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Mechanisms of Development 85 (1999) 3–14

Review article

Protein-tyrosine phosphatases in development

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Accepted 9 April 1999

Abstract

One of the most important mechanisms of eukaryotic signalling is protein phosphorylation on tyrosine residues, which plays a pivotal role in development by regulating cell proliferation, differentiation and migration. Cellular phosphotyrosine (P.Tyr) levels are regulated by the antagonistic activities of the protein-tyrosine kinases (PTKs) and protein-tyrosine phosphatases (PTPs). We have good insight into the function of PTKs at the molecular level and into the role of PTK-mediated signalling in development. Intuitively, PTPs and PTKs are equally important in development. Over the past decade, much emphasis has been placed on elucidation of the function of PTPs, which has led to good insights into the mechanism of PTP-mediated dephosphorylation. Although still relatively little is known about the role of PTPs in cell signalling and development, evidence is now emerging that several PTPs are crucial for proper development. Here I will introduce PTP-mediated signalling and discuss recent findings regarding the function of PTPs in development.

Keywords: Protein-tyrosine phosphatases; Development; Phosphotyrosine

1. Introduction

Protein phosphorylation on tyrosine residues is reversible. Protein-tyrosine kinases (PTKs) mediate phosphorylation of cellular proteins, and protein-tyrosine phosphatases (PTPs) dephosphorylation. Initially, PTPs were thought to be mere household-enzymes that were ubiquitously expressed and had low substrate specificity. Following the purification and sequencing of the first PTP, PTPIB (Tonks et al., 1988a), and subsequent identification of CD45 as a transmembrane PTP (Charbonneau et al., 1988; Tonks et al., 1988b), it became clear that the PTPs are a diverse family of enzymes with many members that have different substrate specificity, are regulated differently, and are differentially expressed. By now, more than 75 PTPs have been cloned from many different species, ranging from bacteria to man. In fact, it has been estimated, based on random sequencing projects, that the human genome contains around 500 PTPs (Neel and Tonks, 1997). Since the enzymatic counterparts of the PTPs, the PTKs, play an important role in development and since there is a wide variety of PTPs, it has been speculated that PTPs have an important function in development as well. By now, several PTPs have been shown to be crucial for proper embryonic development of invertebrates and vertebrates. These PTPs and their function will be described here.

2. Protein-tyrosine phosphatases

The PTP superfamily is defined by the PTP 'fingerprint'-sequence ([I/V]HCXAGXXR[S/T]G) (Fischer et al., 1991) with the absolutely conserved catalytic site cysteine. This cysteine is essential for catalysis, since during catalysis an enzyme-cysteinyl-phosphate intermediate is formed (Guan and Dixon, 1991; Pot and Dixon, 1992). The PTP-superfamily consists of classical PTPs, dual specificity phosphatases (DSPs) and low molecular weight PTPs (Neel and Tonks, 1997). Many DSPs have important functions, like MAPK phosphatase-1 (MKP-1) (Sun et al., 1993) and the tumour suppressor gene PTEN (Li et al., 1997; Li and Sun, 1997; Steck et al., 1997). Recent evidence suggests that PTEN also exhibits lipid phosphatase activity (Maehama and Dixon, 1998), which is apparently responsible for the tumour suppressing activity (Myers et al., 1998). Despite the fact that the DSPs are interesting, I will focus on the classical PTPs here.

Both cytoplasmic and transmembrane PTPs, tentatively called Receptor PTPs, have been identified. The cytoplasmic PTPs and transmembrane PTPs all contain one or two catalytic PTP domain(s) of approximately 240 amino acids, harbouring the fingerprint sequence. Comparison of the

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0925-4773/99/$ - see front matter
PII: S0925-4773(99)00089-1

crystal structures of the PTPs that have been solved to date demonstrates that the PTP domains are not only conserved in sequence, but also in structure (Denu et al., 1996; Fauman and Saper, 1996). The sequences outside the catalytic domain are diverse and may regulate PTP activity and/or function in both the cytoplasmic and receptor PTPs.

2.1. Cytoplasmic protein-tyrosine phosphatases

Cytoplasmic PTPs contain a single catalytic PTP domain. Apart from the PTP domain, the cytoplasmic PTPs do not share sequence homology and the sequences outside the PTP domains have various functions (Mauro and Dixon, 1994) (Fig. 1). For example, a hydrophobic region was identified in PTP1B that directs PTP1B to the endoplasmic reticulum (ER) (Frangioni et al., 1992). Activation of the protease calpain cleaves the catalytic domain off of the ER-localization domain, releasing active PTP1B from the ER into the cytoplasm, where it may dephosphorylate target proteins (Frangioni et al., 1993). Therefore, the hydrophobic region in PTP1B is involved in subcellular localization and may play a role in regulation of PTP1B activity. *Drosophila* DPTP61F contains a hydrophobic C-terminus, similar to PTP1B. In addition, an alternatively spliced form of DPTP61F was identified with a nuclear localization signal that drives nuclear localization of DPTP61F. The catalytic activity of the two splice isoforms is indistinguishable, suggesting that the C-termini are involved in subcellular localization, rather than regulation of activity (McLaughlin and Dixon, 1993).

Shp-1 and Shp-2 contain two Src Homology-2 (SH2) domains that bind specifically to P.Tyr in target proteins. These SH2 domains may thus be involved in substrate-recognition (reviewed by Feng and Pawson, 1994; Van Vactor et al., 1998). The recently solved crystal structure of Shp-2 demonstrates that unliganded Shp-2 is inactive, due to steric hindrance of the catalytic site by the N-terminal SH2 domain. Binding of P.Tyr-containing peptides or proteins induced a conformational change, thereby directly activating the PTP activity of Shp-2. Therefore, the N-terminal SH2 domain acts as a molecular switch, turning Shp-2 on or off, depending on the presence of P.Tyr-containing peptides (Hof et al., 1998). Taken together, the SH2 domains in Shp-2 (and presumably also in Shp-1) have dual functions in regulating the catalytic activity of these PTPs, and in directing the PTP activity to target proteins.

Not only SH2 domains, but also other protein-protein interaction domains have been identified in cytoplasmic PTPs that may direct these PTPs to specific subcellular locations. The band 4.1 domain in PTP-BL may mediate binding to cytoskeletal proteins, and the PDZ-domains binding to specific hydrophobic sequences in the C-termini of target proteins (Saras and Heldin, 1996). The PEST-domains may mediate rapid degradation of PEST-containing PTPs, although the half-life of these PTPs reportedly is not extremely short (Charest et al., 1995). Alternatively, the PEST sequences may provide proline rich binding sites for SH3 or WW domain containing proteins. For instance, PTP-PEST was found to associate with its substrate, $p130^{Cas}$, through a proline-rich sequence in PTP-PEST and the SH3 domain in $p130^{Cas}$ (Garton et al., 1997). In conclusion, the function of sequences outside the catalytic domain in cytoplasmic PTPs are three-fold: (1) regulation of activity, (2) subcellular localization, and (3) interaction with other proteins, including substrates.

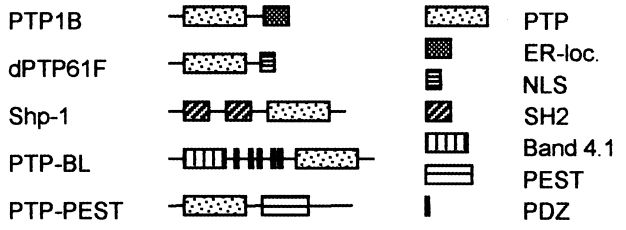

Fig. 1. Cytoplasmic protein-tyrosine phosphatases. Typical examples of cytoplasmic PTPs are depicted. The PTP domains are indicated, as well as the different structural domains outside the PTP domain that may be involved in subcellular localization, in protein-protein interactions and/or in PTP/substrate interactions. The identity of the different domains is indicated in the key on the right. PTP, Protein-Tyrosine Phosphatase; ER-loc., endoplasmic reticulum localization; NLS, nuclear localization signal; SH2, Src Homology 2; PEST, Pro-, GIu-, Ser- and Thr-rich; PDZ, conserved domain initially found in PSD-95, DlgA and ZO-1 that binds to C-terminal hydrophobic sequences in target proteins.

2.2. Receptor protein- tyrosine phosphatases

The receptor PTPs have been classified on the basis of structural differences, mainly in the extracellular domain, and at least six distinct types have been identified (Fig. 2A). The receptor PTPs contain one or two PTP domains, of which the membrane proximal domain (D1) contains the majority of the activity, while the membrane distal domain (D2) of several PTPs has been demonstrated to exhibit some PTP activity (Wang and Pallen, 1991; Wu et al., 1997). However, some D2s lack residues that are essential for catalysis, including the catalytic site cysteine, and these D2s do not display any PTP activity (e.g. HPTPγ, Krueger et al., 1990). Nevertheless, D2s are highly conserved and mutation of only two residues in RPTPα D2 potentiates PTP activity to levels that are comparable to D1 (Lim et al., 1998; Buist et al., 1999), suggesting that RPTP-D2s have an important function. However, in view of the low PTP activity of RPTP-D2s it is unlikely that this residual activity is physiologically relevant. Instead, RPTP-D2s may be involved in regulation of RPTP-D1 activity, in protein-protein interactions with other RPTPs (Wallace et al., 1998; Felberg and Johnson, 1998), or with potential effector proteins (den Hertog et al., 1994; Serra-Pagès et al., 1995), or even in defining substrate specificity by binding to substrates, thereby presenting them to catalytically active RPTP-D1s (Hunter, 1998).

