Physiol Rev
83: 1–24, 2003; 10.1152/physrev.00016.2002.

Receptor Protein Tyrosine Phosphatases in Nervous System Development

KARL G. JOHNSON AND DAVID VAN VACTOR

Department of Cell Biology, Harvard Medical School, Boston, Massachusetts

Johnson, Karl G., and David Van Vactor. Receptor Protein Tyrosine Phosphatases in Nervous System Development. *Physiol Rev* 83: 1–24, 2003; 10.1152/physrev.00016.2002.—Receptor protein tyrosine phosphatases (RPTPs) are key regulators of neuronal morphogenesis in a variety of different vertebrate and invertebrate systems, yet the mechanisms by which these proteins regulate central nervous system development are poorly understood. In the past few years, studies have begun to outline possible models for RPTP function by demonstrating in vivo roles for RPTPs in axon outgrowth, guidance, and synaptogenesis. In addition, the crystal structures of several RPTPs have been solved, numerous downstream effectors of RPTP signaling have been identified, and a small number of RPTP ligands have been described. In this review, we focus on how RPTPs transduce signals from the extracellular environment to the cytoplasm, using a detailed comparative analysis of the different RPTP subfamilies. Focusing on the roles RPTPs play in the development of the central nervous system, we discuss how the elucidation of RPTP crystal structures, the biochemical analysis of phosphatase enzyme catalysis, and the characterization of complex signal transduction cascades downstream of RPTPs have generated testable models of RPTP structure and function.

I. INTRODUCTION

A. Overview

The regulation of protein phosphorylation, whether on serine, threonine, or tyrosine residues, plays a pivotal role in virtually all aspects of eukaryotic development. From the regulation of the cell cycle to cellular proliferation and differentiation, the delicate balance between the phosphorylation activity of kinases and the dephosphorylation activity of phosphatases controls the outcome of countless signal transduction cascades. In the past decade, numerous phosphorylation-dependent signaling mechanisms have been characterized, and detailed signal transduction cascades are being assembled. The rate at which new phosphorylation-dependent signaling mechanisms have been identified, the diversity of cellular contexts in which these mechanisms function, and the conservation of similar signaling mechanisms in a wide variety of organisms make this a very exciting and dynamic frontier in cell biology.

The complex interactions that take place between cells and tissues during the formation of the central nervous system (CNS) have also been well characterized. A neuron in the developing CNS undergoes complex morphogenetic changes, including process outgrowth, guidance, and synapse formation, as the nervous system changes from a collection of undifferentiated cells into an integrated, functional network. Not surprisingly, many of the signaling cascades involved in the orderly formation of the CNS require the proper function of both kinases and phosphatases. Although great strides have been made in understanding the roles of kinases in proper CNS development, until recently relatively little was known about the roles that phosphatases play. In this review we focus on recent studies that have begun to illustrate how a specific subclass of phosphatases, the receptor protein tyrosine phosphatases, function to regulate the proper development of the nervous system.

B. Historical Perspective

The first evidence that phosphorylation and dephosphorylation of proteins was a critical mechanism regulating protein activity came in the 1950s with the discovery that the enzymatically active phosphorylase *a* and the enzymatically inactive phosphorylase *b* are phosphorylated and dephosphorylated forms of the same enzyme (reviewed in Ref. 83). Following this discovery, numerous proteins were shown to be activated or inactivated by serine or threonine phosphorylation, including contractile proteins, cytoskeletal proteins, and cell membrane proteins (reviewed in Ref. 83). However, serine and threonine are not the only sites for protein phosphorylation, confirmed by the identification of the first tyrosine kinases: $pp60^{src}$ and the epidermal growth factor (EGF) receptor (68, 165). The subsequent identification of numerous tyrosine and serine/threonine kinases indicated that the regulation of protein phosphorylation is a common posttranslational mechanism for controlling enzymatic activity. Adding credence to this hypothesis is the observation that ~30% of all cellular proteins are substrates for protein kinases (reviewed in Ref. 35).

While the evidence implicating tyrosine kinases in specific signal transduction cascades continued to mount, it took nearly a decade after the characterization of the first tyrosine kinases before the first protein with tyrosine phosphatase activity, PTP1B (163), was identified. Almost immediately after the discovery of PTP1B, the first receptor protein tyrosine phosphatase (RPTP) was identified. Sequence analysis of CD45 showed that CD45 shares a high degree of similarity to PTP1B, and subsequent biochemical characterization of CD45 showed that it too is a tyrosine phosphatase (162). Shortly after the identification of CD45 came a series of low-stringency screens with the cytoplasmic domains of CD45 in an attempt to identify other members of the RPTP family. This rapid method for RPTP identification resulted in the initial characterization of six separate classes and more than 30 different individual RPTPs in a wide variety of vertebrate and invertebrate species (reviewed in Refs. 17, 25). Recent analysis of structural and evolutionary relationships between RPTPs has expanded the number of distinct RPTP subfamilies from six to eight (2).

II. ENZYME CATALYSIS

A. Biochemical Analysis of Enzyme Kinetics

With the identification of RPTPs both in vertebrates (27, 121, 150) and in invertebrates (152, 159, 181) came the observation that within the tandem phosphatase domains exist a variety of highly conserved sequences that are required for enzyme catalysis. In vitro biochemical analysis using thiol-reducing agents suggested that cysteine may play an important role in catalyzing the dephosphorylation reaction (121, 151, 152). To examine which cysteines may be catalytically important, a highly conserved sequence in the first and second phosphatase domains [$HC(X)_5RT$] was the target of site-directed mutagenesis (152). In the first phosphatase domain of LAR, a cysteine to serine (C-S) mutation in this sequence resulted in the loss of 99% of catalytic activity, suggesting that not only is this cysteine a catalytically important amino acid, but also that at least 99% of catalytic activity of LAR is dependent on the first phosphatase domain (121, 152). Further evidence for this cysteine's role in enzyme catalysis came from experiments showing that labeled iodoacetate, a

potent inhibitor of phosphatase activity, specifically binds to this catalytic cysteine (120).

Biochemical studies on phosphatase enzyme catalysis (35–37, 186) have outlined the general mechanism for the tyrosine dephosphorylation reaction. A substrate protein with a phosphorylated tyrosine enters the active site of the enzyme and is stabilized by an interaction between two oxygen atoms in the phosphoryl group and a conserved active site arginine (reviewed in Ref. 35). The tyrosine phosphoryl group is then transferred to the catalytic cysteine via a nucleophilic attack on the phosphate, generating a thiol phosphate intermediate (29). The dephosphorylated tyrosine is ejected from the active site following protonation by a conserved aspartic acid residue, and the thiol phosphate intermediate is hydrolyzed by a water molecule, returning the enzyme to its original state (37, 186).

B. Analysis of Phosphatase Domain Crystal Structures

The first crystal structures of tyrosine phosphatases were conducted on the cytoplasmic human PTP1B (10) and on Yop51, a secreted dual-specificity phosphatase from *Yersinia* (153). While these two phosphatases share only 20% amino acid identity in the catalytic domain, the crystal structures are highly similar, suggesting a shared catalytic mechanism. Shortly after the crystal structures for these non-receptor protein tyrosine phosphatases were characterized, the first crystal structure for a RPTP was solved. Examination of the crystal structure of mouse protein tyrosine phosphatase (PTP)-α revealed that although the overall folding of the phosphatase domains of both receptor and cytoplasmic phosphatases is highly similar, the quaternary structure of PTP-α indicated that this protein is most stable in a dimerized or multimerized state. Furthermore, PTP-α has a helix-turn-helix domain that forms a structural wedge, sterically blocking the catalytic site of the opposing monomer (16). Recent studies using fluorescence resonance energy transfer have suggested that cellular PTP-α exists in such a dimerized state (157). These observations have led Bilwes et al. (16) to propose a model in which RPTPs may be inactivated as dimers due to a steric hindrance of the catalytic site by the opposing monomer.

The crystal structures of the phosphatase domains of several other RPTPs have recently been solved, including the first phosphatase domains of PTP-μ (16, 64) and the tandem phosphatase domains of LAR (111). Comparative analysis of the quaternary structures of these domains suggests that while PTP-α crystallizes as a dimer, the crystal structure of the first phosphatase domain of PTP-μ lacks the interaction between this helix-turn-helix domain and the active site of the first phosphatase domain on the opposing monomer (64). The phosphatase domains of LAR also appear to crystallize as monomers without any extended contact surfaces between independent molecules (111). In fact, analysis of LAR's crystal packing showed that the helix-turn-helix motif that blocks the catalytic site in PTP-α crystals could not interact with the active site of the opposing monomer due to steric hindrance by the second phosphatase domain (111).

As a result of the differences between the quaternary structures of LAR, PTP-μ, and PTP-α crystals, a consensus model for RPTP structure and function cannot yet be constructed. Although it is possible that the second phosphatase domain of PTP-α might also sterically block the helix-turn-helix interaction with the active site of the opposing PTP-α phosphatase domain, until the crystal structure of the tandem phosphatase domains of PTP-α is resolved this can only be hypothesized. Thus the crystal structures for both PTP-μ and LAR suggest that the model in which an inhibitory loop sterically blocks the active site of the opposing monomer is not true for all RPTPs.

III. COMPARATIVE ANALYSIS OF TYPE IIA RECEPTOR PROTEIN TYROSINE PHOSPHATASE SEQUENCES

A. Cytoplasmic Domains

The first (membrane proximal) phosphatase domain is responsible for >99% of catalytic activity, while the second phosphatase domain appears to bind multiple downstream partners (33, 134, 175). As might be expected for enzymatically active domains, the cytoplasmic phosphatase domains are the most highly conserved domains in these proteins, containing multiple stretches of perfect conservation between different RPTP subfamilies and a high overall level of amino acid identity. This high degree of amino acid identity between the phosphatase domains would also suggest that the RPTP crystal structures are highly conserved. For the crystal structures solved to date, this appears to be the case (16, 64, 70, 111) at least at the level of tertiary protein structure.

Although the second phosphatase domain of type IIa RPTPs has not been shown to have catalytic activity on any in vitro substrates, it can be converted to a catalytically active phosphatase simply by changing two amino acids (111). When these two amino acids (KNRLVN and WPEQGVP) were changed to match the corresponding amino acids in the catalytically active first phosphatase domain (KNRYVN and WPDQGVP), the second phosphatase domain showed similar catalytic activity to the wild-type first phosphatase domain.

These studies brought up the exciting possibility that the second phosphatase domain of RPTPs may have catalytic activity on a particular class of substrates in vivo,

despite being inactive on the substrates tested in vitro. In most RPTPs, the majority of catalytic amino acids are present in the second phosphatase domain. Perhaps D2 is only active on a specific substrate or requires a cofactor for catalysis. Support for this hypothesis comes from studies in *Drosophila* using phosphatases with a catalytically inactive D-A mutation. The loss of function phenotype of the *Drosophila* RPTP DPTP69D can be rescued using a transgene with a D-A mutation in the first phosphatase domain, but not by a transgene with a D-A mutation in both the first and the second phosphatase domains (51). It would appear then that catalytic activity is not only present in the second phosphatase domain, but also that this activity is required for DPTP69D function. However, catalytic activity of the second phosphatase domain is unlikely to be a general characteristic of RPTPs, as several RPTPs (including PTP-ζ and PTP-γ) lack the essential catalytic cysteine in the second phosphatase domain.

B. Extracellular Domains

While the structure of the cytoplasmic domains of RPTPs is conserved across all RPTP subfamilies, the extracellular domains are highly divergent and consist of a wide variety of different structural motifs (Fig. 1). Some RPTPs have large and complex extracellular domains, whereas others have short extracellular domains with no known function (reviewed in Refs. 66, 128). On the basis of sequence conservation and analysis of RPTP family trees, this large and diverse family of proteins has been grouped into six (17, 25) or, more recently, eight (2) subfamilies. The structure and function of the four subfamilies of RPTPs implicated in nervous system development (types IIa, IIb, III, and V) are described in section IV.

IV. ANALYSIS OF RECEPTOR PROTEIN TYROSINE PHOSPHATASE STRUCTURE AND FUNCTION

A. Type IIa RPTPs

The type IIa subfamily of RPTPs are cell adhesion molecule-like proteins and are the most well-characterized family of RPTPs. This family includes *Drosophila* LAR (Dlar) and DPTP69D, *Caenorhabditis elegans* PTP-3, *Hirudo medicinalis* HmLAR1 and HmLAR2, as well as three vertebrate homologs: LAR, PTP-σ, and PTP-δ. These RPTPs have large extracellular domains consisting of multiple immunoglobulin (Ig) domains and two to nine fibronectin type III (FNIII) domains, as well as two cytoplasmic phosphatase domains. The first suggestion that type IIa RPTPs may be involved in CNS development was the demonstration that type IIa RPTPs are expressed specifically in the CNS of *Drosophila* (40, 159), leech (54), and several vertebrates (72, 124, 144, 146, 149, 180) during periods of axon guidance and synapse formation.

1. Roles of type IIa RPTPs in Drosophila nervous system development

The only type IIa RPTPs in *Drosophila* (Dlar and DPTP69D) are expressed exclusively in the nervous system and on subsets of CNS axons (40, 85, 159, 181). DPTP69D or Dlar mutants have partially penetrant motor axon guidance defects whereby motor axons either stop short of or grow past their normal synaptic targets (38, 39, 85), as well as more highly penetrant photoreceptor guidance defects in the visual system (30, 51, 101, 112).

