their data suggest that pTyr789 may participate selectively in enhancing the dephosphorylation of Src by RPTPζ, exerting no effect on more general PTP substrates, such as Raytide and myelin basic protein. The role of Grb2 binding to RPTPζ in this model remains unclear. Grb2 is best known for its ability to bind to the Ras exchange protein, Sos, but interestingly, RPTPζ-bound Grb2 does not bind to Sos. Instead, it may modulate the ability of pTyr789 of RPTPζ to engage the SH2 domain of Src. If so, it is unclear how the SH2 domain of Src, which has a lower apparent affinity for pTyr789 than does the Grb2 SH2 domain [122**], can displace Grb2, perhaps other mechanisms for enhancing the local concentration of Src and RPTPζ are involved. It will be interesting to see whether similar mechanisms operate in other systems in which pTyr residues are engaged intramolecularly by SH2 domains, for example in the dephosphorylation of Stats.

Although the targets of most RPTPs remain to be identified, genetic studies clearly indicate that even closely related RPTPs have distinct biological effects. For example, gene knockouts of individual members of the LAR family of mammalian RPTPs, which include LAR, RPTPθ and RPTPα, yield dramatically different phenotypes. Disruption of the LAR gene results in defective mammary gland development, owing to impaired terminal differentiation of alveoli during pregnancy [123], some defects in forebrain size and hippocampal organization [124], and possibly, defects in glucose homeostasis [125]. However, the latter effects have not been observed in other studies (W Hendriks, NPMoller, personal communication). In contrast, deletion of RPTPθ affects hippocampal long-term potentiation and learning [126**].
RPTPs-deficient mice display defects in brain development, including reduction in the size of the hypothalamus and olfactory bulb. In addition, the pituitary gland is smaller than normal, possibly arising from defects in the proliferation of cells of Rathke's pouch [127*, 128**]. Similarly, in Drosophila, disruption of particular RPTPs leads to specific defects in neuronal migration and the innervation of muscle targets (reviewed in [59,129]). Although different patterns of expression may account for some of the differences in the phenotypes of LAR family knockout mice and flies, intrinsic differences in their substrate specificities and/or their ligand binding properties almost certainly contribute. Further studies are needed to define the regulatory proteins and the substrate specificity of such closely related PTPs, thereby allowing us to understand how they exert such distinct effects in vivo.

Conclusions and perspectives
At the time that the first PTPs were identified, it was thought that there would be a small number of these enzymes, each with a broad specificity and serving a housekeeping function. As illustrated by the examples described above, this initial view of the field was far from the truth. The PTPs are a large, structurally diverse family of signal transducing enzymes that have the potential to manifest exquisite specificity in their effects in vivo. However, this is more than an issue of academic interest. Recent demonstrations of links between various members of the PTP family and human diseases have generated much excitement. For example, it now seems clear that an inhibitor of PTP1B would have therapeutic benefits for the treatment of diabetes and obesity. The demonstration of specificity in the effects of PTPs in vivo, and the recent progress in understanding the molecular mechanisms that underlie such specificity, has given confidence to the suggestions that inhibitors of defined PTPs would exert selective effects on signal transduction processes in vivo. Thus, the PTPs are now recognized as a novel platform for therapeutic intervention in human disease. We hope that the next few years will not only witness further advances in defining how specificity is achieved in the regulation and function of members of the PTP family but also yield the first PTP-based therapeutics.

Acknowledgements
The authors thank members of their laboratories for helpful discussions, Janik Andersen for help with Figure 1, and David Barford for help with Figure 2. Work in the authors’ laboratories is supported by National Institutes of Health grants R01 CA49152, R01 DK50603, P01 DK50654 and P50 HL56993 to BGN and R01 CA45508 to NKT and the Mellam Family Foundation to NKT.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
•• of outstanding interest


•• See annotation [11*].


•• See annotation [11**].


These three excellent reviews [9*, 10**, 11**] provide comprehensive information on the phosphatases that dephosphorylate MAP kinase family members.


•• See annotation [17**].


These two papers [16*, 17**] show that the same dual specificity phosphatase, Vaccinia V1L, specifically dephosphorylates seryl residues in some substrates and throsyl residues in others.