The extracellular domains of RPTPs are diverse, ranging

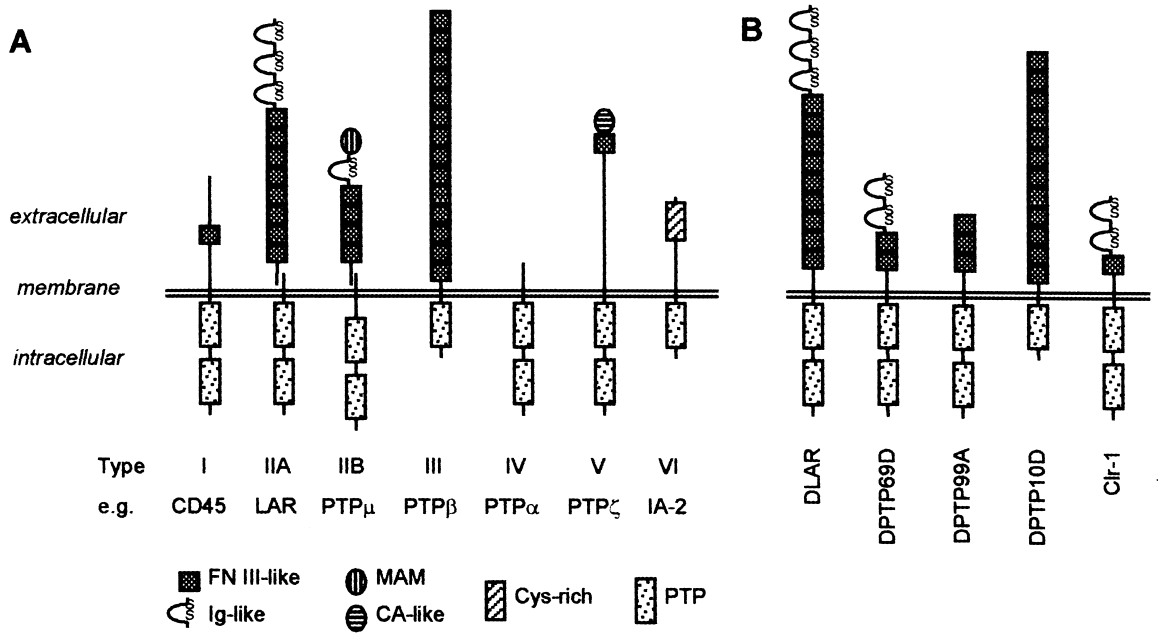

Fig. 2. Receptor Protein-Tyrosine Phosphatases. (A) Vertebrate RPTPs. At least six different subtypes of transmembrane RPTPs have been identified. Classification is done, mainly based on the sequences in the extracellular domain. (B) Invertebrate RPTPs. Clr-1 is a *C. elegans* RPTP, the other four are *Drosophila* RPTPs. The different domains are indicated in the key at the bottom. FNIII-like, fibronectin type III-like; Ig-like, immunoglobulin-like; MAM, conserved domain, initially identified in Meprin, A5 and Mu proteins; CA-like, carbonic anhydrase-like; PTP, Protein-Tyrosine Phosphatase.

from very short, e.g. RPTPε (27 residues), to very extensive. Many RPTPs display features in their extracellular domains of cell adhesion molecules (CAMs) with multiple fibronectin type III-like repeats and immunoglobulin domains. In addition, carbonic anhydrase-like domains, MAM (Meprin-Xenopus A2-Mu) domains or cysteine rich regions have been identified in RPTPs (Fig. 2A). It is tempting to speculate that the RPTPs actually function as receptors, transducing extracellular signals across the membrane by binding extracellular ligands. The subfamily of MAM-domain containing RPTPs has been demonstrated to engage in homophilic interactions when expressed at high levels on apposing cells (Brady-Kalnay et al., 1993; Gebbink et al., 1993; Sap et al., 1994), which is mediated by the MAM domain and the immunoglobulin domain (Brady-Kalnay and Tonks, 1994; Zondag et al., 1995). The extracellular domain of PTPβ/ζ functions as a ligand for the neuronal receptor complexes Contactin/Caspr and Contactin/Nr-CAM and both soluble as well as membrane-bound RPTPβ/ζ ectodomain induced neurite outgrowth of tectal neurons. The carbonic anhydrase domain and the FNIII-like domain are required for binding to the Contactin-protein complexes (Peles et al., 1995; 1997; Sakurai et al., 1997). Recently, the extracellular domain of a specific isoform of LAR was identified as a receptor for the laminin/nidogen complex, and binding of laminin/nidogen to LAR may affect the actin cytoskeletal structure, resulting in morphological changes of cells (O'Grady et al., 1998). However, even though cellular changes have been observed upon ligand binding to RPTPs, it remains to be determined whether intracellular PTP activity is affected by ligand binding.

Whether and, if so, how RPTPs are regulated is an extremely important question that remains to be answered definitively. In analogy to the receptor PTKs, RPTPs may be regulated by dimerization (Weiss and Schlessinger, 1998). It has been demonstrated that chimeric Epidermal Growth Factor Receptor (EGFR)/CD45 is functionally inactivated by ligand-induced dimerization. CD45-D1 activity is essential for potentiation of T cell receptor (TCR) signalling, in that expression of CD45 in T cells that lack endogenous CD45 is sufficient to restore otherwise defective TCR signalling (Desai et al., 1994). Similarly, expression of chimeric EGFR/CD45 restored TCR signalling, but ligand-induced dimerization of EGFR/CD45 abolished restoration of TCR signalling, suggesting that CD45 activity is inhibited by dimerization (Desai et al., 1993). Dimerization-induced inactivation of RPTPs is supported by structural evidence. RPTPα-D1 forms dimers in two independent crystals. These dimers are presumably inactive, since an amino-terminal helix-turn-helix wedge-like structure inserts into the catalytic site of a dyad-related monomer, thereby blocking the catalytic site (Bilwes et al., 1996). The sequences that are involved in dimerization of RPTPα-D1 are conserved in other RPTPs, suggesting that RPTP-dimerization is a general phenomenon. Support for this model comes from binding studies in which CD45-D1 was found to form homodimers in solution (Felberg and Johnson, 1998). Moreover, the wedge-like structure is essential for RPTPσ-D1/RPTPδ-D2 heterodimerization, in

that deletion of the wedge in RPTPσ-D1 abolished binding to RPTPδ-D2 (Wallace et al., 1998). In contrast, RPTPμ-D1 did not form dimers in crystals, like RPTPα-D1 (Hoffmann et al., 1997). However, RPTPμ has an unusually long juxtamembrane region, suggesting that either RPTPμ is an exception that does not dimerize, or that the experimental conditions were not favouring RPTPμ-D1 dimer formation in the crystals. Recent evidence strongly supports the hypothesis that RPTPs are inactivated by dimerization, since mutation of a single residue in the EGFR/CD45 chimaera, corresponding to a residue that is involved in dimerization in RPTPα-D1 crystals, abolished dimerization-induced inactivation (Majeti et al., 1998). In conclusion, evidence is accumulating that dimerization negatively regulates RPTP activity. The model that dimerization inhibits RPTP activity is especially appealing because it is exactly the inverse of the enzymatic counterpart of RPTPs, the RPTKs, that are activated by dimerization. Whether ligand binding is required for RPTP dimerization, similar to the RPTKs, or whether ligands induce monomerization of otherwise dimeric RPTPs remains to be determined.

3. Function of PTPs in invertebrates

Genetic screens have provided detailed insight into the function of PTPs in lower eukaryotic systems. In yeast, much work has been done on PTPs that regulate MAPKs, and thus are involved in multiple signalling pathways (reviewed by Van Vactor et al., 1998). In *Dictyostelium discoidum*, three PTPs have been identified that have distinct effects on growth rate and development (Howard et al., 1992; Gamper et al., 1996). Here, I will focus on the role of PTPs in development of multicellular organisms. Forward and reverse genetic screens using flies and worms have contributed tremendously to our insight into the role of PTPs in development. In fact, we are beginning to unravel the genetic pathways in which PTPs are involved, and biochemical evidence provides definitive proof for some of the genetic interactions. These analyses in invertebrates provide valuable insights into the function of PTPs, and it is tempting to extrapolate the genetic data obtained in flies and worms to vertebrates.

3.1. SH2-domain containing PTPs

One of the first indications that PTPs play an important role in development came from the identification of the gene that is responsible for the *corkscrew* phenotype in *Drosophila* as an SH2-domain containing PTP (Perkins et al., 1992). Csw is most homologous to mammalian Shp-2, although Csw contains an insert in its catalytic domain. *Csw* is maternally required for normal determination of cell fates at the termini of the embryo and the *csw* mutant embryos are twisted or U-shaped and die during pupal stages (Perkins et al., 1992). Analysis of double mutants

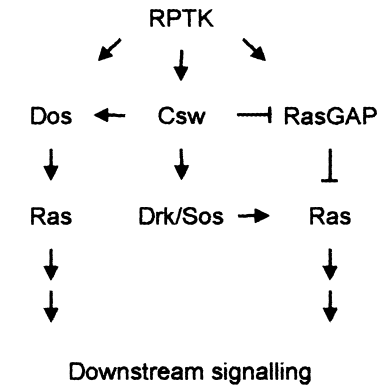

Fig. 3. Csw may play a central role in RPTK to Ras signalling. Csw-mediated dephosphorylation of the adaptor protein Dos leads to activation of Ras. In addition, Csw itself becomes phosphorylated on tyrosine upon binding to activated RPTKs, thereby providing binding sites for the SH3-SH2-SH3 adaptor protein Drk. Binding of Drk with its associated protein, Sos, a guanine nucleotide exchange factor for Ras, leads to activation of Ras. Moreover, binding of Csw to activated RPTKs may lead to dephosphorylation of specific binding sites in RPTKs. For instance, Csw dephosphorylates the binding site for the negative regulator of Ras, RasGAP, in the Torso RPTK, thereby prohibiting RasGAP activation. In conclusion, Csw is a modulator of RPTK signalling and is essential for several developmental processes in the *Drosophila* embryo, including eye development (mediated by the RPTK Sevenless), and specification of embryonic terminal cells (mediated by the RPTK Torso).

indicated that *csw* is genetically downstream of the RPTK *torso*, and in parallel with *D-Raf*. Csw is not only required for Torso signalling, but also for signalling by other RPTKs, including Sevenless, Breathless and DER (Perkins et al., 1996; Allard et al., 1996).