In *Drosophila*, motor axons project laterally from the CNS in a segmentally repeated pattern. After exiting the CNS, the motor axons sort into five fascicles: the intersegmental nerve (ISN), intersegmental nerve b (ISNb), and segmental nerves a, c, and d (SNa, SNc, and SNd, respectively). When labeled using an antibody to Fasciclin II, a highly stereotyped pattern of innervation is observed for each fascicle. The most carefully studied branch is ISNb, which exits the CNS along the ISN fascicle. ISNb defasciculates from ISN, turning internally near muscle 28 to project to its ventral muscle targets (Fig. 2*A*). ISNb axons form synapses on muscles 6/7, 12, 13, 30, 14, and 28 (87).

In Dlar or DPTP69D mutants, two axon pathfinding defects are observed for ISNb. In many segments, ISNb axons exhibit either a "full bypass" phenotype in which all ISNb axons fail to leave the ISN pathway and project in parallel with the ISN toward inappropriate dorsal targets, or a "partial bypass" phenotype in which only a fraction of ISNb axons innervate their proper ventral targets (85). Although phenotypically similar, Dlar and DPTP69D have a subtle but distinct difference in their bypass phenotypes; Dlar exhibits a "parallel bypass" phenotype in which ISNb follows the path of ISN as a separate fascicle (85), while DPTP69D exhibits a "fusion bypass" phenotype in which ISNb remains fasciculated with ISN (38). These observations suggest that Dlar is involved in the recognition of the ventral muscle field, while DPTP69D is required for the defasciculation of ISNb from ISN.

In addition to the bypass phenotypes described above, Dlar or DPTP69D mutant ISNb axons exhibit other misrouting defects, including "U-turn," "split-detour," and "split-stall" phenotypes (38, 39). Double mutants in both DPTP69D and Dlar have more penetrant and severe ISNb axon guidance defects, suggesting that Dlar and DPTP69D cooperate during motor axon guidance in *Drosophila* to allow ISNb to reach its appropriate synaptic targets (38, 39).

The axon guidance defects in Dlar or DPTP69D mu-

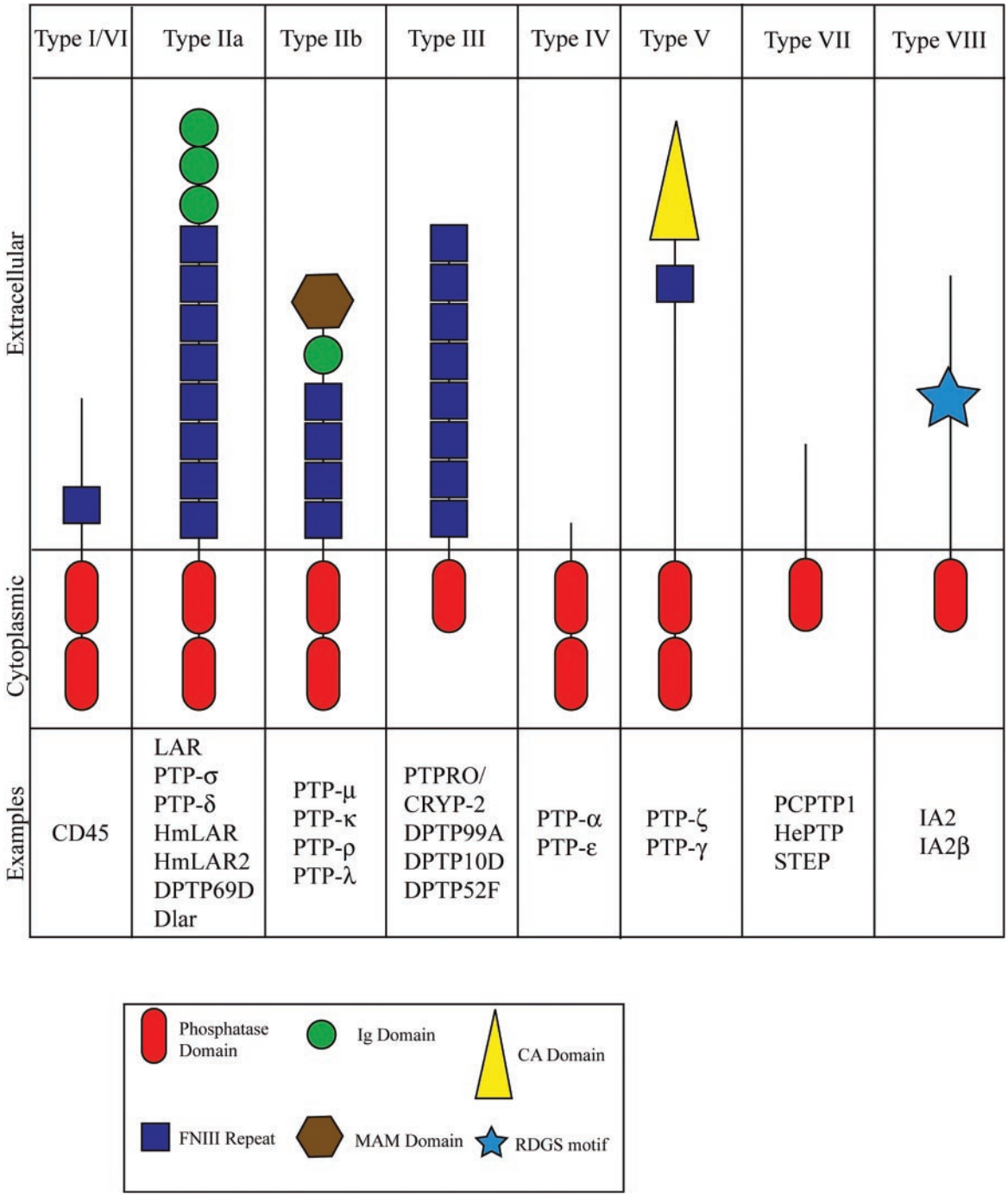

FIG. 1. Schematic diagram of the eight subfamilies of receptor protein tyrosine phosphatases (RPTPs) classified to date. Type I RPTPs contain a single fibronectin type III domain (FNIII) extracellularly and two cytoplasmic phosphatase domains. The type IIa RPTPs have large extracellular domains consisting of three NH_2-terminal immunoglobulin-like (Ig) domains and eight FNIII domains. Type IIb RPTPs have an extracellular meprin-A5-PTP-μ (MAM) domain, a single Ig domain, and multiple FNIII domains. Type III RPTPs have a series of FNIII domains extracellularly but are unusual in that they only have one cytoplasmic phosphatase domain. Type IV RPTPs have the shortest extracellular domains, which are often heavily glycosylated, while type V RPTP extracellular domains have a carbonic anhydrase domain, linked to a single FNIII domain. Type VII RPTPs have one cytoplasmic phosphatase domain and a short extracellular domain, while type VIII RPTPs (thought to be catalytically inactive) contain a RDGS adhesion recognition motif. Members of the type IIa, type IIb, type III, and type IV subfamilies have been implicated in the regulation of neuronal morphogenesis. (Note that these are highly schematized diagrams and do not reflect the exact structure of each RPTP listed below, but rather a stereotyped structure for the family; for example, Dlar has 9 instead of 8 FNIII domains while DPTP99A has 2 instead of 1 phosphatase domain.)

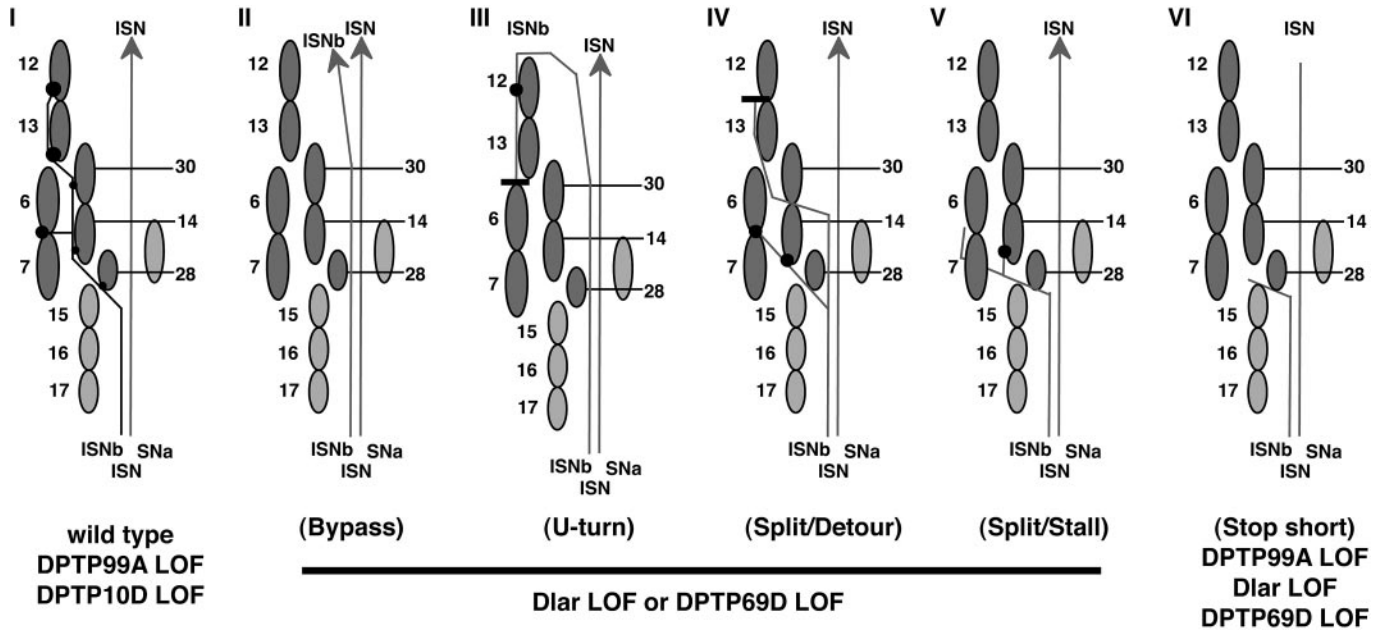

FIG. 2. Motor axon and central nervous system defects observed in *Drosophila* RPTP mutants. The ISNb motor axon guidance defects observed in RPTP mutants are diverse. *Panel I* shows the wild-type pattern of innervation in which the ISNb bundle defasciculates from the ISN near muscle 28 and projects interiorly to innervate muscles 28, 14, 6/7, 30, 13, and 12, while ISN and SNa project to more dorsal targets. *Panels II–V* show examples of the loss-of-function phenotypes observed in Dlar or DPTP69D mutants. *Panel VI* shows the loss-of-function phenotype for the triple knock-out in DPTP99A, DPTP69D, and Dlar, where ISNb motor axons frequently stop short of their proper targets.

tants, however, are not limited to motor neurons. In the *Drosophila* visual system, DPTP69D and Dlar mutants also exhibit highly penetrant defects in photoreceptor axon guidance (30, 51, 101, 112). The *Drosophila* compound eye consists of ~800 individual ommatidia, each containing eight photoreceptors (R1–R8). R1–R6 extend axons to the optic lamina, whereas R7 and R8 grow past the lamina to terminate in the medulla (Fig. 3*A*). Axons from the R cells in an individual ommatidium enter the optic lobe in a highly stereotyped spatial order; a fascicle of R1–R7 axons surrounding the R8 axon, as well as in a highly stereotyped temporal order; R8 entering first, followed by R1–R6 and finally R7. Within the lamina, R1–R6 axons remain as a single fascicle that terminates in a tight cluster until midpupal development, when growth cones extend from this bundle to reach stereotyped targets across the surface of the lamina plexus (Fig. 3*B*).

In DPTP69D mutant embryos, R1–R6 axons frequently fail to terminate in the lamina, and instead follow the R8 axon into the medulla (Fig. 3*A*) (51). In addition, nearly 50% of DPTP69D mutant R7 photoreceptor axons terminate inappropriately in the R8 recipient layer instead of projecting on to the R7 layer (112) (Fig. 3*A*). Using transgenes to rescue these phenotypes, Garrity et al. (51) demonstrated that the FNIII domains of DPTP69D, as well as an intact phosphatase domain, are essential for normal R1–R6 target recognition in the lamina, yet expression of DPTP69D in R7 or R8 does not retarget these axons to the lamina. These data demonstrate that DPTP69D expression is not sufficient to target photoreceptor axons to the lamina, but suggests a model by which DPTP69D acts as a permissive rather than an instructive cue, allowing the R1–R7 photoreceptors to defasciculate from the R8 axon and navigate independently to their appropriate termination zones, rather than directly initiating a signal that targets individual photoreceptor axons to their appropriate target layers (112, 145).