This paper, along with [33]


110. Elchebly et al. [49] studying mice bearing a deletion of PTP1B exon 5. Furthermore, the resistance to diet-induced obesity is found to be a consequence of a marked decrease in fat cell mass but not fat cell number. Consistent with the absence of insulin receptor hyper-phosphorylation in fat, PTP1B−/− mice exhibit increased insulin-stimulated glucose uptake in muscle, but not fat. Finally, energy expenditure is increased in the mutant mice, providing a possible explanation for their resistance to obesity.
This study provides a molecular explanation for the selectivity of PTP1B for the activated insulin receptor. Crystal structure analysis reveals a novel, "second" pocket that allows dual interaction between PTP1B and two phospho-proteins. This study provides a molecular explanation for the selectivity of PTP1B for the activated insulin receptor. Crystal structure analysis reveals a novel, "second" pocket that allows dual interaction between PTP1B and two phospho-proteins. This study provides a molecular explanation for the selectivity of PTP1B for the activated insulin receptor. Crystal structure analysis reveals a novel, "second" pocket that allows dual interaction between PTP1B and two phospho-proteins. This study provides a molecular explanation for the selectivity of PTP1B for the activated insulin receptor. Crystal structure analysis reveals a novel, "second" pocket that allows dual interaction between PTP1B and two phospho-proteins. This study provides a molecular explanation for the selectivity of PTP1B for the activated insulin receptor. Crystal structure analysis reveals a novel, "second" pocket that allows dual interaction between PTP1B and two phospho-proteins. This study provides a molecular explanation for the selectivity of PTP1B for the activated insulin receptor. Crystal structure analysis reveals a novel, "second" pocket that allows dual interaction between PTP1B and two phospho-proteins.


This paper reports the crystal structures of complexes of the catalytic domain of SHP-1 with two different phosphotyrosyl peptides from Src-1/Src2. The authors suggest regions within SHP-1 PTP domain that may be important for substrate selectivity. However, the WPD loop is not fully closed in either structure, raising questions as to whether bona fide catalytic intermediates have been visualized.


This paper presents the first structure of both PTP domains of a two-domain RPTP. In the structure, the two PTP domains are oriented orthogonally, in a manner that would preclude the type of dimer-induced inhibition by the RPTP juxtamembrane domain that was observed in the structure of the amino-terminal PTP domain of RPTPalpha (see [104]). Reference [109], which presents the structure of domain 1 of RPTPalpha, also is inconsistent with the 'inhibitory wedge' model.


This review summarizes our current understanding of CD45. In particular, it emphasizes how more recent studies have overturned the notion that CD45 only targets the carboxy-terminal inhibitory tyrosyl residues of Src family kinases. Instead, it is now clear that both the activating and inhibitory tyrosyl residues can be targeted by CD45.


These two papers [105,106] present biological and biochemical evidence in favor of the 'dimer-induced inhibition' model proposed earlier (see [104]). However, no direct evidence of inhibition of RPTPalpha upon dimerization is presented.


This paper shows that generating a mutation in the presumptive 'inhibitory wedge' of CD45 (see [107] for additional details) in the mouse, using a 'knock-in' strategy, results in the development of a lupus-like syndrome. These findings are consistent with the dimerization/inhibition model.


These two papers [117,118] report the phenotypes of RPTPalpha-deficient mice and cells. In particular, RPTPalpha-deficient fibroblasts have decreased SFK activity, which correlates with hyperphosphorylation of their inhibitory carboxy-terminal
tyrosyl residues. These findings confirm earlier predictions, from overexpression studies (see [116]), that RTPα targets src family kinases


This paper suggests a novel role for tyrosyl phosphorylation of RTPα (see [120,121]) in the dephosphorylation of Src family kinases by this enzyme. Binding of the SH2 domain of a src family kinase to tyrosyl phosphorylated Grb2 is suggested to ‘pry open’ the closed (i.e. inhibited) form of the kinase, thereby allowing RPTPα to dephosphorylate the carboxy-terminal tyrosyl residue.


See annotation [128**].


See annotation [128**].


These three papers, along with earlier work on LAR knockout mice (see [123–125]) and studies of Drosophila RPTPs (see [59] and [129] for reviews) emphasize the diverse biological functions of members of the LAR sub-family of RPTPs.