Csw may play a central role in RPTK to Ras signalling (Fig. 3). Recent evidence suggests that Csw binds to Torso P.Tyr630 through its SH2 domain(s) and directly dephosphorylates Torso P.Y918, the binding site for RasGAP. In addition, Csw acts as an adaptor protein, coupling Torso to Ras activation via Drk and Sos. Therefore, Csw counteracts RasGAP in two ways, by prohibiting RasGAP activation and by counteracting RasGAP activity (Fig. 3). The opposing activities of Csw and RasGAP modulate the strength of the Torso RPTK signal, thereby establishing the fine-tuning of *Drosophila* terminal development (Cleghon et al., 1998). RasGAP is not the only downstream component in Csw-mediated signalling and other substrates are involved in the function of Csw as well. For instance, Dos is an adaptor protein that is genetically downstream of Csw in the Sevenless signalling pathway and Dos was identified as a substrate of Csw, using a 'substrate-trapping' procedure (Fig. 3) (Herbst et al., 1996; Raabe et al., 1996). In conclusion, Csw may be a regulator of RPTK to Ras signalling that acts at multiple sites.

The homolog of Shp-2 in *C. elegans*, PTP-2, is essential for proper oogenesis and although it is not essential for vulval development, mutation of PTP-2 affects vulval phenotypes caused by several other genes. Like in *Drosophila*, PTP-2 is genetically downstream of RPTKs, in paral-

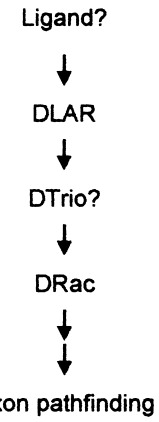

Fig. 4. Model for DLAR signalling in axon pathfinding. Genetic evidence suggests that DRac is downstream of DLAR. Biochemical evidence from mammalian systems suggests that the large multi-domain protein Trio mediates DLAR-induced DRac activation, since Trio binds to LAR and contains a Rac-specific guanine nucleotide exchange factor domain. The axon pathfinding phenotype of the DLAR mutant *Drosophila* embryos suggests that the extracellular domain and/or ligands are involved in DLAR function. However, to date such ligands have not been identified yet.

lel with or upstream of Ras (Gutch et al., 1998), suggesting a similar role for *C. elegans* PTP-2 as for *Drosophila* Csw.

3.2. Receptor PTPs

The first indication that RPTPs play an important role during development also came from work in *Drosophila*. The expression pattern of four RPTPs in *Drosophila*, DLAR, DPTP10D, DPTP99A and DPTP69D, with high and specific expression in motor neuron axons, suggested that these RPTPs are involved in axon guidance (Tian et al., 1991; Yang et al., 1991; Desai et al., 1996). These three RPTPs belong to the type IIa and type III subfamilies of RPTPs, which are characterized by multiple immunoglobulin-like and FNIII-like domains (DLAR and DPTP69D), or only FNIII-like domains (DPTP99A and DPTP10D) in the extracellular domain (Fig. 2B). Recently, genetic evidence confirmed the importance of RPTPs in axon guidance, since *Drosophila* mutants were identified with mutations in RPTPs that showed defects in axonal pathfinding (Krueger et al., 1996; Desai et al., 1996; 1997). The phenotype of these RPTP mutant embryos have been discussed in excellent recent reviews (Chien, 1996; Van Vactor et al., 1998; Van Vactor, 1998). Briefly, in DLAR mutant embryos, the segmental nerve b (SNb) axons bypass their normal muscle target region and continue to extend distally along the common pathway, following the intersegmental nerve (ISN) (Krueger et al., 1996). DPTP69D mutant embryos show a complex axon pathfinding phenotype, in that the SNb axon stops growing before reaching its target muscle, or it follows incorrect pathways and bypasses its target. DPTP99A mutants have no obvious axon pathfinding phenotype. However, the function of DPTP69D and DPTP99A may be partially redundant, since the penetrance and the severity of the SNb defects in mutant embryos that lack both DPTP69D and DPTP99A is enhanced compared to DPTP69D mutant embryos (Desai et al., 1996). Analysis of mutants that lack DLAR, DPTP69D and DPTP99A demonstrates that specific RPTPs are required for pathway decisions along the trajectories of the ISN and SNb motor nerves. In addition, only in the triple mutant the SNb axon does not progress beyond the first branch point, indicating that any of the three RPTPs is sufficient for progression beyond the first branch point. It is noteworthy that these three RPTPs are only required for pathway decisions of particular axons, since the SN root branches (SNa and SNc) are relatively normal in embryos that lack all three RPTPs (Desai et al., 1997).

Although it is clear that RPTPs play an important role in axon pathfinding in *Drosophila*, the molecular mechanism underlying the involvement of these RPTPs remains to be determined. It has been demonstrated that the small GTP binding protein Rac is genetically downstream of DLAR (Kaufmann et al., 1998). This interaction may be mediated by Trio, a large multi-domain protein that binds to human LAR and contains a Rac guanine nucleotide exchange factor domain (Debant et al., 1996). It is noteworthy that *C. elegans* UNC-73, which is closely related to vertebrate Trio, specifically activates Rac and is required for proper cell and growth cone migration, presumably through regulation of cytoskeletal rearrangements (Steven et al., 1998). Whether a *Drosophila* homolog of Trio couples DLAR to Rac remains to be determined, but this notion has led to a model for the involvement of DLAR in axon pathfinding (Fig. 4). This linear model is far from complete, since other signalling proteins that interact with the cytoplasmic domain of LAR, including LIP-1 and Liprins (Serra-Pagès et al., 1995; 1998), may be involved in axon pathfinding as well. Interestingly, recently the PTK Abl and its substrate Enabled (Ena) were found to play important roles in axon pathfinding, which may be due to direct interactions with the DLAR pathway, since Abl and Ena associate with the cytoplasmic domain of DLAR, and are dephosphorylated by DLAR (Wills et al., 1999). In view of the axon pathfinding phenotype, it is highly likely that LAR ligands are involved in LAR-mediated axon pathfinding. There is evidence that the extracellular domain of LAR is involved in pathfinding in the leech, *Hirudo medicinalis*. HmLAR2, the leech homolog of DLAR, is specifically expressed on growth cones of transient neuron-like template cells, Comb cells, when these cell processes grow out rapidly. Injection of antibodies, directed at the extracellular domain of HmLAR2, leads to partial internalization of HmLAR2. Concomitantly, the processes of the HmLAR2-expressing cells project aberrantly and extend over shorter distances than normal, while the growth cones appear normal. These data suggest that the extracellular domain of HmLAR2 transduces extracellular signals that are required for normal growth cone pathfinding (Gershon et al., 1998).

The first transmembrane PTP that was identified in *C.*

elegans is Clr-1, which contains two immunoglobulin-like and one FNIII-like domain (Fig. 2B) (Kokel et al., 1998). Mutant embryos with mutations in *clr-1* express the severe *clear* phenotype that is characterized by extremely short, immobile and infertile animals. In addition, their pharynx and intestine appears to float within the pseudocoelom. Animals that are homozygous for the most severe alleles die during larval development, but most animals survive. Wild type *clr-1* completely rescued the phenotype, but mutant *clr-1* with a mutation in the catalytic site cysteine in D1 did not, demonstrating that catalytic activity of Clr-1-D1 is essential for its function. *Clr-1* was identified in a screen for suppressors of the *egl-15* hypomorphic mutant phenotype. *Egl-15* encodes a *C. elegans* RPTK, a fibroblast growth factor receptor (FGFR) (DeVore et al., 1995). *Clr-1* and *egl-15* mutually suppress each other, suggesting that this RPTP and this RPTK have opposing activities. Using null alleles, it was clear that the *clear* phenotype of *clr-1* mutants is dependent on integrity of *egl-15*, while the phenotype of *egl-15* mutants is not dependent on *clr-1*. Since catalytic activity of Clr-1 is required for its function, it is highly likely that Clr-1-mediated dephosphorylation acts as a negative regulator of *egl-15* signalling (Kokel et al., 1998).

4. The function of PTPs in vertebrates

Forward genetics experiments in invertebrates have provided insight into the function of several PTPs, as described above. Homologous PTPs have been identified in vertebrates and it is tempting to speculate that these vertebrate PTPs have similar functions as their invertebrate counterparts. However, until now, such extrapolations have proved to be very difficult. In fact, still relatively little is known about the function of PTPs in vertebrate development, despite many extensive PTP expression studies, revealing complex spatio-temporal expression patterns. Many PTPs, in particular RPTPs, are highly and specifically expressed during brain development (reviewed by Stoker and Dutta, 1998), suggesting that PTPs play an important role in neural development. Gene ablation studies have provided some insight into the function of PTPs in vertebrate development, although knock-outs of many PTPs disappointingly did not result in phenotypes, which is presumably due to redundancy. Double or triple knock-outs of closely related subfamily members may be required to obtain insight into the function of these subfamilies of PTPs. Much work has been done, focussed at analysis of the function of PTPs in cell signalling, which has provided important clues as to the biological function of these PTPs. However, the most important question regarding PTP-signalling – what are their substrates – remains elusive in most cases. Here, I will discuss the (possible) role of several PTPs in vertebrate development.