More recently, two elegant papers describe distinct and highly penetrant photoreceptor axon guidance defects in Dlar mutants. Instead of R1–R6 spreading out from the terminal bundle in the lamina during the midpupal stages, Dlar mutant growth cones maintain their tight association (30) and fail to reach their appropriate synaptic targets (Fig. 3*B*). In addition, Dlar mutants exhibit a highly penetrant R7 phenotype, where Dlar mutant R7 axons retract from the normal R7 recipient layer and inappropriately target the R8 recipient layer (30, 101). Using Dlar and DPTP69D transgenes and fusion proteins to rescue the Dlar and DPTP69D mutant phenotypes, Maurel-Zaffran et al. (101) demonstrated that although the intracellular domains of Dlar and DPTP69D are interchangeable and appear to share common signaling mechanisms [as suggested by their genetic redundancy in motor axons (39) and their ability to bind the same signaling proteins (175)], the extracellular domain of Dlar is

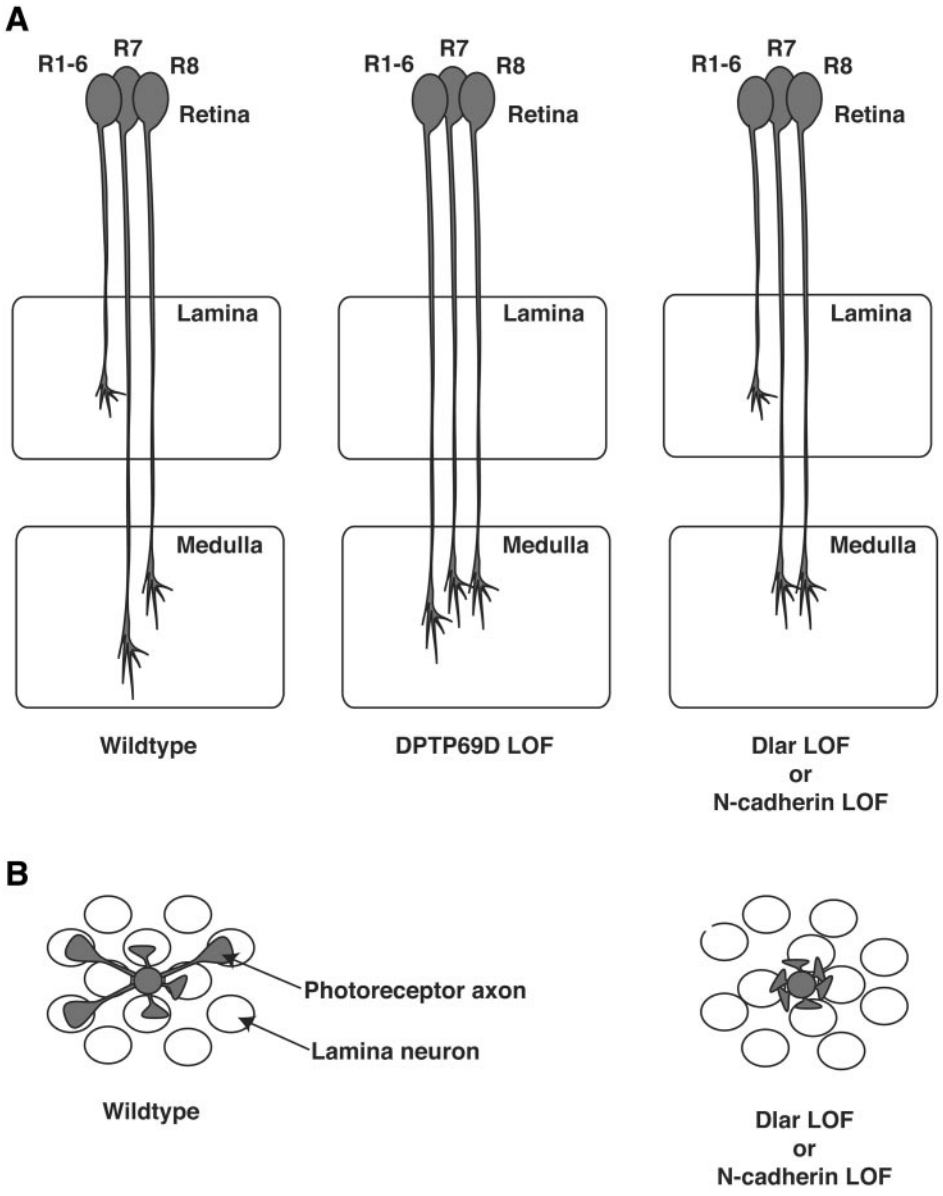

FIG. 3. Photoreceptor axon guidance defects are observed in *Drosophila* RPTP mutants. *A*, *left*: wild-type pattern of axonal connectivity in which photoreceptor (R)1–6 axons project to the lamina, while R7 and R8 project to separate layers of the medulla. *Middle*: photoreceptor axon guidance defects observed in DPTP69D mutants, in which R1–6 frequently bypass their normal laminar targets and project to the medulla while R7 aberrantly terminates in the R8 layer. *Right*: targeting defects of Dlar mutant photoreceptors, in which R7 axons fail to maintain their association with proper targets in the medulla and retract to the R8 recipient layer. This mistargeting of R7 photoreceptors to the R8 layer is also seen when N-cadherin is specifically lost in R7 photoreceptors, but when all other photoreceptors are wild type. *B*: defects in R1–R6 targeting within the lamina are also seen in Dlar mutants. *Left*: normal pattern of R1–R6 connectivity in the lamina, in which R1–R6 axons defasciculate from the central position and innervate distinct laminar targets. *Right*: failure of R1–R6 axons to defasciculate observed in both Dlar and N-cadherin mutants.

uniquely able to recognize and interact with specific extracellular molecules required for proper R7 targeting.

The inability of Dlar mutant R7 photoreceptors to maintain their appropriate termination zone can be rescued either by expressing a full-length Dlar transgene in either R7 or R8 or by expressing the extracellular domains of Dlar in R8 (101). These data suggest that Dlar functions in both a cell-autonomous and -nonautonomous manner during R7 development, acting as both a receptor for R7 axons for which catalytic activity is required and as a ligand expressed by R8. In a similar manner, during the organization of the *Drosophila* follicular epithelium (12, 49), Dlar also appears to mediate cell-nonautonomous effects. Dlar mutant oocytes have severe disruptions in the organization of actin filaments. In mosaic oocytes where follicle cells lacking Dlar are surrounded by wild-type cells, the disruptions in actin filament morphology in Dlar mutant clones extends to the cells immediately surrounding these clones (12, 49). However, it is not known whether these cell-nonautonomous phenotypes are a direct or an indirect consequence of Dlar loss of function. Thus, although both studies demonstrate a cell-nonautonomous function for Dlar, it is not yet known how Dlar mediates these effects, or what extracellular proteins interact with Dlar.

In *Drosophila*, loss of Dlar function also results in reduced synapse size and decreased terminal branch complexity (75). Ultrastructural analysis at the *Drosophila* neuromuscular junction also reveals a 2.5-fold increase in active zone size in Dlar mutants, while electrophysiological characterization of Dlar mutants shows a parallel reduction in evoked, presynaptic neurotransmit-

ter release without a change in postsynaptic sensitivity (75). Thus, not only does Dlar regulate axon guidance to the proper synaptic target, but it also plays a crucial role in the morphogenesis of the developing synapse.

2. *Roles of type IIa homologs in CNS development in leech and C. elegans*

Following the work on type IIa RPTPs in *Drosophila*, two LAR homologs were identified in leech (HmLAR1 and HmLAR2). These RPTPs are expressed at high levels on the processes and growth cones of comb cells, two unusual cells that send out ~70 parallel axonlike processes (53). To perturb the function of HmLAR homologs, Gershon et al. (53) used in vivo addition of antibodies to the extracellular domain of HmLAR2. This resulted in dramatic alterations in the projection pattern of comb cell processes, in which the normally parallel comb cell processes frequently extended filopodia that contacted neighboring processes and occasionally crossed over sibling comb cell processes (53) (Fig. 4).

Recent studies have shown that tagged HmLAR2 extracellular domains not only bind to comb cell processes, but also increase comb cell process contacts (9), resulting in a phenotype similar to the addition of antibodies to the extracellular domain (53). Furthermore, injections of double-stranded RNA to knock down endogenous HmLAR2 function (RNAi) resulted in a similar phenotype to the in vivo addition of HmLAR2 antibodies or the HmLAR2 extracellular domain. These results, and the suggestion that HmLAR2 may bind homophilically in vitro (9), led the authors to propose a mechanism by which HmLAR2 regulates self-repulsion of sibling comb cell processes, promoting the outgrowth of parallel processes. Their model suggests that when sibling process filopodia contact one another, HmLAR2 homophilic binding induces filopodial retraction (reviewed in Ref. 8), maintaining parallel process outgrowth (Fig. 4).

Recent studies have demonstated that the *C. elegans* LAR homolog PTP-3 also plays an important role in embryonic development (61). Using RNAi and genetic loss of function of PTP-3, mild defects in epidermal morphology were observed that looked highly similar to the loss of function of the *C. elegans* Eph receptor VAB-1. Indeed, VAB-1 mutant phenotypes were severely enhanced by the loss of PTP-3 function (61), suggesting that these proteins (a tyrosine kinase and a tyrosine phosphatase) function synergistically during *C. elegans* epidermal morphogenesis. Unlike the

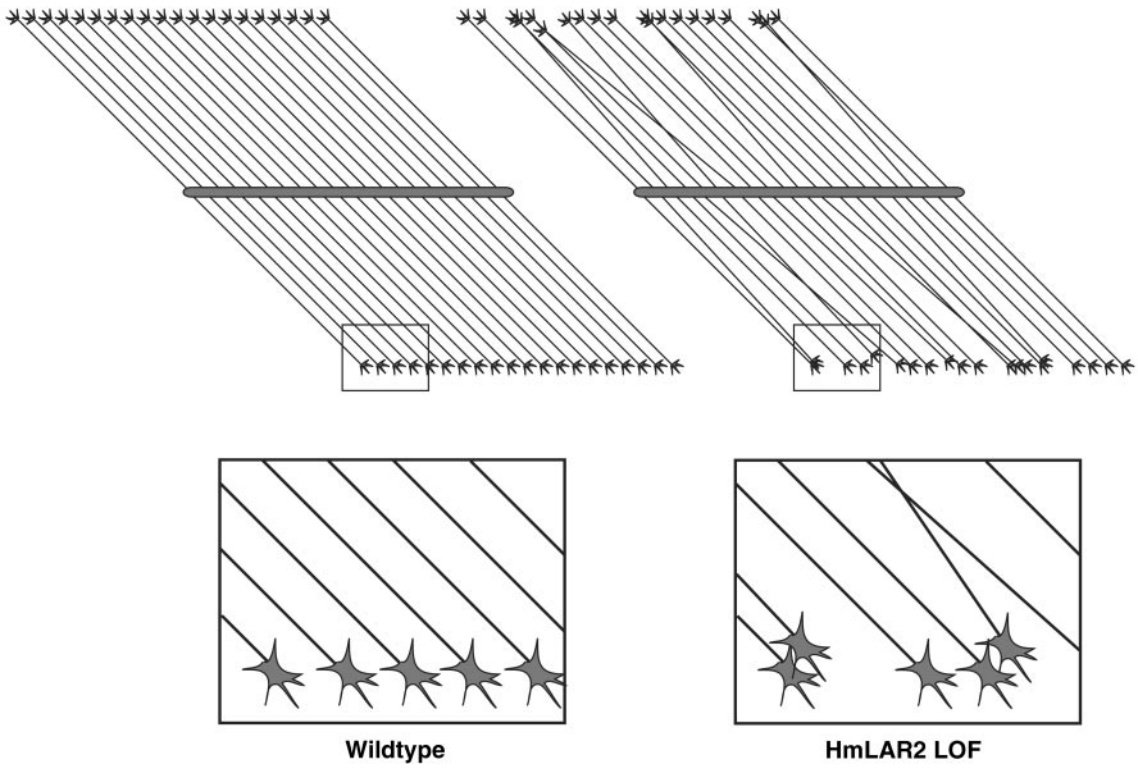

FIG. 4. Type IIa RPTPs are involved in process outgrowth in the leech. Wild-type comb cells send out numerous parallel processes that do not overlap (*left*). *Right*: defects observed after inhibition of HmLAR2 function, by injecting either HmLAR2 antibodies or the extracellular domain of HmLAR2, or by focal injection of HmLAR2 RNAi. After HmLAR2 perturbations, sibling comb cell processes cross each other, and comb cell growth cones display an increased frequency of filopodial contacts. Bottom panels show higher magnification schematics of the top panels.

Drosophila and leech LAR homologs, however, PTP-3 does not appear to have a CNS phenotype.

3. Roles of type IIa RPTPs in CNS development in vertebrates

Three type IIa RPTPs have been identified in vertebrates: LAR (152), PTP-σ (144, 180), and PTP-δ (123), each of which is thought to be a vertebrate homolog of Dlar, possibly arising through gene duplication (66). Surprisingly, DPTP69D appears to have no vertebrate homologs. LAR, PTP-σ, and PTP-δ are expressed in overlapping but distinct patterns in the CNS (72, 91, 130) and appear to be concentrated in the growth cones of elongating processes (53, 144, 146, 184), suggesting that these RPTPs may play a conserved role in the development of the vertebrate nervous system.

Vertebrate type IIa RPTP proteins exhibit complex spatial and temporal expression patterns of differentially spliced transcripts (124, 144, 184, 185). Alternative splicing occurs most frequently within the FNIII domains, and although the functional significance of alternative splicing is largely unknown, differential splicing appears to regulate ligand binding specificity. For example, the fifth FNIII domain of LAR can bind the extracellular laminin-nidogen complex only if exon 13 is spliced out (116). Inclusion of this small exon, or exclusion of the fifth FNIII domain, completely blocks the ability of LAR to bind laminin. In addition, alkaline phosphatase (AP)-tagged proteins representing the two neurally expressed PTP-σ isoforms exhibit distinct binding patterns in the chick retinotectal system (57), suggesting that the alternatively spliced extracellular domains interact differentially with ligands in the retinotectal system.

Members of this family of RPTPs are proteolytically cleaved in the membrane-proximal part of the extracellular domain, but the cleavage products remain tightly associated. LAR, for example, has a penta-arginine cleavage site near the transmembrane domain, in which a single arginine to alanine substitution can abrogate cleavage (136). The tight association between the cleaved halves of LAR is only present in growing cells; once confluence is reached, the NH_2-terminal subunit is released, whereas the COOH-terminal subunit is degraded (183). Proteolytic processing can be triggered by the addition of a calcium iontophore and appears to depend on the function of protein kinase C (1). The functional relevance of this juxtamembrane cleavage has yet to be determined, but an appealing model proposes that binding and release of associated extracellular domains may help regulate the adhesivity of certain cell-cell contacts.

Although numerous studies have examined the spatial and temporal patterns of expression of this family of RPTPs, only recently have functional studies shed light on how these RPTPs may be involved in CNS development. The first such studies done in vertebrates addressed the roles of PTP-σ in axon outgrowth and guidance in the developing chick retina. Chick retinal ganglion cells (RGCs) express a variety of RPTPs, including PTP-σ, CRYP2, PTP-μ, PTP-α, and PTP-γ in overlapping but distinct patterns during retinal development (91). Putative ligands for PTP-σ, detected using alkaline phosphatase-PTP-σ fusion proteins as probes, are expressed on basement membranes of the optic fiber layer in the developing retina (57), specifically on the Mueller glia endfeet which make up part of this layer (90). Blocking PTP-σ/ligand interactions, either at the receptor level using function-blocking antibodies or at the ligand level by adding the PTP-σ extracellular domain, induces a more filopodial-like growth cone and reduces the average length of RGC axons on intact basement membranes, but not on laminin or matrigel (90). These data strongly suggest that PTP-σ acts as a receptor for a growth-promoting cue present on the glial endfeet of the retinal basement membrane and that signaling through PTP-σ enhances the rate of axon elongation as well as the formation of a more lamellipodial growth cone in vitro (90, 107). The identity of at least one ligand for PTP-σ has recently been determined (see sect. VI*A*).

PTP-δ, like HmLAR2, is a homophilic cell adhesion molecule, but in contrast to HmLAR2, PTP-δ homophilic interactions serve to promote neurite outgrowth and adhesion for forebrain neurons in vitro (172). In fact, a soluble gradient of the extracellular domain of PTP-δ can mediate attractive turning of forebrain neurons (155), suggesting that while the extracellular domains of HmLAR2 and PTP-δ both serve as homophilic cell adhesion molecules, the signal transduction cascades activated by these homologous proteins generate opposing effects on outgrowth. While HmLAR2 mediates repulsive turning away from sites of homophilic interactions and prevents the fasciculation of sibling comb cell processes (9), PTP-δ mediates attractive responses toward sites of homophilic contact and may enhance the fasciculative outgrowth of axons expressing this receptor (172).

Possible in vivo roles for all three type IIa RPTPs (LAR, PTP-σ, and PTP-δ) were recently examined in the developing *Xenopus* visual system. The expression of catalytically inactive LAR, PTP-σ, and PTP-δ constructs [consisting of the cytoplasmic phosphatase domains with a cysteine to serine (C-S) mutation in the first phosphatase domain] caused specific and significant changes in the rate of axon elongation (73). Expression of C-S PTP-δ decreased the rate of retinal ganglion cell axon elongation, either in vivo or on retinal basement membranes in vitro. Expression of C-S PTP-σ caused a significant increase in the rate of axon outgrowth on retinal basement membranes, whereas expression of C-S LAR had no effect either in vitro or in vivo. Interestingly, while expression of these C-S RPTPs significantly altered the rate of retinal

ganglion cell axon outgrowth, they did not appear to affect retinal ganglion cell axon guidance. Retinal ganglion cells expressing C-S LAR, C-S PTP-δ, or C-S PTP-σ, or even all three C-S RPTP constructs, were able to navigate properly from the retinal ganglion cell layer to the optic tectum (73). These studies suggest that while the rate of RGC axon extension is influenced by type IIa RPTPs, RGC axon guidance was not affected after expression of catalytically inactive RPTP constructs.

The analysis of RPTP knock-out mice has also enhanced our understanding of how type IIa RPTPs function in the developing CNS. Mice homozygous for a deletion in LAR exhibit subtle phenotypes consisting of a reduced cholinergic innervation of the dentate gyrus and a reduction in the size of basal forebrain cholinergic neurons (182). The subtle defects observed in these mice may not necessarily reflect a true loss of LAR function, because low levels of LAR expression were seen in these knock-out mice (139, 182). LAR is also required for proper peripheral nerve regeneration, because LAR knock-out mice exhibit reduced regrowth of the sciatic nerve after a lesion (167). In fact, the expression of multiple type IIa RPTPs (including LAR and PTP-σ) is responsive to sciatic nerve crush, although the magnitude and direction of altered expression is debatable; one study finds a 50% increase in the levels of PTP-σ mRNA and a 50 and 20% decrease in LAR and PTP-α (62), whereas another study showed increased LAR protein expression following sciatic nerve crush (178).

Mutant mice lacking either PTP-σ or PTP-δ have more obvious neurological effects than mice lacking LAR. Mice lacking PTP-σ exhibit motor coordination deficits, pituitary abnormalities, delayed development, and hypomyelination (46, 170), whereas PTP-δ mutant mice have memory deficits and a hyperpotentiation of CA1 and CA3 hippocampal synapses (164). None of these defects is as severe as the homozygous lethal defects seen when *Dlar* is mutated in *Drosophila*, suggesting that in vertebrates the loss of one of the vertebrate LAR family members may be partially compensated for by the function of other vertebrate LAR family members (reviewed in Ref. 17). Double and triple mutant mice may help address whether the type IIa RPTPs have overlapping functions in the development of the vertebrate CNS.

One of the most surprising aspects of the LAR subfamily of RPTPs is that these homologous proteins appear to function so differently from one other. Despite sharing 66–71% amino acid identity, and interacting with common cytoplasmic effectors like liprin-α, LAR, PTP-σ, and PTP-δ appear not to exhibit homologous functions. Some type IIa RPTPs appear to mediate homophilic binding while others interact with heterotypic ligands; ligand-receptor interactions for some RPTPs mediate repulsion and defasciculation and for others mediate attraction and fasciculation. As such, any detailed model outlining the function of the type IIa RPTPs, let alone the entire RPTP family, seems unlikely to be correct; rather, it appears that the type IIa RPTPs may play both overlapping and nonoverlapping roles in CNS development.

B. Type IIb RPTPs

The type IIb family of RPTPs consists of at least five identified members in vertebrates: PTP-μ, PTP-κ, PTP-ψ, PTP-λ, and PTP-ρ, but none in *Drosophila*. These RPTPs are characterized based on the presence of a meprin-A5-PTP-μ (MAM) domain at the NH_2 terminus of the protein, one Ig domain, and multiple FNIII domains in the extracellular domain. Type IIb RPTPs are expressed in distinct patterns during development, with high levels of expression in the developing CNS (71, 102, 129, 179) including expression in retinal ganglion cells (26, 72, 91, 147). These RPTPs, like the type IIa RPTPs discussed earlier, are cleaved in the membrane-proximal extracellular domain and appear to maintain tight association between the cleaved fragments (71).

The most extensively studied members of this family are PTP-μ and PTP-κ, both of which are homophilic, neurite outgrowth-promoting, cell adhesion molecules (21, 23–25, 44, 52, 189). Homophilic binding of these RPTPs (see sect. VI*B*) is independent of phosphatase activity, as catalytically inactive C-S mutant RPTPs, or constructs lacking the phosphatase domains entirely, retain homophilic adhesive properties (52). PTP-μ associates with and appears to mediate its axon outgrowth-promoting effects through interactions with cadherins (22, 26). Downregulation of PTP-μ expression using antisense oligonucleotides, or expression of a catalytically inactive C-S mutant PTP-μ, can decrease retinal ganglion cell axon outgrowth on an N-cadherin substrate (26). This demonstrates that catalytic activity is essential for PTP-μ function and confirms that PTP-μ is a key regulator of N-cadherin-mediated neurite outgrowth.

C. Type III RPTPs

The type III subfamily of RPTPs consist of four *Drosophila* proteins (DPTP99A, DPTP10D, DPTP52F and DPTP4E) and five vertebrate RPTPs, including PTP-β, DEP1, SAP1, PTPS31, and a vertebrate gene with homologs called CRYP-2 in chick (20), GLEPP-1 in rabbits (158), RPTP-BK in rats (156), mGLEPP/mPTPRO in mice (161, 173), and PTP-U2/GLEPP1 in humans (133, 174). Although an attempt has been made to unify the nomenclature and call these homologous genes PTPRO (142), the presence of a nonhomologous human type IIb RPTP already named PTPRO (4) suggests that another name may be more well suited. Nevertheless, because we will

refer exclusively to studies on the chick homolog, we will follow convention and call this gene CRYP-2/cPTPRO.

Type III RPTPs are characterized by the presence of several extracellular FNIII domains and a single cytoplasmic phosphatase domain (with the exception of PTP99A which has two cytoplasmic phosphatase domains). PTP99A, PTP52F, and PTP10D are expressed selectively in the CNS of *Drosophila* (131, 181), whereas PTP4E appears to be broadly expressed early in development (117). The most extensively studied vertebrate type III RPTP is CRYP-2/cPTPRO, which is expressed by retinal ganglion cells (20, 91) during periods of axonogenesis and axon guidance from the retina to the tectum.

Although no phenotype has been observed for flies lacking DPTP99A, this RPTP genetically interacts with the type IIa RPTPs, Dlar and DPTP69D. Double mutants in DPTP69D and DPTP99A exhibit more highly penetrant motor axon guidance defects than in DPTP69D alone, causing >85% of ISNb nerves to exhibit bypass, detour, or stall defects (38) (Fig. 2). The bypass phenotype observed in DPTP69D/DPTP99A double mutants is subtly different from that observed in Dlar loss of function (as described above), in that DPTP69D/DPTP99A mutants exhibit a failure to defasciculate from the ISN, while Dlar mutants frequently bypass the ventral muscle field as a separate but parallel fascicle to the ISN (85).

The motor axon guidance phenotypes for Dlar, DPTP69D, and DPTP99A mutations, as well as double and triple mutations in these genes, strongly support the hypothesis that these RPTPs do not function in isolation to recognize guidance cues at unique choice points; rather, guidance defects observed in RPTP mutants are more pleiotrophic. This might suggest a role for RPTPs in regulating the fidelity of choice point navigation; the loss of RPTP function results in an increased frequency of guidance errors in a certain set of axons, while the loss of function of multiple RPTPs enhances the frequency of guidance errors. One might hypothesize then that with each additional RPTP mutated, one would observe an increase in axon guidance errors. Although this appears to explain many of the double and triple mutant phenotypes, the guidance defects of multiple RPTP mutants are not strictly additive. Analysis of double and triple mutants in DPTP69D, DPTP99A, and Dlar showed that these RPTPs act both cooperatively and competitively during motor axon guidance in *Drosophila*, sometimes enhancing and sometimes suppressing the phenotypes of individual RPTP mutants (39). For example, mutations in DPTP99A suppress the ISNb bypass phenotype in Dlar but enhance the Dlar ISN stop-short phenotype (39). These data demonstrate that the function of RPTPs is not strictly redundant and illuminate the fact that genetic interactions between different RPTP subfamilies are likely to be complex.

Type III RPTPs are also involved in axon guidance at the midline of the *Drosophila* CNS. Although single mutations in DPTP10D and DPTP99A alone have no obvious CNS phenotype, double, triple, and quadruple RPTP mutations have severe and highly penetrant CNS defects. DPTP69D loss of function causes breaks in the third (outermost) longitudinal fascicle and also results in the inappropriate growth of longitudinal axons across the midline (154). These phenotypes are dramatically enhanced in DPTP69D/DPTP10D double mutants and are much more severe in triple or quadruple (Dlar, DPTP69D, DPTP99A, DPTP10D) RPTP mutants, in which all detectable longitudinal tracts are converted into commissural pathways (154). These data suggest that tyrosine phosphorylation controls the responsiveness of growth cones to the repulsive cues at the CNS midline in *Drosophila*. Adding credence to this hypothesis was the recent discovery that DPTP69D and DPTP10D interact genetically with genes known to regulate axon guidance at the *Drosophila* midline, such as Roundabout (Robo), Slit, and Commisureless (Comm) (79, 80, 132). From these studies, a model has emerged in which DPTP10D and DPTP69D function as positive regulators of Slit/Roundabout repulsive signaling that prevents the longitudinal tracts from crossing midline of the *Drosophila* CNS (154).

Mutations in DPTP52F cause multiple CNS and motor axon guidance defects. DPTP52F loss of function causes breaks in the third longitudinal fascicle as well as the failed branching of SNa (131). Surprisingly, Dlar mutations are able to rescue the DPTP52F CNS phenotype, without having an effect on the ISN or SNa phenotypes, while genetic interactions of DPTP52F with either DPTP10D or DPTP69D are entirely synergistic (131). These data provide further evidence for both competitive and cooperative interactions between the different *Drosophila* RPTPs and confirm that individual guidance choices are likely to involve the integration of information from several RPTP signaling pathways.