4.1. SH2 domain-containing PTPs

In vertebrates, two SH2-containing PTPs have been identified, Shp-1 and Shp-2. Shp-1 is predominantly expressed in haematopoietic cells, while Shp-2 is more broadly expressed. Shp-1 was identified as the gene that is disrupted in the *motheaten* mutant mice (Shultz et al., 1993). Two *motheaten* alleles have been identified with varying severity, *motheaten* (*me/me*) and *motheaten viable* (me^v/me^v). *Me/me* homozygous mice develop systemic autoimmune disease and die within 3–9 weeks after birth. The defects in these mice are linked to developmental or functional defects in the haematopoetic system (Shultz et al., 1993), consistent with exclusive expression of Shp-1 in the haematopoetic system (Yi et al., 1992). Shp-1 binds to specific P.Tyr residues in target proteins through its SH2 domains. Many potential Shp-1 substrates have been identified, including haematopoietic receptors, e.g. the interleukin-3 (IL-3) receptor, associated kinases, e.g. ZAP-70, and adaptor proteins (reviewed by Frearson and Alexander, 1997). However, which of these substrates are essential for the function of Shp-1 remains to be determined.

Gene targeting of Shp-2 in mice was done by deletion of exon 2 or exon 3 in two independent studies (Arrandale et al., 1996; Saxton et al., 1997). Both groups report that targeting of Shp-2 led to embryonic lethality at day 10.5 and 8.5, respectively. Whether the exon 2 deletion is a null mutation remains to be determined, but as it turns out the exon 3 deletion gives rise to reduced (approximately 25% of wild type) expression of mutant Shp-2 with a deletion in the C-terminal SH2 domain. This mutant Shp-2 is not functional, since it does not bind to its normal targets (Saxton et al., 1997). Targeting of Shp-2 exon 3 led to improper gastrulation and Shp-$2^{exon3-/-}$ embryos died at mid-gestation with defects in the node, the notochord and in posterior elongation (Saxton et al., 1997). Analysis of MAP kinase activation in fibroblasts, derived from the Shp-$2^{exon3-/-}$ embryos, indicated that Shp-2 is essential for proper MAP kinase activation, since FGF-induced MAP kinase activation is transient in Shp-$2^{exon3-/-}$ cells and sustained in wild type cells. Moreover, MAP kinase activation is differentially affected, since PDGF-induced MAP kinase activation is identical in Shp-$2^{exon3-/-}$ and wild type fibroblasts (Saxton et al., 1997). Conceivably, the function of Shp-2 in signalling of some RPTKs, including the FGFR, is essential for normal early vertebrate development. Overexpression of dominant negative Shp-2 caused severe posterior truncations in *Xenopus* embryos. In addition, in ectodermal explants, dominant negative Shp-2 blocked FGF-induced mesoderm induction and MAP kinase activation (Tang et al., 1995), again suggesting that Shp-2 acts as a positive component in FGFR-signalling. Taken together, all of the observed phenotypes, including the ones in invertebrates, are consistent with the large body of biochemical evidence that Shp-2 is required for proper RPTK signalling.

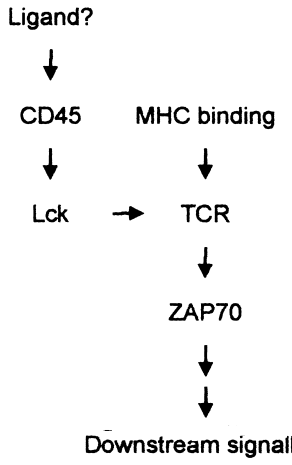

Fig. 5. CD45 potentiates T cell receptor (TCR) signalling. CD45 dephosphorylates and activates the PTK Lck (and Fyn, not depicted here), which phosphorylates multiple subunits of the TCR on tyrosine upon MHC binding to the TCR. These phosphorylated TCR subunits provide binding sites for downstream kinases, including ZAP70, which is phosphorylated by Lck and/or Fyn and becomes activated, resulting in downstream signalling. In the absence of CD45, Lck and Fyn are not activated, and MHC binding to the TCR does not lead to tyrosine phosphorylation of TCR subunits, nor to downstream signalling. It is not clear whether ligand binding to CD45 is involved in TCR potentiation.

4.2. Other cytoplasmic PTPs

Relatively little is known about the role of the other cytoplasmic PTPs in vertebrate development. Several PTPs have been knocked out by gene targeting, or by gene trapping. For instance, a gene trap insertion in PTP-BL produced a fusion between PTP-BL and β-galactosidase, allowing detailed analysis of PTP-BL expression during development (Thomas et al., 1998). PTP-BL is highly expressed in epithelial cells and in the peripheral nervous system, suggesting that PTP-BL plays an important role there. However, specific gene targeting by homologous recombination of PTP-BL did not induce obvious phenotypes in PTP-BL$^{-/-}$ mice, presumably due to redundancy (W. Hendriks, personal communication). Gene targeting of the T cell PTP, TC-PTP, which is highly expressed in haematopoietic cells led to specific defects in bone marrow, B cell lymphopoiesis and erythropoiesis, as well as in T and B cell functions. Deletion of TC-PTP is not embryonic lethal, but the TC-PTP$^{-/-}$ mice die 3–5 weeks after birth. The phenotype of the TC-PTP$^{-/-}$ mice demonstrates that TC-PTP plays an important role in both haemopoiesis and immune function (You-Ten et al., 1997). Recently, PTP1B was knocked out and PTP1B$^{-/-}$ mice developed normally. However, PTP1B$^{-/-}$ mice displayed enhanced sensitivity to insulin and were resistant to weight gain, consistent with a role for PTP1B in insulin receptor dephosphorylation (Elchebly et al., 1999a). Finally, PTP-PEST has been knocked out (Côté et al., 1998), which induces embryonic lethality around 8.5 days p.c. PTP-PEST$^{-/-}$ embryos display several abnormalities, from the central nervous system to the development of some cell lineages, derived from the endoderm (M. Tremblay, personal communication).

Interestingly, embryonic fibroblasts, derived from the PTP-PEST$^{-/-}$ embryos, facilitated identification of p130^{Cas}, and p130^{Cas}-family members, Hef1 and Sin as substrates of PTP-PEST (Côté et al., 1998). In addition, the PTP-PEST$^{-/-}$ fibroblasts provided evidence that PTP-PEST is involved in the breakdown of focal adhesions (Augers-Loustau et al., 1999), which may be why PTP-PEST is essential for early development. Taken together, we are beginning to get some insight into the role of PTPs in development, and since deletion of many cytoplasmic PTPs leads to embryonic lethality, it appears that these PTPs are essential for normal early development.

4.3. CD45

CD45 was the first RPTP to be identified, based on sequence homology to PTP1B in the intracellular domain (Tonks et al., 1988b; Charbonneau et al., 1988). The extracellular domain contains one FNIII-like domain (Fig. 2). Bona fide CD45 ligands are not known, and therefore it is not clear whether the CD45 ectodomain acts as a ligand binding domain. Many splice isoforms of CD45 have been identified, all of which are expressed exclusively in the haematopoietic system. Gene targeting of exon 6 was aimed at deletion of a specific isoform of CD45, but instead abolished expression of all CD45 isoforms in B cells and most T cells (Kishihara et al., 1993). T cell development was severely affected in CD45$^{exon6-/-}$ mice, while development of B cells appeared normal (Kishihara et al., 1993). However, significant defects in B cell maturation and signalling have been identified (Cyster et al., 1996). CD45-null mice were generated by targeting of the invariant exon 9, and here T cell development was disrupted even more severely at the CD4$^-$CD8$^-$ to CD4$^+$CD8$^+$ transition and later when CD4$^+$CD8$^+$ cells develop into CD4$^+$ or CD8$^+$ thymocytes (Byth et al., 1996).

CD45 potentiates T cell receptor (TCR) and B cell receptor (BCR) activation and over the past decade much emphasis has been placed on elucidation of the mechanism by which CD45 is involved in TCR and BCR potentiation, leading to detailed insight into the process (Fig. 5) (reviewed by Frearson and Alexander, 1997). CD45 dephosphorylates the inhibitory C-terminal phosphorylation site in Lck and Fyn, thereby activating these Src-family PTKs (Mustelin et al., 1989; Ostergaard et al., 1989; Shiroo et al., 1992). Upon ligation of the TCR, activated Lck and possibly also Fyn phosphorylate components of the TCR, including CD3-ε and TCR-ζ on Immunoreceptor Tyrosine-based Activation Motifs (ITAMs). Phosphorylated ITAMs provide binding sites for SH2-containing signalling molecules, including the PTK, ZAP-70, leading to subsequent intracellular signalling (reviewed by Qian and Weiss, 1997). In thymocytes that lack CD45, Lck and Fyn are not

activated and engagement of the TCR does not induce tyrosine phosphorylation of the TCR, nor any of the downstream events, demonstrating that CD45 is essential for TCR signalling. Although it has not been proven formally, it is tempting to speculate that CD45-mediated potentiation of the TCR is involved in T cell development.