The chicken type III RPTP CRYP-2/cPTPRO is also expressed strongly in the CNS, including expression on RGCs during periods of axon outgrowth and guidance (20) as well as in the target for RGC axons, the optic tectum (91). CRYP-2/cPTPRO is alternatively spliced in the juxtamembrane region, similar to the type IIa and type IIb RPTPs. With the use of Fc-CRYP-2/cPTPRO fusion proteins, putative ligands for CRYP-2/cPTPRO were identified on numerous retinal cell types, including RGCs (142). Recently, elegant studies have shown that CRYP-2/cPTPRO functions as a repulsive molecule for RGC axons in vitro, inducing RGC growth cone collapse, inhibiting retinal neurite outgrowth, and acting as a chemorepulsive cue in a growth cone turning assay (142). Although a model is emerging suggesting that CRYP-2/cPTPRO receptor ligand interactions may be involved in the formation of the retinotectal projection, the in vivo roles of

CRYP-2/cPTPRO are only beginning to be understood, because a receptor for CRYP-2/cPTPRO is not yet known.

D. Type V RPTPs

The final family of RPTPs implicated in axon guidance and outgrowth has two identified family members (PTP-ζ/RPTP-β and PTP-γ) that are expressed in the developing CNS (11, 84). These RPTPs are chondroitin sulfate proteoglycans with secreted extracellular domains containing a carbonic anhydrase-like domain and a single FNIII domain (74) and are expressed at high levels in the CNS during development, including expression on cortical cells (60, 91, 114, 137, 138, 168) during periods of migration and process initiation.

In vertebrates, the secreted ECD of PTP-ζ/RPTP-β is also known as phosphacan and can bind a number of neurite outgrowth-promoting cell adhesion molecules including tenascin, axonin-1, contactin, F3, NCAM, NrCAM, and NgCAM (reviewed in Ref. 17). Interactions between phosphacan and these extracellular matrix molecules seem to promote axon outgrowth and cortical cell migration, demonstrated by the fact that the addition of soluble phosphacan can inhibit outgrowth on NgCAM, presumably by blocking the interactions of endogenous PTP-ζ/RPTP-β with its ligand(s) (105) and the observation that pleiotrophin-PTPζ/RPTPβ interactions can promote the migration of rat cortical neurons in vitro (97). However, because the vast majority of the extracellular domain of PTPζ/RPTPβ in the brain is found as soluble phosphacan, competition for ligands between phosphacan and PTPζ/RPTPβ is almost certain to exist (100). The potential in vivo role of ligand masking by phosphacan is only beginning to be addressed.

Recent studies have also implicated PTP-α, a type IV RPTP, in CNS development. With the use of morpholinos to knock down PTP-α expression, dramatic changes in retinal morphology were observed, including defects in retinal lamination and cell fate determination (166). The molecular mechanisms underlying these defects are, as yet, poorly understood.

V. SIGNAL TRANSDUCTION DOWNSTREAM OF RECEPTOR PROTEIN TYROSINE PHOSPHATASES

Unlike receptor tyrosine kinases (RTKs) which conveniently tag their substrates with phosphate groups in response to ligand binding, no simple labeling strategy and few biologically relevant ligands have been available to dissect signaling events downstream of RPTP family members. Despite these limitations, the advent of yeast interaction trap technology, PTP substrate-trapping mutations, and genetic analysis has opened the door to an emerging picture of RPTP signal transduction pathways. Although the picture is far from complete, one can now trace a path from cell surface to intracellular effectors for several RPTPs.

A. The Ableson Tyrosine Kinase as a Partner for LAR RPTPs

One simple prediction for any catalytically active RPTP is that signaling will require an intimate functional relationship with at least one protein tyrosine kinase (PTK). Indeed, genetic studies of Dlar identified the Ableson protooncogene (Abl) PTK as a regulator of the Dlar signaling pathway (175). Abl and Dlar display a potent antagonistic relationship in vivo. Reduction of Abl gene dose by half suppresses the axon guidance phenotype of multiple Dlar mutant backgrounds (175). Conversely, overexpression of Abl in postmitotic neurons results in a Dlar-like phenotype dependent on an intact kinase active site. Coexpression of wild-type Abl and Dlar reverses this effect, suggesting that Dlar is capable of dephosphorylating the relevant phosphoprotein substrates. Loss of Abl alone disrupts axon pathway formation, demonstrating that both Dlar and Abl are essential for axonal development (176). Although genetic interaction assays do not prove direct interactions, the Dlar cytoplasmic domain can recruit Abl from cellular extracts, binds to purified Abl, and can dephosphorylate the kinase in vitro (175). These findings suggest that Dlar and Abl mediate a phosphorylation-dependent switch that controls axon guidance behavior (Fig. 5).

Although vertebrate Abl homologs have been studied extensively in the context of cancer biology, cell proliferation, and DNA damage response (reviewed in Ref. 140), relatively little is known about the role of Abl in controlling cell motility and cell shape. However, a growing body of evidence suggests that Abl and related genes also control cellular morphogenesis and the assembly of actin cytoskeleton (82, 89). But what intracellular effectors mediate this set of Abl functions? Genetic screens to unravel the Abl pathway in *Drosophila* identified several potential substrates, including Enabled, Disabled, and others (reviewed in Ref. 63). Because the tyrosine phosphorylation state of Enabled (Ena) is dramatically reduced in Abl mutants (55), Ena is a prime candidate. Indeed, phenotypic analysis of Ena mutants reveals a Dlar-like axon guidance phenotype (175). Like Abl, Ena binds to the Dlar (and DPTP69D) phosphatase domains, is phosphorylated by Abl, and dephosphorylated by Dlar in vitro (175), consistent with the overlap in axonal phenotypes. Indeed, it would appear that Ena serves as a molecular switch, turned on and off by the relative activities of both Abl and Dlar.

The Ena family of proteins includes three vertebrate

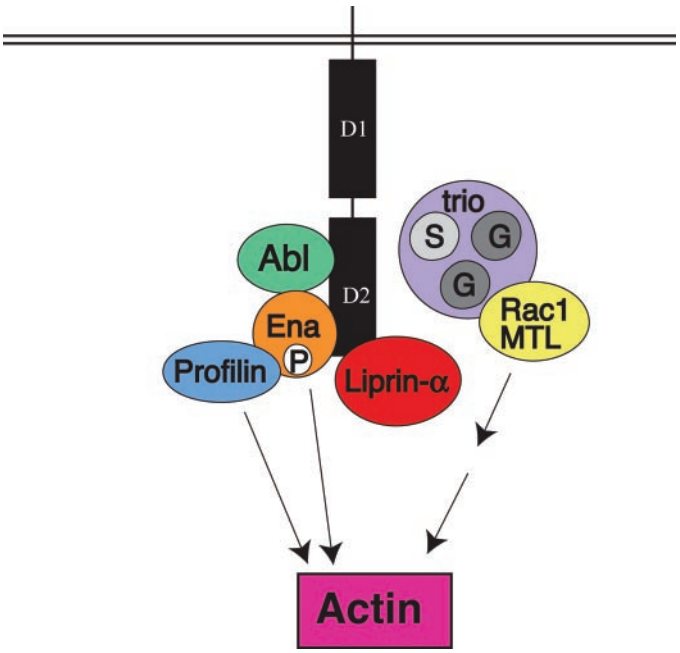

FIG. 5. Schematic diagram representing signal transduction cascades downstream of Dlar. Dlar has been shown to interact genetically with Trio, a protein that activates the Rho family of small GTPases. Dtrio exhibits GEF activity on Drac, which can indirectly promote the formation of new actin filaments. The cytoplasmic domains of Dlar also bind to Liprin-α, a regulator of synapse formation. Dlar can also directly bind and dephosphorylate Abl and Ena. Ena is a shared substrate for Dlar and Abl and appears to form a phosphorylation-dependent switch controlled by the activity of Dlar and Abl.

homologs: mammalian Ena (Mena), the vasodilator stimulated phosphoprotein (VASP), and Ena-VASP-like (Evl; Ref. 56). Analysis of Mena and VASP function in mammalian cells has shown these proteins to be key regulators of actin cytoskeletal assembly and cell motility (reviewed in Ref. 88). In fibroblasts, Mena acts to promote actin assembly by regulating the average length of actin polymers, providing a means of regulating the velocity of cell movement (14, 15; Bear et al., unpublished data). Although Ena contains a number of protein interaction motifs, leading edge cell motility depends primarily on an actin-binding domain that competes with actin-capping protein to allow polymer growth (15). Consistent with this model, injection of cytochalasin D (a small molecule that binds to the barbed ends of microfilaments) at doses that mimic an increase in capping protein results in an axon guidance phenotype very similar to loss of Ena or Dlar (76). These data suggest a model where Dlar and Ena cooperate to control the rate of growth cone motility and leading edge exploration at key choice points to ensure a high fidelity in axon guidance decisions.

In addition to actin, Ena family members bind to several intracellular partners, including the actin-binding protein profilin (125). Like Abl and Ena, profilin is expressed abundantly in the developing nervous system (169, 176). Interestingly, genetic analysis of profilin in *Drosophila* reveals axon outgrowth defects identical to those found in Abl mutants (176), suggesting that Abl and profilin cooperate. Potent genetic interactions between Abl and profilin in vivo support this hypothesis (176). Profilin has been shown to promote and antagonize actin assembly in different contexts (reviewed in Ref. 65); however, the precise role of profilin in the Abl pathway is still a mystery. Other Abl-associated proteins are also likely to contribute to the Abl pathway, including the adenylyl cyclase-associated protein (CAP), which binds to actin monomers and inhibits polymer assembly. Although *Drosophila* CAP does cooperate with Abl in controlling certain axon guidance behaviors, and associates with Abl, Ena, and profilin in *Drosophila* cells (Wills et al., unpublished data), the role of CAP in motor axon guidance and the Dlar pathway is unknown.

Interestingly, Dlar and DPTP69D appear to share at least some of the same signaling machinery during axon guidance in *Drosophila* (101). The Abl pathway may represent part of this overlap, since Enabled binds directly to the cytoplasmic domain of DPTP69D (175). However, genetic interaction experiments have not been performed to test the functional relationship between DPTP69D and the Abl pathway.

B. Small GTPases and Trio in the LAR Pathway

Although Abl and Ena provide direct links between Dlar and cytoskeletal effectors, the pathway seems to be far more complex. Studies of the cytoplasmic domain of mammalian LAR identified an interacting protein called Trio, which contains two Dbl oncogene-homologous guanine nucleotide exchange factor (GEF) domains, the motifs that activate GTPases in the Rho subfamily (33). Characterization of the mammalian Rho family GTPases, Rac, Rho, and Cdc42, in nonneuronal cells showed that these proteins control a variety of actin-dependent cell motility behaviors (reviewed in Ref. 59). Moreover, analysis of Rho family function in *Drosophila* and *C. elegans* showed that these GTPases play crucial roles in axonal and cell migration (76, 95, 188). In particular, overexpression of dominant negative Rac1 in postmitotic neurons results in a Dlar-like axon guidance phenotype, consistent with a model where Trio acts downstream of LAR to activate Rac in motor growth cones (76). Accordingly, Rac1 and Dlar display specific, dose-sensitive genetic interactions indicative of a cooperative relationship in vivo (76).

The analysis of genetic loss of function of Rac1, Rac2, and Mtl genes in *Drosophila* development is only beginning to be examined. However, these alleles have demonstrated that the dominant negative Rac constructs do not necessarily recapitulate the loss-of-function allele. For example, the dominant negative isoforms of Rac have

been shown to regulate planar cell polarity in both the wing and the retina (45, 47), but the triple loss of function of Rac1, Rac2, and Mtl has no similar phenotype in these tissues (58). As a result, the exact role of the Rho family of GTPases in motor axon guidance has yet to be determined.

Although embryonic lethal mutations in vertebrate Trio exist (115), axon pathfinding in these mutants has not been examined. However, the neuronal functions of this GEF protein have been extensively studied in both *C. elegans* and *Drosophila*. This analysis shows that loss of Trio function yields striking defects in axon guidance, outgrowth, and cell migration (6, 13, 93, 113, 143). These axon pathway defects are widespread throughout the nervous system, suggesting that Trio function is not limited to the Dlar pathway. However, dose-sensitive genetic interactions exist between Trio and Dlar, supporting the hypothesis that Trio mediates some of Dlar's downstream pathway (13). Further support for this model comes from the fact that Trio was independently identified as a genetic enhancer of Abl that also interacts with Ena in vivo (93).

While multiple studies implicate Rac1 downstream of Trio, the role of Rho in the pathway is controversial. Although in vitro nucleotide exchange factor assays with mammalian Trio confirm that GEF domain 2 can activate RhoA (33), parallel assays of the invertebrate genes show little if any catalytic activity in this domain (113, 143). Site-directed mutations in both GEF domains also show that retinal axons do not seem to require catalysis by GEF domain 2 (113). However, analysis of Trio function in the *Drosophila* brain suggests a potential overlap with Rho (5). Interestingly, in the *Drosophila* oocyte where Dlar regulates actin microfilament bundle polarity and helps define the shape of the developing egg, loss of Rho activity yields a phenotype highly reminiscent of Dlar loss of function (12).

C. Liprins and LAR Localization

In addition to intracellular proteins with catalytic activities, LAR family RPTPs recruit a group of proteins called liprins that seem to provide a scaffold linking LAR to a network of other proteins (Fig. 5). Liprins fall into related α- and β-subtypes that are highly conserved from worm to fly to human (75, 134, 135). Liprins are small proteins composed of NH_2-terminal coiled-coil domains and COOH-terminal domains that contain steryl alpha motif (SAM) repeats and are expressed in the developing nervous system (75, 134, 135, 187). Liprin-α proteins bind directly to the second phosphatase domain of LAR family members and associate with all members of the vertebrate LAR family (LAR, PTP-δ, and PTP-σ; Ref. 124).

Although the functions of vertebrate liprins are unknown, genetic analysis in *C. elegans* and *Drosophila* have shown that liprin-α plays a crucial role in nervous system function. In a screen for *C. elegans* lacking normal synapse structure, mutations in a liprin-α ortholog (syd-2) were identified (187). In these mutants, neurotransmitter-filled synaptic vesicles fail to cluster appropriately at neuromuscular junctions (NMJs). Ultrastructural analysis reveals that the organizing centers for neurotransmitter release (active zones) are increased in size by roughly twofold (187). Behavioral defects in syd-2 mutants are consistent with a functional deficit in synaptic transmission.

In *Drosophila*, loss of liprin-α results in a reduction in both synapse size and terminal branch complexity (75), an identical phenotype to Dlar loss of function at the synapse (75). Ultrastructural analysis at the *Drosophila* NMJ shows a 2.5-fold increase in active zone dimensions in both liprin-α and Dlar mutants, confirming that the role of liprins has been well-conserved. Moreover, electrophysiological characterization of both liprin and Dlar mutants exhibit a parallel reduction in evoked, presynaptic neurotransmitter release without a change in postsynaptic sensitivity (75).

Although liprin-α proteins associate with LAR RPTPs, their contribution to the LAR mechanism is largely unknown. In this regard, it is interesting that genetic epistasis analysis in *Drosophila* shows that liprin-α is required for Dlar function (75). However, different models can explain this relationship. One model for liprin function is that these intracellular components deliver or recruit LAR receptors to sites of cell-cell interaction, such as focal adhesions (134). Alternatively, liprins may be scaffolding proteins that physically link LAR receptors to targets of PTP regulation. Although liprins lack catalytic domains, signaling motifs, and tyrosine phosphorylation, accumulated evidence suggests that liprins interact with a network of other proteins at the synapse, including the multiple PDZ-domain containing protein GRIP (177).

D. Catenins as Partners of Multiple RPTPs

A number of studies have shown that LAR family RPTPs localize to sites of cell-cell and cell-substrate interactions in nonneuronal cells. For example, liprin-α and LAR localize to focal adhesions (FAs) in fibroblasts (134), and Dlar colocalizes with Enabled and integrin receptors at FA-like junctions in epithelial cells surrounding the *Drosophila* oocyte (12). One additional type of cell-cell contact abundant in LAR protein is the adherens junction that forms a stable bond between neighboring cells. Here, LAR appears to associate with cadherins, the major cell adhesion molecule (CAM) responsible for the formation of adherens junctions (1). Like other CAMs, cadherin function is dependent on a linkage to the cortical actin cytoskeleton. To accomplish this, cadherins recruit mem-

bers of the β-catenin/Armadillo family of proteins (94). Studies in both epithelial and neuronal cell lines reveal that LAR receptors associate with catenins (86, 108), forming a protein complex likely to link LAR to different cadherins. Interestingly, tyrosine phosphorylation of β-catenin plays a key role in the initiation of cell migration by increasing the free intracellular pool of the protein (108). LAR is capable of dephosphorylating β-catenin, preventing the release of catenins and thus blocking the movement of epithelial cells (108). While the importance of the relationship between LAR and β-catenin during axon guidance is not known, the proteins do associate in PC12 cells (86). It is known, however, that neuronal cadherins play an important role in the formation of axon pathways in the *Drosophila* embryo (69) and in vertebrates (18, 43, 99, 118, 126, 148). Moreover, the β-catenin of *Drosophila* (Armadillo) appears to be important for some aspects of axonogenesis in the same system, where it also interacts with mutations in the Abl protein tyrosine kinase (94). These observations suggest that LAR receptors may modulate cadherin function through a partnership between Abl and β-catenin; however, this has yet to be demonstrated.

Interestingly, the retinal phenotypes observed for Dlar loss of function are nearly indistinguishable from the phenotypes observed for N-cadherin loss of function. In both cases, R1-R6 photoreceptor axons reach the lamina, but once in the lamina fail to extend out of the ommatidial bundle (Fig. 3). In addition, R7 axons also fail to terminate in their appropriate position, and instead project to the R8 recipient layer (92). These data, together with the demonstration that vertebrate LAR associates with cadherins, provide strong circumstantial evidence that Dlar and N-cadherin function together to regulate photoreceptor axon guidance. However, subtle phenotypic differences, such as the observation that N-cadherin is required for proper topographic map formation in the lamina and medulla, while Dlar plays no role, suggest that these genes also have independent roles during photoreceptor axon guidance.

Interestingly, multiple RPTP subfamilies interact with catenin-cadherin complexes. PTP-μ has been shown to associate with cadherins and both α- and β-catenins in a variety of tissues, including brain (23). Both PTP-μ and PTP-κ can bind β-catenin, and PTP-κ can catalyze the dephosphorylation of β-catenin in vitro (50). The PTP-μ interaction appears to be mediated by direct binding of the receptor cytoplasmic domains. Consistent with a functional interplay between PTP-μ and cadherins in the developing nervous system, axon outgrowth on cadherin substrates has been shown to require PTP-μ activity in cultured neurons (26).

The type V RPTP PTPζ/RPTPβ has been shown to interact with a variety of extracellular ligands, and studies have recently begun to identify several cytoplasmic substrates. The phosphatase domains of PTPζ/RPTPβ bind to β-catenin and can catalyze the dephosphorylation of β-catenin in vitro (103). Interactions of the extracellular domains of PTPζ/RPTPβ with pleiotrophin cause an inhibition of PTP-ζ catalytic activity and a concomitant increase of β-catenin phosphorylation (103). This is the best experimental evidence supporting the model that ligand binding can regulate the catalytic activity of RPTPs and prompted the authors to propose a ligand-induced receptor inactivation model, in which ligand binding causes an inhibition in receptor enzymatic activity (103).

PTPζ/RPTPβ also binds to the postsynaptic density scaffold protein PSD-95/SAP90 (78), as well as the G protein-coupled receptor kinase-interactor 1/Cool-associated, tyrosine-phosphorylated 1 (GIT1/Cat-1) (77). However, the functional relevance of these interactions has yet to be uncovered.

E. The Src Tyrosine Kinase and DPTP10D

Although relatively little is known about the intracellular partners of other RPTPs, there are a few hints. For example, biochemical experiments in *Drosophila* identified a substrate for DPTP10D called gp150 (160). This leucine-rich repeat bearing transmembrane glycoprotein forms a stable complex with DPTP10D in cultured *Drosophila* cells, where gp150 is phosphorylated by a member of the Src PTK family and can be dephosphorylated by either DPTP10D or DPTP99A (48). Interestingly, Src and a 40-kDa phosphoprotein form a complex with gp150 in this context. While gp150 is a good substrate for the v-Abl PTK in vitro, the in vivo function of gp150 remains unknown.

Interactions of RPTPs with the Src tyrosine kinase family are not limited to DPTP10D. Previous studies have demonstrated an interaction between CD45 and the tyrosine kinases Lck and Fyn (109, 110). In contrast to the notion that RPTPs could serve strictly to negatively regulate PTKs, CD45 can dephosphorylate an inhibitory COOH-terminal phosphotyrosine, thereby activating these tyrosine kinases (109, 110).

F. Clr2 and Fibroblast Growth Factor Receptor

Although ample evidence suggests that RPTP signaling involves intracellular PTKs, RTKs are also likely to participate in RPTP pathways as well. One nice example comes from the analysis of the fibroblast growth factor receptor (FGFR) ortholog in *C. elegans* (42, 81). Genetic analysis in *C. elegans* identified the type II RPTP Clr-1 as an antagonist of the FGFR ortholog egl-15 (81). In this system, the Clr-1 phenotype is suppressed by loss of egl-15 and can be mimicked by overexpression of egl-15 alone (81). Because Clr-1 requires catalytic PTP activity to function during *C. elegans* development, it is likely that

FGFR and Clr-1 share a set of functionally relevant substrate proteins. Although FGFR is known to play a role in axon outgrowth and guidance, as well as in transducing signals from other CAMs (such as N-cadherin), the identity of shared substrates for Clr-1 and the FGFR is still unknown.

G. PKC-δ Signaling Downstream of PTP-μ

Recent studies have outlined two players in a novel signaling mechanism downstream of PTP-μ. With the use of the first phosphatase domain of PTP-μ in a yeast two-hybrid screen, the receptor for activated protein kinase C (RACK1) was identified as a candidate interactor (106). This scaffolding protein has been implicated in shuttling proteins to the plasma membrane, suggesting that PTP-μ may use RACK1 to recruit other signaling proteins to sites of cell-cell contact (106). Indeed, PKC-δ has recently been shown to be required for neurite outgrowth downstream of PTP-μ and is also present in a protein complex with PTP-μ and RACK1 (127). Together, these data suggest that PTP-μ signals through RACK1, which recruits PKC-δ and results in the promotion of neurite outgrowth (127).

VI. THE SEARCH FOR RECEPTOR PROTEIN TYROSINE PHOSPHATASE LIGANDS

Although the analysis of signal transduction cascades downstream of several RPTPs has resulted in the characterization of numerous downstream effectors, few ligands for RPTPs have been identified. Despite concerted effort in *Drosophila* and in many vertebrate systems, in no organism have ligand-receptor interactions been demonstrated to play an important role in neural morphogenesis in vivo. In the past few years, in vitro studies have demonstrated that extracellular binding partners exist for at least three families of RPTPs and that receptor-ligand interactions may regulate axon growth and guidance for multiple families of RPTPs.

A. Ligands for Type IIa RPTPs

The first identified ligand for a type IIa RPTP was the laminin-nidogen complex (116). This complex extracellular matrix protein binds to a specific splice form of the fifth FNIII domain of LAR that lacks exon 13 (as described above). Laminin is well known for its action in promoting axon outgrowth (31, 122) and has recently been identified as a potential modulator of growth cone responses to diffusible guidance factors (67). Outside the CNS the predominant LAR isoform would be predicted to interact with the laminin-nidogen complex, although the majority of LAR transcripts expressed in the CNS include exon 13 and would therefore not bind laminin. As a result, the in vivo significance of laminin-LAR interactions has yet to be elucidated.

The purified extracellular domains of human PTP-δ and leech HmLAR2 have both been shown to bind homophilically in vitro (9, 172). Although the domains responsible for homophilic interactions have not yet been accurately mapped, it appears that at least for HmLAR2, the Ig domains play an important role (9). Interestingly, homophilic binding of HmLAR2 and PTP-δ appears to have opposing biological effects. As described above, homophilic PTP-δ interactions serve to promote neurite outgrowth and mediate attractive turning responses in cultured forebrain neurons (172), while HmLAR2 interactions mediate repulsion between sibling comb cell processes (9).

Heterotypic ligands for the third vertebrate type IIa RPTP, PTP-σ, have recently been identified in chick. With the use of fusion proteins consisting of the extracellular domain of PTP-σ linked to AP, receptor binding activity had been detected in the optic tectum and on the retinal basement membrane (57). At least one putative ligand was proposed to be a heparan sulfate proteoglycan, because binding activity in the retinal basement membrane was lost after treatment with heparinase III while chondroitinase treatment had no effect (3). Indeed, the extracellular domain of PTP-σ was found to bind heparan-albumin with high affinity and can also bind agrin and collagen XVIII (two major retinal HSPGs). Site-directed mutagenesis of the extracellular domains of PTP-σ has demonstrated that the heparan-binding interactions are regulated by highly conserved basic amino acids (KKXKK) in the first Ig domain of PTP-σ (3). Interestingly, this KKGKK motif is perfectly conserved in the first Ig domain of human LAR, PTP-δ and PTP-σ, as well as in the mouse homologs of these genes, suggesting that other vertebrate RPTPs may share the ability to bind HSPGs. The first Ig domain of Dlar, however, contains the sequence KNGKK at this site; the change in net charge (+2 vs. +4) raises the question of whether HSPG binding will be conserved in *Drosophila*.

Although no ligands have been identified for *Drosophila* RPTPs, there is mounting evidence that the extracellular domains are required for proper RPTP function. Support for this hypothesis came from studies examining the role of DPTP69D and Dlar in photoreceptor axon guidance. As described in section IV*A*, the DPTP69D loss of function phenotype can be rescued by expressing a DPTP69D transgene that lacks the Ig domains, but not by a transgene that lacks the FNIII domains, suggesting that the FNIII domains are required for DPTP69D function (51). In addition, the Dlar loss of function phenotype in the retina can be rescued by a transgene encoding a chimeric protein consisting of Dlar's extracellular domain and DPTP69D's cytoplasmic domain, but not by a chi-

meric protein consisting of DPTP69D's extracellular domain and Dlar's cytoplasmic domain (101). This suggests that not only does Dlar interact with a unique extracellular ligand, but also that the cytoplasmic signal transduction mechanisms may be shared between DPTP69D and Dlar.