4.4. LAR subfamily

The mammalian LAR subfamily consists of three members, LAR, RPTPδ and RPTPσ. Due to alternative splicing, many different isoforms exist of these three RPTPs that may have different functions (Pulido et al., 1995). During mouse development, these three RPTPs are differentially expressed. LAR is highly expressed in the basal lamina-associated epithelial tissues of (neuro)ectodermal, neural crest/ectomesenchyme and endodermal origin. RPTPδ and RPTPσ expression largely overlap with high expression in (neuro)ectodermal, neural crest- and mesodermal-derived tissues (Schaapveld et al., 1998). Since RPTPδ and RPTPσ are highly expressed in the developing nervous system, these RPTPs may be the homologs of DLAR, rather than mammalian LAR itself. Further evidence, supporting this hypothesis is that gene targeting of LAR, using a gene trap approach did not induce an obvious neuronal phenotype (Skarnes et al., 1995). Close examination of mice with a gene trap insertion in the LAR gene, in which expression of full length LAR is greatly reduced, indicated that the size of cholinergic neurons was significantly reduced and that cholinergic innervation of the dentate gyrus was markedly decreased (Yeo et al., 1997). Schaapveld et al. (1997) generated mice lacking the sequences encoding both LAR PTP domains by gene targeting. $LAR^{PTP-/-}$ mice are viable and do not display an obvious neuronal phenotype. In fact, these mice develop normally, without obvious histological abnormalities. However, $LAR^{PTP-/-}$ females have mammary gland defects during gestation, due to impaired terminal differentiation of alveoli at late pregnancy, resulting in defective milk production (Schaapveld et al., 1997). From these $LAR^{PTP-/-}$ mice it is clear that LAR PTP activity is not essential for proper development of the mammalian nervous system (Schaapveld et al., 1997). However, RPTPδ and RPTPσ may take over LAR's function in $LAR^{-/-}$ mice. $RPTP\delta^{-/-}$ mice are viable and appear to display growth retardation, early mortality, abnormal posture and dramatic motor defects (Uetani et al., 1997), while $RPTP\sigma^{-/-}$ mice display pituitary dysplasia, defects in the olfactory lobes and a reduction in the total central nervous system size and cell number (Elchebly et al., 1999b; Wallace et al., 1999). It is likely that the highly homologous LAR family members have redundant functions. In fact, all three family members associate with LAR-interacting proteins, including LIP-1 (Pulido et al., 1995), and Liprins (Serra-Pagès et al., 1998), suggesting that indeed downstream signalling is similar. In order to obtain insight into the function of the LAR subfamily, double or even triple knock-out mice will have to be generated.

4.5. MAM-domain containing RPTPs

The MAM-domain containing RPTPs, RPTPμ, RPTPκ and PCP2 are differentially expressed during vertebrate development with distinct and complementary expression patterns, suggesting that these RPTPs play a role in organogenesis and in tissue formation (Fuchs et al., 1998). However, functional inactivation of RPTPκ (Skarnes et al., 1995) and gene targeting of RPTPμ (M.F.B.G. Gebbink, personal communication) did not induce obvious phenotypes. Perhaps the phenotypes are subtle and much work is needed to elucidate the in vivo function of these RPTPs. Alternatively, redundancy may be responsible for the lack of phenotypes, although this is unlikely since the type IIb RPTPs are not co-expressed in the same cells (Fuchs et al., 1998).

Cell biological experiments indicated that the extracellular domains of RPTPμ and RPTPκ engage in homophilic interactions, suggesting that RPTPμ and RPTPκ are involved in cell-cell interactions (Brady-Kalnay et al., 1993; Gebbink et al., 1993; Sap et al., 1994). In addition, further evidence suggests that the MAM-subfamily of RPTPs interacts with cadherins and catenins. For instance, RPTPκ binds to β-catenin and plakoglobin (γ-catenin) in vivo and in vitro, and β-catenin may be a substrate of RPTPκ (Fuchs et al., 1996). Initially, such an interaction between a RPTP and the cadherin-catenin complex was demonstrated for the archetypical MAM-domain containing PTP, RPTPμ (Brady-Kalnay et al., 1995). The interaction between RPTPμ and cadherins has been the subject of debate (Zondag et al., 1996), but recent evidence strongly suggests that RPTPμ interacts with cadherins (Brady-Kalnay et al., 1998). Whether RPTPμ is involved in dephosphorylation of the cadherin-catenin complex remains to be determined. Although the function of the MAM-domain containing RPTPs in development remains elusive at the moment, it is highly likely that this subfamily of RPTPs plays a role in cell-cell interactions, which may be mediated by the interaction with the cadherin-catenin complex.

4.6. RPTPα

The RPTPα-RPTPε subfamily of RPTPs is characterized by a very short extracellular domain. RPTPε has the shortest extracellular domain (27 residues), while RPTPα (123 residues) is the best characterized of the two. RPTPα mRNA and protein are highly expressed in mouse dorsal root ganglia and cranial sensory ganglia. In addition, RPTPα expression is enhanced in the adrenal gland, indicating that RPTPα is highly expressed in derivatives of the neural crest (den Hertog et al., 1996). During chicken development, RPTPα mRNA expression is enhanced in pre-migratory and migrating granule cells, as well as in Bergmann glia and their radial processes (Fang et al., 1996). A detailed analysis of

RPTPα expression in the retinotectal system of the chick indicates that RPTPα expression is restricted to Muller glia cells and radial glia of the retina and of the tectum (Ledig et al., 1999). These expression data suggest that RPTPα has a function in derivatives of the neural crest and/or in glia cells.

Gene targeting of RPTPα did not result in obvious developmental defects (J. Sap, personal communication). RPTPε knock-out mice do not have an obvious developmental phenotype, although they may display subtle physiological defects (A. Elson, personal communication). The lack of obvious developmental phenotypes in RPTPα and RPTPε knock-out mice suggests that the function of these RPTPs is taken over by related RPTPs (possibly by each other) or that the phenotypes are rather subtle. A double knock-out with a deletion of both RPTPα and RPTPε may provide insight into the role of this subfamily of RPTPs in development.

One of the first indications that RPTPα is involved in developmental processes, came from in vitro studies using pluripotent P19 embryonal carcinoma cells. During neuronal differentiation of P19 cells, RPTPα expression is transiently induced with highest levels of expression preceding the neuronal phenotype. Overexpression of RPTPα altered the differentiation fate of P19 EC cells, in that retinoic acid induced neuronal differentiation instead of endodermal and mesodermal differentiation. Neuronally differentiated RPTPα-expressing P19 cells formed bona fide terminally differentiated neurons, illustrated by their electrical excitability (den Hertog et al., 1993).

The mechanism by which RPTPα is involved in neuronal differentiation remains to be determined. The PTK Src may be a component of the RPTPα-signalling pathway. Src is negatively regulated by Csk-mediated tyrosine phosphorylation in its C-terminus (at position 527) (Nada et al., 1991). Overexpression of RPTPα leads to dephosphorylation and thus activation of Src in Rat Embryo Fibroblasts (REFs) and P19 EC cells (Zheng et al., 1992; den Hertog et al., 1993). In addition, RPTPα dephosphorylates Src P.Tyr527 in vitro, suggesting that the effect observed in REFs and in P19 cells is direct (den Hertog et al., 1993). Moreover, Src activity is reduced in RPTPα$^{-/-}$ embryonic fibroblasts, strongly suggesting that Src is a substrate of RPTPα (Ponniah et al., 1999; Su et al., 1999).

The adaptor protein GRB2 has been identified to bind to tyrosine phosphorylated RPTPα in vivo and in vitro, and thus may act as an effector protein of RPTPα (den Hertog et al., 1994; Su et al., 1994). However, GRB2-binding appears not to be involved in the RPTPα-induced phenotype in P19 cells, since mutant RPTPα-Y789F that does not bind GRB2 still alters the differentiation fate of P19 cells in favour of neuronal differentiation (J. den Hertog, unpublished data). Not only the GRB2 SH2 domain, but also the C-terminal SH3 domain is involved in binding to RPTPα (den Hertog and Hunter, 1996; Su et al., 1996). The region in RPTPα that is involved in binding of the SH3 domain suggests that GRB2 may be involved in negative regulation of RPTPα activity, since the C-terminal SH3 domain presumably blocks access to the catalytic site of RPTPα-D1. Future work will provide insight into the role of GRB2-binding in the function of RPTPα.

5. Conclusion

Much progress has been made over the past couple of years in elucidation of the function of PTPs in development. However, the function of many PTPs still remains elusive. From the functional analyses that have been done with PTPs to date, using mutant flies and worms, and using knock-out mice, a common theme is emerging that cytoplasmic PTPs play a role early during development with severe defects upon inactivation of these genes, while the RPTPs have a more subtle function later during development, which is redundant in most cases as well. As a result, especially the function of the RPTPs in vertebrate development is unclear at the moment and insight may require generation of mice with multiple targeted RPTP genes, knocking out whole subfamilies of RPTPs.

Acknowledgements

I would like to thank Jan Sap, Ari Elson, Michel Tremblay, Martijn Gebbink and Wiljan Hendriks for discussing the phenotype of their knock-out mice prior to publication, and Arjan Buist, Christophe Blanchetot and Astrid van der Sar for critical reading of the manuscript. Work in my laboratory is supported by grants from the Dutch Cancer Society (NKB/KWF) and from the Earth and Life Sciences Foundation/Netherlands Organization for Scientific Research (ALW/NWO).

References

Allard, J.D., Chang, H.C., Herbst, R., McNeill, H., Simon, M.A., 1996. The SH2-containing tyrosine phosphatase corkscrew is required during signaling by sevenless. Ras1 and Raf. Development 122, 1137–1146.

Angers-Loustau, A., Cote, J.F., Charest, A., Dowbenko, D., Spencer, S., Lasky, L.A., Tremblay, M.L., 1999. Protein tyrosine phosphatase-PEST regulates focal adhesion disassembly, migration, and cytokinesis in fibroblasts. J. Cell Biol. 144, 1019–1031.

Arrandale, J.M., Gore-Willse, A., Rocks, S., Ren, J.M., Zhu, J., Davis, A., Livingston, J.N., Rabin, D.U., 1996. Insulin signaling in mice expressing reduced levels of Syp. J. Biol. Chem. 271, 21353–21358.

Bilwes, A.M., den Hertog, J., Hunter, T., Noel, J.P., 1996. Structural basis for inhibition of receptor protein-tyrosine phosphatase-alpha by dimerization. Nature 382, 555–559.

Brady-Kalnay, S.M., Tonks, N.K., 1994. Identification of the homophilic binding site of the receptor protein tyrosine phosphatase PTP mu. J. Biol. Chem. 269, 28472–28477.

Brady-Kalnay, S.M., Flint, A.J., Tonks, N.K., 1993. Homophilic binding of PTP mu, a receptor-type protein tyrosine phosphatase, can mediate cell-cell aggregation. J. Cell Biol. 122, 961–972.

Brady-Kalnay, S.M., Rimm, D.L., Tonks, N.K., 1995. Receptor protein tyrosine phosphatase PTP mu associates with Cadherins and Catenins in vivo. J. Cell Biol. 130, 977–986.

Brady-Kalnay, S.M., Mourton, T., Nixon, J.P., Pietz, G.E., Kinch, M., Chen, H., Brackenburry, R., Rimm, D.L., Del Vecchio, R.L., Tonks, N.K., 1998. Dynamic interactions of PTP mu with multiple cadherins in vivo. J. Cell Biol. 141, 287–296.

Buist, A., Zhang, Y.L., Keng, Y.F., Wu, L., Zhang, Z.Y., den Hertog, J., 1999. Restoration of potent protein-tyrosine phosphatase activity in the membrane distal domain of Receptor Protein-Tyrosine Phosphatase alpha. Biochemistry 38, 914–922.

Byth, K.F., Conroy, L.A., Howlett, S., Smith, A.J., May, J., Alexander, D.R., Holmes, N., 1996. CD45-null transgenic mice reveal a positive regulatory role for CD45 in early thymocyte development, in the selection of CD4 + CD8 + thymocytes, and B cell maturation. J. Exp. Med. 183, 1707–1718.

Charbonneau, H., Tonks, N.K., Walsh, K.A., Fischer, E.H., 1988. The leukocyte common antigen (CD45): a putative receptor-linked protein tyrosine phosphatase. Proc. Natl. Acad. Sci. USA 85, 7182–7186.

Charest, A., Wagner, J., Shen, S.H., Tremblay, M.L., 1995. Murine protein tyrosine phosphatase-PEST, a stable cytosolic protein tyrosine phosphatase. Biochem. J. 308, 425–432.

Chien, C.B., 1996. PY in the fly. Receptor-like tyrosine phosphatases in axonal pathfinding. Neuron 16, 1065–1068.

Cleghon, V., Feldmann, P., Ghiglione, C., Copeland, T.D., Perrimon, N., Hughes, D.A., Morrison, D.K., 1998. Opposing actions of CSW and RasGAP Modulate the strength of Torso RTK Signaling in the Drosophila terminal pathway. Mol. Cell 2, 719–727.

Côté, J.F., Charest, A., Wagner, J., Tremblay, M.L., 1998. Combination of gene targeting and substrate trapping to identify substrates of protein tyrosine phosphatases using PTP-PEST as a model. Biochemistry 37, 13128–13137.

Cyster, J.G., Healy, J.I., Kishihara, K., Mak, T.W., Thomas, M.L., Goodnow, C.C., 1996. Regulation of B lymphocyte negative and positive selection by tyrosine phosphatase CD45. Nature 381, 325–328.

Debant, A., Serra-Pagès, C., Seipel, K., O'Brien, S., Tang, M., Park, S.H., 1996. The multidomain protein Trio binds the LAR transmembrane tyrosine phosphatase, contains a protein kinase domain, and has separate rac- specific and rho-specific guanine nucleotide exchange factor domains. Proc. Natl. Acad. Sci. USA 93, 5466–5471.

den Hertog, J., Hunter, T., 1996. Tight association of GRB2 with receptor protein tyrosine phosphatase alpha is mediated by the SH2 and C-terminal SH3 domains. EMBO J. 15, 3016–3027.

den Hertog, J., Pals, C.E., Peppelenbosch, M.P., Tertoolen, L.G., de Laat, S.W., Kruijer, W., 1993. Receptor protein tyrosine phosphatase alpha activates pp60c-src and is involved in neuronal differentiation. EMBO J. 12, 3789–3798.

den Hertog, J., Tracy, S., Hunter, T., 1994. Phosphorylation of receptor protein-tyrosine phosphatase alpha on Tyr789, a binding site for the SH3-SH2-SH3 adaptor protein GRB-2 in vivo. EMBO J. 13, 3020–3032.

den Hertog, J., Overvoorde, J., de Laat, S.W., 1996. Expression of receptor protein-tyrosine phosphatase alpha mRNA and protein during mouse embryogenesis. Mech. Dev. 58, 89–101.

Denu, J.M., Stuckey, J.A., Saper, M.A., Dixon, J.E., 1996. Form and function in protein dephosphorylation. Cell 87, 361–364.

Desai, D.M., Sap, J., Schlessinger, J., Weiss, A., 1993. Ligand-mediated negative regulation of a chimeric transmembrane receptor tyrosine phosphatase. Cell 73, 541–554.

Desai, D.M., Sap, J., Silvennoinen, O., Schlessinger, J., Weiss, A., 1994. The catalytic activity of the CD45 membrane-proximal phosphatase domain is required for TCR signaling and regulation. EMBO J. 13, 4002–4010.

Desai, C.J., Gindhart Jr, J.G., Goldstein, L.S., Zinn, K., 1996. Receptor tyrosine phosphatases are required for motor axon guidance in the Drosophila embryo. Cell 84, 599–609.

Desai, C.J., Krueger, N.X., Saito, H., Zinn, K., 1997. Competition and cooperation among receptor tyrosine phosphatases control motoneuron growth cone guidance in Drosophila. Development 124, 1941–1952.

DeVore, D.L., Horvitz, H.R., Stern, M.J., 1995. An FGF receptor signaling pathway is required for the normal cell migrations of the sex myoblasts in C. elegans hermaphrodites. Cell 83, 611–620.

Elchebly, M., et al., 1999a. Increased insulin sensitivity and obesity resistance in mice lacking the Protein Tyrosine Phosphatase-1B gene. Science 283, 1544–1548.

Elchebly, M., Wagner, J., Kennedy, T.E., Lanctot, C., Michaliszyn, E., Itie, A., Drouin, J., Tremblay, M.L., 1999b. Neuroendocrine dysplasia in mice lacking protein tyrosine phosphatase sigma. Nat. Genet. 21, 330–333.

Fang, K.S., Martins-Green, M., Williams, L.T., Hanafusa, H., 1996. Characterization of chicken protein tyrosine phosphatase alpha and its expression in the central nervous system. Brain Res. Mol. Brain Res. 37, 1–14.

Fauman, E.B., Saper, M.A., 1996. Structure and function of the protein tyrosine phosphatases. Trends Biochem. Sci. 21, 413–417.

Felberg, J., Johnson, P., 1998. Characterization of recombinant CD45 cytoplasmic domain proteins. Evidence for intramolecular and intermolecular interactions. J. Biol. Chem. 273, 17839–17845.

Feng, G.S., Pawson, T., 1994. Phosphotyrosine phosphatases with SH2 domains: regulators of signal transduction. Trends Genet. 10, 54–58.

Fischer, E.H., Charbonneau, H., Tonks, N.K., 1991. Protein tyrosine phosphatases: a diverse family of intracellular and transmembrane enzymes. Science 253, 401–406.

Frangioni, J.V., Beahm, P.H., Shifrin, V., Jost, C.A., Neel, B.G., 1992. The nontransmembrane tyrosine phosphatase PTP-1B localizes to the endoplasmic reticulum via its 35 amino acid C-terminal sequence. Cell 68, 545–560.

Frangioni, J.V., Oda, A., Smith, M., Salzman, E.W., Neel, B.G., 1993. Calpain-catalyzed cleavage and subcellular relocation of protein phosphotyrosine phosphatase 1B (PTP-1B) in human platelets. EMBO J. 12, 4843–4856.

Frearson, J.A., Alexander, D.R., 1997. The role of phosphotyrosine phosphatases in haematopoietic cell signal transduction. Bioessays 19, 417–427.

Fuchs, M., Mueller, T., Lerch, M.M., Ullrich, A., 1996. Association of human protein tyrosine phosphatase kappa with members of the armadillo family. J. Biol. Chem. 271, 16712–16719.

Fuchs, M., Wang, H., Ciossek, T., Chen, Z., Ullrich, A., 1998. Differential expression of MAM-subfamily protein tyrosine phosphatases during mouse development. Mech. Dev. 70, 91–109.

Gamper, M., Howard, P.K., Hunter, T., Firtel, R.A., 1996. Multiple roles of the novel protein tyrosine phosphatase PTP3 during Dictyostelium growth and development. Mol. Cell Biol. 16, 2431–2444.

Garton, A.J., Burnham, M.R., Bouton, A.H., Tonks, N.K., 1997. Association of PTP-PEST with the SH3 domain of p130cas: a novel mechanism of protein tyrosine phosphatase substrate recognition. Oncogene 15, 877–885.