B. Ligands for Type IIb RPTPs

The RPTPs PTP-μ and PTP-κ have been shown to bind homophilically in numerous studies (see sect. IV*B*). Recently, the homophilic binding regions of PTP-μ and PTP-κ were explored using chimeric proteins consisting of the MAM domain of PTP-κ and the rest of the extracellular domain of PTP-μ (189). These proteins did not bind either PTP-μ or PTP-κ, but instead interacted homophilically. In addition, homophilic binding activity of both PTP-μ and PTP-κ is lost when the MAM domain is deleted. These experiments demonstrate that the MAM domain is necessary, but not sufficient, for homophilic interactions; rather, homophilic binding is mediated by both the MAM domain (52, 189) and the Ig domain (24) of these RPTPs, and despite high levels of sequence conservation, heterophilic interactions between PTP-μ and PTP-κ do not occur (189). The most logical explanation of these observations is that MAM-MAM and Ig-Ig homophilic interactions cooperate to generate the homophilic binding of PTP-μ and PTP-κ, but no data that directly support this hypothesis are available.

Recently, several other members of the type IIb family of RPTPs have been identified. These include PTP-ρ, PTP-ψ, PTP-*o*, and PTP-λ and PTP-π (4, 28, 32, 102, 141). Although structural conservation would suggest that these RPTPs interact homophilically, no experimental evidence has demonstrated homophilic binding for any type IIb RPTPs except for PTP-μ and PTP-κ, and no heterotypic ligands have been identified for any type IIb RPTP.

C. Ligands for Type V RPTPs

Recently, several ligands for the type V RPTP PTPζ/RPTPβ have been identified. The extracellular domain of PTPζ/RPTPβ (phosphacan) binds a variety of extracellular ligands, including N-CAM, Ng-CAM, tenascin (105), contactin (119), pleiotrophin, and midkine (96). Two of these ligands (pleiotrophin and midkine) have been demonstrated to exert their effects, at least in part, through regulation of PTPζ/RPTPβ phosphatase activity.

The most well-characterized ligand-receptor interactions for any RPTP are the ligand-receptor interactions that take place between PTPζ/RPTPβ and its extracellular ligand pleiotrophin. Pleiotrophin-PTPζ/RPTPβ interactions promote the migration of rat cortical neurons in vitro (97) and inhibit the phosphatase activity of PTPζ/RPTPβ both in vitro and in vivo. Pleiotrophin binding to PTPζ/RPTPβ inhibits its phosphatase activity, demonstrated by the fact that an increase in the level of phosphorylated tyrosine on one of PTPζ/RPTPβ's endogenous substrates (β-catenin) after exogenous application of pleiotrophin was observed (103). These observations led the authors to propose a "ligand-induced receptor inactivation" model as a possible mechanism for RPTP function (see sect. VII*C*).

VII. MODELS FOR RECEPTOR PROTEIN TYROSINE PHOSPHATASE FUNCTION

One of the fundamental goals in RPTP biology is to understand how extracellular interactions regulate the activity of the cytoplasmic phosphatase domains. Although there does not appear to be one model capable of describing the function of all RPTPs, there are four possible mechanisms that outline how an individual RPTP may function to transduce extracellular signals into cytoplasmic effects: *1*) some RPTPs may not have specific extracellular ligands, *2*) the binding of extracellular ligands for some RPTPs may not regulate phosphatase activity, *3*) ligand binding could enhance or inhibit phosphatase activity, and *4*) a combination of the above models in which different ligands exert different biological effects through the same RPTP. Although the data described above show that the ligand-induced receptor inactivation model accurately describes the function of some RPTPs, we will discuss each possibility and the data supporting each model.

A. RPTPs May Not Interact With Specific Ligands

Families of RPTPs are organized based on the overall structure of the extracellular domains. These extracellular domains are highly conserved not only between species, but also between proteins within subfamilies, suggesting that the extracellular domains play conserved roles in development. However, this conserved role does not necessarily indicate a conserved interaction with ligands. For example, the extracellular domains of some RPTPs (such as PTP-ϵ and PTP-α) are so small that it is conceivable that their only function is to tether tyrosine phosphatase activity to the plasma membrane. Alternatively, the extracellular domains of other phosphatases could function to sterically block other transmembrane proteins from getting close enough to interact with the cytoplasmic phosphatase domains. It is also possible that RPTPs are simply part of a large signaling complex and that the extracellular domains are responsible for modulating interactions in *cis* to form such a complex.

B. Ligand Binding May Not Regulate Phosphatase Activity

Just because RPTPs bind to extracellular partners and have catalytically active cytoplasmic phosphatase domains does not necessarily mean that ligand binding regulates phosphatase activity. Instead, the extracellular domains of these RPTPs could be functioning simply as CAMs, or as part of a receptor complex that operates independently of the cytoplasmic phosphatase domains. The activity of the phosphatase domains, for example, may be regulated exclusively by cytoplasmic partners [such as the oxidative state of the enzyme (104)], while the extracellular domains could control proper localization of the protein to specific subcellular structures (such as FAs).

Phosphatase activity is required for the normal function of many RPTPs. DPTP69D loss-of-function phenotype in photoreceptors cannot be rescued with a catalytically inactive DPTP69D construct (51), and phosphatase activity is required for a *C. elegans* Clr-1 transgene to rescue the loss-of-function phenotype (81). In addition, overexpression of catalytically inactive PTP-μ inhibits axon outgrowth on an N-cadherin substrate, mimicking PTP-μ loss of function by RNAi (26). Clearly phosphatase activity is required for the normal function of these proteins, but none of these studies directly addresses whether enzymatic activity is regulated by ligand binding.

C. Ligand Binding Could Enhance or Inhibit Phosphatase Activity

Largely because few specific ligands have been identified for RPTPs, it is not known whether ligand binding induces receptor dimerization or monomerization, or whether different ligands may function in opposing manners. Several lines of evidence lead to an appealing model in which ligand binding to the extracellular domains of an RPTP induces receptor dimerization and that dimerization of RPTPs results in an inactivation of the catalytic phosphatase activity, but only one set of studies examining the interactions between endogenous ligands, receptors, and substrates has been conducted.

Data suggesting that dimerization of the cytoplasmic phosphatase domains results in an inhibition of phosphatase activity have come from several sources. In the absence of EGF, a chimeric receptor containing the extracellular domain of the EGF receptor and the cytoplasmic domains of CD45 can restore the function of CD45 (41). However, dimerization of this chimeric protein following addition of EGF functionally inhibits the activity of the EGFR-CD45 chimera (41). In addition, disulfide-bonded PTP-α homodimers have been demonstrated to lack catalytic phosphatase activity (70). Indeed, the crystal structures of some (but not all) RPTPs show the presence of an inhibitory helix-turn-helix structure in the first phosphatase domain that has been proposed to bind and inhibit the first phosphatase domain of the opposing monomer (Fig. 6). Following site-directed mutagenesis on the inhibitory structure of CD45, severe lymphoproliferation and autoimmune nephritis occur, suggesting that this structure is indeed a key natural inhibitor of CD45 activity (98). Because other RPTPs lack this structure, or have three-dimensional conformations that would prevent this structure from interacting with the active site of the opposing monomer, the mechanism by which dimerization might result in catalytic inhibition is still debated.

Although the mechanism by which ligand-induced receptor inactivation works may be contested, evidence continues to mount that this mechanism accurately describes the regulation of enzymatic activity for multiple RPTPs. The recent studies described above on the interactions of pleiotrophin and its natural receptor PTPζ/RPTPβ have demonstrated that an endogenous ligand can induce the inactivation of its receptor both in vitro and in vivo (103). Furthermore, the expression of a catalytically inactive PTP-σ construct in *Xenopus* retinal ganglion cells increases the rate of process outgrowth (73), while blocking PTP-σ ligand/receptor interactions decreases the rate of process outgrowth in chick (90). Together, these data suggest that PTP-σ receptor/ligand interactions also promote axon outgrowth by inactivating PTP-σ enzymatic activity, similar to the mechanism described for PTPζ/RPTPβ.

D. A More Complex Combination of the Above Models

Although the ligand-induced receptor inactivation model explains a great deal of the biochemical and functional data on RPTP function, several studies suggest that the regulation of RPTP activity is more complex than this model would indicate. Two studies have recently shown that there appears to be cross-talk between the different RPTPs and that this cross-talk may also regulate the enzymatic activity of these receptors.

The first evidence for cross-talk between RPTPs demonstrated that the second phosphatase domain of PTP-δ can bind to, and inhibit, the first phosphatase domain of PTP-σ (171). This inhibitory interaction is dependent on the helix-turn-helix wedge present in the second phosphatase domain of PTP-δ. Similar wedge-dependent cross-talk between RPTPs has also been shown for PTP-α, which interacts with the second phosphatase domains of PTP-σ, PTP-α, LAR, PTP-δ, and PTP-μ (19). Although the functional relevance of such interactions has yet to be determined, this certainly opens the possi-

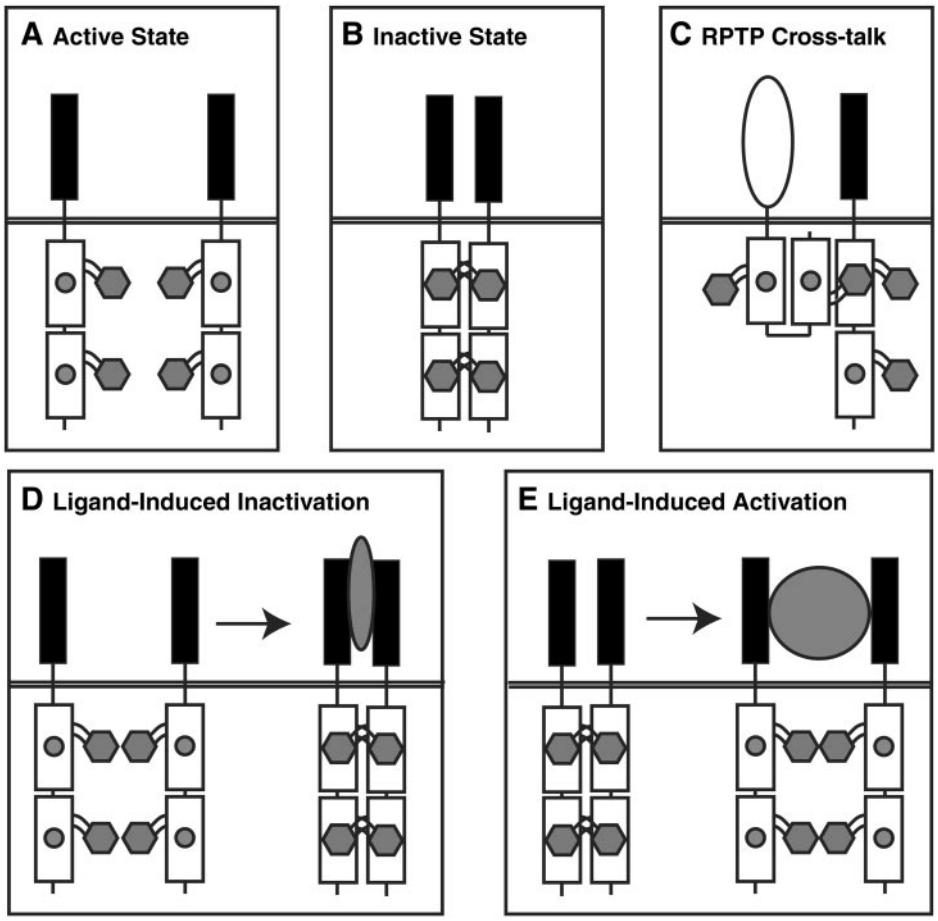

FIG. 6. Schematic diagram of models for the regulation of RPTPs enzymatic activity. *A*: model for the active state of RPTPs. In this model, the active sites of the first (and second) phosphatase domains are available for catalysis. *B*: model for the inactive state of RPTPs in which the helix-turn-helix domain of a second RPTP sterically blocks the catalytic site of its opposing monomer. *C*: potential for cross-talk between different RPTPs, with the helix-turn-helix domain of one RPTP blocking the catalytic site of a different RPTP. *D*: model for ligand-induced inactivation based on ligand-induced dimerizaion. *E*: model for ligand-induced activation based on a ligand-induced monomerization of the RPTPs. Hexagons, helix-turn-helix inhibitory domain; small circles, catalytic domain.

bility that signaling downstream of RPTPs may require the integration of multiple receptor signals.

Further evidence for cross-talk between RPTPs comes from the studies examining the interactions between different RPTPs during motor axon guidance in *Drosophila*. In this system, DPTP99A, DPTP69D, and Dlar have been shown to both cooperate and compete with each other as motor axon growth cones navigate to their appropriate targets. Although these studies do not indicate that these proteins interact directly, it is clear that positive and negative interactions occur between these RPTPs. Whether through positive and negative regulation of shared signal transduction machinery, or through a direct physical interaction, it appears that these RPTPs function in concert to regulate the fidelity of axon guidance decisions.

It has also been demonstrated that the cytoplasmic phosphatase domains of several RPTPs are themselves phosphorylated (34). Unfortunately, although some binding partners for these phosphorylation sites have been identified (GRB2 binding Tyr-789 of PTP-α for example, Ref. 34), the functional significance of RPTP phosphorylation has yet to be elucidated.