Gebbink, M.F., Zondag, G.C., Wubbolts, R.W., Beijersbergen, R.L., van Etten, I., Moolenaar, W.H., 1993. Cell-cell adhesion mediated by a receptor-like protein tyrosine phosphatase. J. Biol. Chem. 268, 16101–16104.

Gershon, T.R., Baker, M.W., Nitabach, M., Macagno, E.R., 1998. The leech receptor protein tyrosine phosphatase HmLAR2 is concentrated in growth cones and is involved in process outgrowth. Development 125, 1183–1190.

Guan, K.L., Dixon, J.E., 1991. Evidence for protein-tyrosine-phosphatase catalysis proceeding via a cysteine-phosphate intermediate. J. Biol. Chem. 266, 17026–17030.

Gutch, M.J., Flint, A.J., Keller, J., Tonks, N.K., Hengartner, M.O., 1998. The Caenorhabditis elegans SH2 domain-containing protein tyrosine phosphatase PTP-2 participates in signal transduction during oogenesis and vulval development. Genes Dev. 12, 571–585.

Herbst, R., Carroll, P.M., Allard, J.D., Schilling, J., Raabe, T., Simon, M.A., 1996. Daughter of sevenless is a substrate of the phosphotyrosine phosphatase Corkscrew and functions during sevenless signaling. Cell 85, 899–909.

Hof, P., Pluskey, S., Dhe-Paganon, S., Eck, M.J., Shoelson, S.E., 1998. Crystal structure of the tyrosine phosphatase SHP-2. Cell 92, 441–450.

Hoffmann, K.M.V., Tonks, N.K., Barford, D., 1997. The crystal structure of domain 1 of receptor protein-tyrosine phosphatase mu. J. Biol. Chem. 272, 27505–27508.

Howard, P.K., Sefton, B.M., Firtel, R.A., 1992. Analysis of a spatially regulated phosphotyrosine phosphatase identifies tyrosine phosphorylation as a key regulatory pathway in Dictyostelium. Cell 71, 637–647.

Hunter, T., 1998. Anti-phosphatases take the stage. Nat. Genet. 18, 303–305.

Kaufmann, N., Wills, Z.P., Van Vactor, D., 1998. Drosophila Rac1 controls motor axon guidance. Development 125, 453–461.

Kishihara, K., Penninger, J., Wallace, V.A., Kundig, T.M., Kawai, K., Wakeham, A., Timms, E., Pfeffer, K., Ohashi, P.S., Thomas, M.L., Furlonger, C., Paige, C.J., Mak, T.W., 1993. Normal B lymphocyte development but impaired T cell maturation in CD45-exon6 protein tyrosine phosphatase-deficient mice. Cell 74, 143–156.

Kokel, M., Borland, C.Z., Delong, L., Horvitz, H.R., Stern, M.J., 1998. clr-1 encodes a receptor tyrosine phosphatase that negatively regulates an FGF receptor signaling pathway in Caenorhabditis elegans. Genes. Dev. 12, 1425–1437.

Krueger, N.X., Streuli, M., Saito, H., 1990. Structural diversity and evolution of human receptor-like protein tyrosine phosphatases. EMBO J. 9, 3241–3252.

Krueger, N.X., Van Vactor, D., Wan, H.I., Gelbart, W.M., Goodman, C.S., Saito, H., 1996. The transmembrane tyrosine phosphatase DLAR controls motor axon guidance in Drosophila. Cell 84, 611–622.

Ledig, M.M., McKinnell, I.W., Mrsic Flogel, T., Wang, J., Mason, I., Alvares, C., Bixby, J.L., Mueller, B.K., Stoker, A.W., 1999. Expression of receptor tyrosine phosphatases during development of the retinotectal projection of the chick. J. Neurobiol. 39, 81–96.

Li, D.M., Sun, H., 1997. TEP1, encoded by a candidate tumor suppressor locus, is a novel protein tyrosine phosphatase regulated by transforming growth factor beta. Cancer Res. 57, 2124–2129.

Li, J., Yen, C., Liaw, D., Podsypanina, K., Bose, S., Wang, S.I., Puc, J., Miliaresis, C., Rodgers, L., McCombie, R., Bigner, S.H., Giovanella, B.C., Ittmann, M., Tycko, B., Hibshoosh, H., Wigler, M.H., Parsons, R., 1997. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 275, 1943–1947.

Lim, K.L., Kolatkar, P.R., Ng, K.P., Ng, C.H., Pallen, C.J., 1998. 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. 273, 28986–28993.

Maehama, T., Dixon, J.E., 1998. The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, Phosphatidylinositol 3,4,5-Trisphosphate. J. Biol. Chem. 273, 13375–13378.

Majeti, R., Bilwes, A.M., Noel, J.P., Hunter, T., Weiss, A., 1998. Dimerization-induced inhibition of receptor protein tyrosine phosphatase function through an inhibitory wedge. Science 279, 88–91.

Mauro, L.J., Dixon, J.E., 1994. 'Zip codes' direct intracellular protein tyrosine phosphatases to the correct cellular 'address'. Trends Biochem. Sci. 19, 151–155.

McLaughlin, S., Dixon, J.E., 1993. Alternative splicing gives rise to a nuclear protein tyrosine phosphatase in Drosophila. J. Biol. Chem. 268, 6839–6842.

Mustelin, T., Coggeshall, K.M., Altman, A., 1989. Rapid activation of the T-cell tyrosine protein kinase pp561ck by the CD45 phosphotyrosine phosphatase. Proc. Natl. Acad. Sci. USA 86, 6302–6306.

Myers, M.P., Pass, I., Batty, I.H., van der Kaay, J., Stolarov, J.P., Hemmings, B.A., Wigler, M.H., Downes, C.P., Tonks, N.K., 1998. The lipid phosphatase activity of PTEN is critical for its tumor supressor function. Proc. Natl. Acad. Sci. USA 95, 13513–13518.

Nada, S., Okada, M., Macauley, A., Cooper, J.A., Nakagawa, H., 1991. Cloning of a complementary DNA for a protein-tyrosine kinase that specifically phosphorylates a negative regulatory site of p60c-src. Nature 351, 69–72.

Neel, B.G., Tonks, N.K., 1997. Protein tyrosine phosphatases in signal transduction. Curr. Opin. Cell Biol. 9, 193–204.

O'Grady, P., Thai, T.C., Saito, H., 1998. The laminin-nidogen complex is a ligand for a specific splice isoform of the transmembrane protein tyrosine phosphatase LAR. J. Cell Biol. 141, 1675–1684.

Ostergaard, H.L., Shackelford, D.A., Hurley, T.R., Johnson, P., Hyman, R., Sefton, B.M., Trowbridge, I.S., 1989. Expression of CD45 alters phosphorylation of the Ick-encoded tyrosine protein kinase in murine lymphoma T-cell lines. Proc. Natl. Acad. Sci. USA 86, 8959–8963.

Peles, E., Nativ, M., Campbell, P.L., Sakurai, T., Martinez, R., Lev, S., Clary, D.O., Schilling, J., Barnea, G., Plowman, G.D., Schlessinger, J., 1995. The carbonic anhydrase domain of receptor tyrosine phosphatase beta is a functional ligand for the axonal cell recognition molecule contactin. Cell 82, 251–260.

Peles, E., Nativ, M., Lustig, M., Grumet, M., Schilling, J., Martinez, R., Plowman, G.D., Schlessinger, J., 1997. Identification of a novel contactin-associated transmembrane receptor with multiple domains implicated in protein-protein interactions. EMBO J. 16, 978–988.

Perkins, L.A., Larsen, I., Perrimon, N., 1992. Corkscrew encodes a putative protein tyrosine phosphatase that functions to transduce the terminal signal from the receptor tyrosine kinase torso. Cell 70, 225–236.

Perkins, L.A., Johnson, M.R., Melnick, M.B., Perrimon, N., 1996. The nonreceptor protein tyrosine phosphatase corkscrew functions in multiple receptor tyrosine kinase pathways in Drosophila. Dev. Biol. 180, 63–81.

Ponniah, S., Wang, D.Z., Lim, K.L., Pallen, C.J., 1999. Targeted disruption of the tyrosine phosphatase PTPalpha leads to constitutive downregulation of the kinases src and Fyn. Curr. Biol. 9, 535–538.

Pot, D.A., Dixon, J.E., 1992. Active site labeling of a receptor-like protein tyrosine phosphatase. J. Biol. Chem. 267, 140–143.

Pulido, R., Serra-Pagès, C., Tang, M., Streuli, M., 1995. The LAR/PTP delta/PTP sigma subfamily of transmembrane protein-tyrosine- phosphatases: multiple human LAR, PTP delta, and PTP sigma isoforms are expressed in a tissue-specific manner and associate with the LAR-interacting protein LIP.1. Proc. Natl. Acad. Sci. USA 92, 11686–11690.

Qian, D., Weiss, A., 1997. T cell antigen receptor signal transduction. Curr Opin. Cell Biol. 9, 205–212.

Raabe, T., Riesgo-Escovar, J., Liu, X., Bausenwein, B.S., Deak, P., Maroy, P., Hafen, E., 1996. DOS, a novel pleckstrin homology domain-containing protein required for signal transduction between sevenless and Ras1 in Drosophila. Cell 85, 911–920.

Sakurai, T., Lustig, M., Nativ, M., Hemperly, J.J., Schlessinger, J., Peles, E., Grumet, M., 1997. Induction of neurite outgrowth through contactin and Nr-CAM by extracellular regions of glial receptor tyrosine phosphatase beta. J. Cell Biol. 136, 907–918.

Sap, J., Jiang, Y.P., Friedlander, D., Grumet, M., Schlessinger, J., 1994. Receptor tyrosine phosphatase R-PTP-kappa mediates homophilic binding. Mol. Cell Biol. 14, 1–9.

Saras, J., Heldin, C.H., 1996. PDZ domains bind carboxy-terminal sequences of target proteins. Trends Biochem. Sci. 21, 455–458.

Saxton, I.M., Henkemeyer, M., Gasca, S., Shen, R., Rossi, D.J., Shalaby, F., Feng, G.S., Pawson, T., 1997. Abnormal mesoderm patterning in mouse embryos mutant for the SH2 tyrosine phosphatase Shp-2. EMBO J. 16, 2352–2364.

Schaapveld, R.Q., Schepens, J.T., Robinson, G.W., Attema, J., Oerlemans, F.T., Fransen, J.A., Streuli, M., Wieringa, B., Hennighausen, L., Hendriks, W.J., 1997. Impaired mammary gland development and function in mice lacking LAR receptor-like tyrosine phosphatase activity. Dev. Biol. 188, 134–146.

Schaapveld, R.Q.J., Schepens, J.T.G., Baechner, D., Attema, J., Wieringa, B., Jap, P.H.K., Hendriks, W.J.A.J., 1998. Developmental expression of the cell adhesion molecule-like protein tyrosine phosphatases LAR, RPTP delta and RPTP sigma in the mouse. Mech. Dev. 77, 59–62.

Serra-Pagès, C., Kedersha, N.L., Fazikas, L., Medley, Q., Debant, A., Streuli, M., 1995. The LAR transmembrane protein tyrosine phosphatase and a coiled-coil LAR-interacting protein co-localize at focal adhesions. EMBO J. 14, 2827–2838.

Serra-Pagès, C., Medley, Q.G., Tang, M., Hart, A., Streuli, M., 1998. Liprins, a family of LAR transmembrane protein-tyrosine phosphatase-interacting proteins. J. Biol. Chem. 273, 15611–15620.

Shiroo, M., Goff, L., Biffen, M., Shivnan, E., Alexander, D., 1992. CD45 tyrosine phosphatase-activated couples the T cell antigen receptor to pathways of diacylglycerol production, protein kinase C activation and calcium influx. EMBO J. 11, 4887–4897.

Shultz, L.D., Schweitzer, P.A., Rajan, T.V., Yi, T., Ihle, J.N., Matthews, R.J., Thomas, M.L., Beer, D.R., 1993. Mutations at the murine motheaten locus are within the hematopoietic cell protein-tyrosine phosphatase (Hcph) gene. Cell 73, 1445–1454.

Skarnes, W.C., Moss, J.E., Hurtley, S.M., Beddington, R.S., 1995. Capturing genes encoding membrane and secreted proteins important for mouse development. Proc. Natl. Acad. Sci. USA 92, 6592–6596.

Steck, P.A., Pershouse, M.A., Jasser, S.A., Yung, W.K., Lin, H., Ligon, A.H., Langford, L.A., Baumgard, M.L., Hattier, T., Davis, T., Frye, C., Hu, R., Swedlund, B., Teng, D.H., Tavtigian, S.V., 1997. Identification of a candidate tumour suppressor gene, MMAC1 at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat. Genet. 15, 356–362.

Steven, R., Kubiseski, T.J., Zheng, H., Kulkarni, S., Mancillas, J., Ruiz Morales, A., Hogue, C.W.V., Pawson, T., Culotti, J., 1998. UNC-73 activates the Rac GTPase and is required for cell and growth cone migrations in C. elegans. Cell 92, 785–795.

Stoker, A., Dutta, R., 1998. Protein tyrosine phosphatases and neural development. Bioessays 20, 463–472.

Su, J., Batzer, A., Sap, J., 1994. Receptor tyrosine phosphatase R-PTP-alpha is tyrosine-phosphorylated and associated with the adaptor protein Grb2. J. Biol. Chem. 269, 18731–18734.

Su, J., Muranjan, M., Sap, J., 1999. Receptor protein tyrosine phosphatase alpha activates src-family kinases and control integrin-mediated responses in fibroplasts. Curr. Biol. 9, 505–511.

Su, J., Yang, L.T., Sap, J., 1996. 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. 271, 28086–28096.

Sun, H., Charles, C.H., Lau, L.F., Tonks, N.K., 1993. MKP-1 (3CH134), an immediate early gene product, is a dual specificity phosphatase that dephosphorylates MAP kinase in vivo. Cell 75, 487–493.

Tang, T.L., Freeman Jr, R.M., O'Reilly, A.M., Neel, B.G., Sokol, S.Y., 1995. The SH2-containing protein-tyrosine phosphatase SH-PTP2 is required upstream of MAP kinase for early Xenopus development. Cell 80, 473–483.

Thomas, T., Voss, A.K., Gruss, P., 1998. Distribution of a murine protein tyrosine phosphatase BL-beta-galactosidase fusion protein suggests a role in neurite outgrowth. Dev. Dyn. 212, 250–257.

Tian, S.S., Tsoulfas, P., Zinn, K., 1991. Three receptor-linked protein-tyrosine phosphatases are selectively expressed on central nervous system axons in the Drosophila embryo. Cell 67, 675–680.

Tonks, N.K., Diltz, C.D., Fischer, E.H., 1988. Purification of the major protein-tyrosine-phosphatases of human placenta. J. Biol. Chem. 263, 6722–6730.

Tonks, N.K., Charbonneau, H., Diltz, C.D., Fischer, E.H., Walsh, K.A., 1988. Demonstration that the leukocyte common antigen CD45 is a protein tyrosine phosphatase. Biochemistry 27, 8695–8701.

Uetani, N., Asano, M., Mizuno, K., Yakura, H., Iwakura, Y., 1997. Targeted disruption of the murine protein tyrosine phosphatase delta (MPTPdelta) gene results in growth retardation and behavioral abnormalities. In: Yakura, H. (Ed.). Kinases and Phosphatases in Lymphocyte and Neuronal Signaling, Springer, Berlin, pp. 313.

Van Vactor, D., O'Reilly, A.M., Neel, B.G., 1998. Genetic analysis of protein tyrosine phosphatases. Curr. Opin. Genet. Dev. 8, 112–126.

Van Vactor, D., 1998. Protein tyrosine phosphatases in the developing nervous system. Curr. Opin. Cell Biol. 10, 174–181.

Wallace, M.J., Fladd, C., Batt, J., Rotin, D., 1998. 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. 18, 2608–2616.

Wallace, M.J., Batt, J., Fladd, C.A., Henderson, J.T., Skarnes, W., Rotin, D., 1999. Neuronal defects and posterior pituitary hypoplasia in mice lacking the receptor tyrosine phosphatase PTPsigma. Nat. Genet. 21, 334–338.

Wang, Y., Pallen, C.J., 1991. The receptor-like protein tyrosine phosphatase HPTP alpha has two active catalytic domains with distinct substrate specificities. EMBO J. 10, 3231–3237.

Weiss, A., Schlessinger, J., 1998. Switching signals on or off by receptor dimerization. Cell 94, 277–280.

Wills, Z., Bateman, J., Korey, C.A., Comer, A., Van Vactor, D., 1999. The tyrosine kinase Abl and its substrate Enabled collaborate with the receptor phosphatase Dlar to control motor axon guidance. Neuron 22, 301–312.

Wu, L., Buist, A., den Hertog, J., Zhang, Z.Y., 1997. Comparative kinetic analysis and substrate specificity of the tandem catalytic domains of the receptor-like protein-tyrosine phosphatase alpha. J. Biol. Chem. 272, 6994–7002.

Yang, X.H., Seow, K.T., Bahri, S.M., Oon, S.H., Chia, W., 1991. Two Drosophila receptor-like tyrosine phosphatase genes are expressed in a subset of developing axons and pioneer neurons in the embryonic CNS. Cell 67, 661–673.

Yeo, T.T., Yang, T., Massa, S.M., Zhang, J.S., Honkaniemi, J., Butcher, L.L., Longo, F.M., 1997. Deficient LAR expression decreases basal forebrain cholinergic neuronal size and hippocampal cholinergic innervation. J. Neurosci. Res. 47, 348–360.

Yi, T.L., Cleveland, J.L., IhIe, J.N., 1992. Protein tyrosine phosphatase containing SH2 domains: characterization, preferential expression in hematopoietic cells, and localization to human chromosome 12p12-p13. Mol. Cell Biol. 12, 836–846.

You-Ten, K.E., Muise, E.S., Itie, A., Michaliszyn, E., Wagner, J., Jothy, S., Lapp, W.S., Tremblay, M.L., 1997. Impaired bone marrow microenvironment and immune function in T cell protein tyrosine phosphatase-deficient mice. J. Exp. Med. 186, 683–693.

Zheng, X.M., Wang, Y., Pallen, C.J., 1992. Cell transformation and activation of pp60c-src by overexpression of a protein tyrosine phosphatase. Nature 359, 336–339.

Zondag, G.C., Koningstein, G.M., Jiang, Y.P., Sap, J., Moolenaar, W.H., Gebbink, M.F.B.G., 1995. Homophilic interactions mediated by receptor tyrosine phosphatases mu and kappa. A critical role for the novel extracellular MAM domain. J. Biol. Chem. 270, 14247–14250.

Zondag, G.C., Moolenaar, W.H., Gebbink, M.F.B.G., 1996. Lack of association between receptor protein tyrosine phosphatase RPTP mu and cadherins. J. Cell Biol. 134, 1513–1517.