VIII. SUMMARY

RPTPs are important yet poorly understood regulators of axon guidance and outgrowth in a variety of experimental systems. Recent studies have outlined signal transduction mechanisms downstream of multiple RPTPs and have resulted in the characterization of several extracellular ligands. Based on the results of these studies, crystal structures of the cytoplasmic phosphatase domains, and biochemical analysis of enzymatic activity, models have emerged describing how RPTPs may transduce extracellular signals into intracellular effects. Although much of the current data favor a model in which ligand binding causes the inactivation of the cytoplasmic phosphatase domains, possibly through dimerization, RPTPs have not truly come of age at a mechanistic level, and the formulation of generalized models with such little experimental evidence is speculative at best.

A number of fundamental questions remain in RPTP biology. What are the in vivo functions of vertebrate RPTPs? What are the ligands for the *Drosophila* RPTPs? Is the ligand-induced receptor inactivation model of RPTP function generally applicable, or does it only apply to a subset of RPTPs? Is steric hindrance by the inhibitory

wedge the sole mechanism underlying ligand-induced receptor inactivation, or are there other mechanisms that explain this obervation? The further characterization of ligands and substrates for these RPTPs and the detailed analysis of phosphatase biochemistry will allow many of these questions to be answered.

This work was supported by National Institute of Neurological Disorders and Stroke Grants NS-40043 and NS-35909. K. G. Johnson is a Helen Hay Whitney postdoctoral fellow, and D. Van Vactor is a Leukemia and Lymphoma Society fellow.

Address for reprint requests and other correspondence: K. G. Johnson, Dept. of Cell Biology, 240 Longwood Ave., Building LHRRB-409, Boston, MA 02446.

REFERENCES

1. Aicher B, Lerch MM, Muller T, Schilling J, and Ullrich A. Cellular redistribution of protein tyrosine phosphatases LAR and PTPσ by inducible proteolytic processing. *J Cell Biol* 138: 681–696, 1997.
2. Andersen JN, Mortensen OH, Peters GH, Drake PG, Iversen LF, Olsen OH, Jansen PG, Andersen HS, Tonks NK, and Moller NP. Structural and evolutionary relationships among protein tyrosine phosphatase domains. *Mol Cell Biol* 21: 7117–7136, 2001.
3. Aricescu AR, McKinnell IW, Halfter W, and Stoker AW. Heparan sulfate proteoglycans are ligands for receptor protein tyrosine phosphatase sigma. *Mol Cell Biol* 22: 1881–1892, 2002.
4. Avraham S, London R, Tulloch GA, Ellis M, Fu Y, Jiang S, White RA, Painter C, Steinberger AA, and Avraham H. Characterization and chromosomal localization of PTPRO, a novel receptor protein tyrosine phosphatase, expressed in hematopoietic stem cells. *Gene* 204: 5–16, 1997.
5. Awasaki T, Saito M, Sone M, Suzuki E, Sakai R, Ito K, and Hama C. The *Drosophila* trio plays an essential role in patterning of axons by regulating their directional extension. *Neuron* 26: 119–131, 2000.
6. Baier H, Klostermann S, Trowe T, Karlstrom RO, Nusslein-Volhard C, and Bonhoeffer F. Genetic dissection of the retinotectal projection. *Development* 123: 415–425, 1996.
7. Baker MW and Macagno ER. RNAi of the receptor tyrosine phosphatase HmLAR2 in a single cell of an intact leech embryo leads to growth-cone collapse. *Curr Biol* 10: 1071–1074, 2000.
8. Baker MW and Macagno ER. The role of a LAR-like receptor tyrosine phosphatase in growth cone collapse and mutual-avoidance by sibling processes. *J Neurobiol* 44: 194–203, 2000.
9. Baker MW, Rauth SJ, and Macagno ER. Possible role of the receptor protein tyrosine phosphatase HmLAR2 in interbranch repulsion in a leech embryonic cell. *J Neurobiol* 45: 47–60, 2000.
10. Barford D, Flint AJ, and Tonks NK. Crystal structure of human protein tyrosine phosphatase 1B. *Science* 263: 1397–1404, 1994.
11. Barnea G, Silvennoinen O, Shaanan B, Honegger AM, Canoll PD, D'eustachio P, Morse B, Levy JB, Laforgia S, and Huebner K. Identification of a carbonic anhydrase-like domain in the extracellular region of RPTP gamma defines a new subfamily of receptor tyrosine phosphatases. *Mol Cell Biol* 13: 1497–1506, 1993.
12. Bateman J, Reddy RS, Saito H, and Van Vactor D. The receptor tyrosine phosphatase Dlar and integrins organize actin filaments in the *Drosophila* follicular epithelium. *Curr Biol* 11: 1317–1327, 2001.
13. Bateman J, Shu H, and Van Vactor D. The guanine nucleotide exchange factor trio mediates axonal development in the *Drosophila* embryo. *Neuron* 26: 93–106, 2000.
14. Bear JE, Loureiro JJ, Libova I, Fassler R, Wehland J, and Gertler FB. Negative regulation of fibroblast motility by Ena/VASP proteins. *Cell* 101: 717–728, 2000.
15. Bear JE, Svitkina TM, Krause M, Schafer DA, Loureiro J, Strasser GA, Maly IV, Chaga OY, Cooper JA, Borisy GG, and Gertler FB. Antagonism between Ena/VASP proteins and actin filament capping regulates fibroblast motility. *Cell* 109: 509–521, 2002.
16. Bilwes AM, Den Hertog J, Hunter T, and Noel JP. Structural basis for inhibition of receptor protein-tyrosine phosphatase-alpha by dimerization. *Nature* 382: 555–559, 1996.
17. Bixby JL. Receptor tyrosine phosphatases in axon growth and guidance. *Neuroreport* 11: R5–R10, 2000.
18. Bixby JL and Zhang R. Purified N-cadherin is a potent substrate for the rapid induction of neurite outgrowth. *J Cell Biol* 110: 1253–1260, 1990.
19. Blanchetot C and Den Hertog J. Multiple interactions between receptor protein-tyrosine phosphatase (RPTP) alpha and membrane-distal protein-tyrosine phosphatase domains of various RPTPs. *J Biol Chem* 275: 12446–12452, 2000.
20. Bodden K and Bixby JL. Cryp-2: a receptor-type tyrosine phosphatase selectively expressed by developing vertebrate neurons. *J Neurobiol* 31: 309–324, 1996.
21. Brady-Kalnay SM, Flint AJ, and Tonks NK. Homophilic binding of PTP mu, a receptor-type protein tyrosine phosphatase, can mediate cell-cell aggregation. *J Cell Biol* 122: 961–972, 1993.
22. Brady-Kalnay SM, Mourton T, Nixon JP, Pietz GE, Kinch M, Chen H, Brackenbury R, Rimm DL, Del Vecchio RL, and Tonks NK. Dynamic interaction of PTPmu with multiple cadherins in vivo. *J Cell Biol* 141: 287–296, 1998.
23. Brady-Kalnay SM, Rimm DL, and Tonks NK. Receptor protein tyrosine phosphatase PTPmu associates with cadherins and catenins in vivo. *J Cell Biol* 130: 977–986, 1995.
24. Brady-Kalnay SM and Tonks NK. Identification of the homophilic binding site of the receptor protein tyrosine phosphatase PTP mu. *J Biol Chem* 269: 28472–28477, 1994.
25. Brady-Kalnay SM and Tonks NK. Protein tyrosine phosphatases as adhesion receptors. *Curr Opin Cell Biol* 7: 650–657, 1995.
26. Burden-Gulley SM and Brady-Kalnay SM. PTPmu regulates N-cadherin-dependent neurite outgrowth. *J Cell Biol* 144: 1323–1336, 1999.
27. Charbonneau H, Tonks NK, Walsh KA, and Fischer EH. The leukocyte common antigen (CD45): a putative receptor-linked protein tyrosine phosphatase. *Proc Natl Acad Sci USA* 85: 7182–7186, 1988.
28. Cheng J, Wu K, Armanini M, O'Rourke N, Dowbenko D, and Lasky LA. A novel protein-tyrosine phosphatase related to the homotypically adhering kappa and mu receptors. *J Biol Chem* 272: 7264–7277, 1997.
29. Cho H, Ramer SE, Itoh M, Kitas E, Bannwarth W, Burn P, Saito H, and Walsh CT. Catalytic domains of the LAR and CD45 protein tyrosine phosphatases from *Escherichia coli* expression systems: purification and characterization for specificity and mechanism. *Biochemistry* 31: 133–138, 1992.
30. Clandinin TR, Lee CH, Herman T, Lee RC, Yang AY, Ovasapyan S, and Zipursky SL. *Drosophila* LAR regulates R1–R6 and R7 target specificity in the visual system. *Neuron* 32: 237–248, 2001.
31. Cohen J, Burne JF, McKinlay C, and Winter J. The role of laminin and the laminin/fibronectin receptor complex in the outgrowth of retinal ganglion cell axons. *Dev Biol* 122: 407–418, 1987.
32. Crossland S, Smith PD, and Crompton MR. Molecular cloning and characterization of PTP pi, a novel receptor-like protein-tyrosine phosphatase. *Biochem J* 319: 249–254, 1996.
33. Debant A, Serra-Pages C, Seipel K, O'Brien S, Tang M, Park SH, and Streuli M. 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, 1996.
34. Den Hertog J, Tracy S, and Hunter T. 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, 1994.
35. Denu JM and Dixon JE. Protein tyrosine phosphatases: mechanisms of catalysis and regulation. *Curr Opin Chem Biol* 2: 633–641, 1998.
36. Denu JM, Lohse DL, Vijayalakshmi J, Saper MA, and Dixon JE. Visualization of intermediate and transition-state structures in protein-tyrosine phosphatase catalysis. *Proc Natl Acad Sci USA* 93: 2493–2498, 1996.
37. Denu JM, Stuckey JA, Saper MA, and Dixon JE. Form and function in protein dephosphorylation. *Cell* 87: 361–364, 1996.
38. Desai CJ, Gindhart JG Jr, Goldstein LS, and Zinn K. Receptor

tyrosine phosphatases are required for motor axon guidance in the *Drosophila* embryo. *Cell* 84: 599–609, 1996.
39. DESAI CJ, KRUEGER NX, SAITO H, AND ZINN K. Competition and cooperation among receptor tyrosine phosphatases control motoneuron growth cone guidance in *Drosophila*. *Development* 124: 1941–1952, 1997.
40. DESAI CJ, POPOVA E, AND ZINN K. A *Drosophila* receptor tyrosine phosphatase expressed in the embryonic CNS and larval optic lobes is a member of the set of proteins bearing the "HRP" carbohydrate epitope. *J Neurosci* 14: 7272–7283, 1994.
41. DESAI DM, SAP J, SCHLESSINGER J, AND WEISS A. Ligand-mediated negative regulation of a chimeric transmembrane receptor tyrosine phosphatase. *Cell* 73: 541–554, 1993.
42. DEVORE DL, HORVITZ HR, AND STERN MJ. An FGF receptor signaling pathway is required for the normal cell migrations of the sex myoblasts in *C. elegans* hermaphrodites. *Cell* 83: 611–620, 1995.
43. DOHERTY P, ROWETT LH, MOORE SE, MANN DA, AND WALSH FS. Neurite outgrowth in response to transfected N-CAM and N-cadherin reveals fundamental differences in neuronal responsiveness to CAMs. *Neuron* 6: 247–258, 1991.
44. DROSOPOULOS NE, WALSH FS, AND DOHERTY P. A soluble version of the receptor-like protein tyrosine phosphatase kappa stimulates neurite outgrowth via a Grb2/MEK1-dependent signaling cascade. *Mol Cell Neurosci* 13: 441–449, 1999.
45. EATON S, WEPF R, AND SIMONS K. Roles for Rac1 and Cdc42 in planar polarization and hair outgrowth in the wing of *Drosophila*. *J Cell Biol* 135: 1277–1289, 1996.
46. ELCHEBLY M, WAGNER J, KENNEDY TE, LANCTOT C, MICHALISZYN E, ITIE A, DROUIN J, AND TREMBLAY ML. Neuroendocrine dysplasia in mice lacking protein tyrosine phosphatase sigma. *Nat Genet* 21: 330–333, 1999.
47. FANTO M, WEBER U, STRUTT DI, AND MLODZIK M. Nuclear signaling by Rac and Rho GTPases is required in the establishment of epithelial planar polarity in the *Drosophila* eye. *Curr Biol* 10: 979–988, 2000.
48. FASHENA SJ AND ZINN K. Transmembrane glycoprotein gp150 is a substrate for receptor tyrosine phosphatase DPTP10D in *Drosophila* cells. *Mol Cell Biol* 17: 6859–6867, 1997.
49. FRYDMAN HM AND SPRADLING AC. The receptor-like tyrosine phosphatase lar is required for epithelial planar polarity and for axis determination within *Drosophila* ovarian follicles. *Development* 128: 3209–3220, 2001.
50. FUCHS M, MULLER T, LERCH MM, AND ULLRICH A. Association of human protein-tyrosine phosphatase kappa with members of the armadillo family. *J Biol Chem* 271: 16712–16719, 1996.
51. GARRITY PA, LEE CH, SALECKER I, ROBERTSON HC, DESAI CJ, ZINN K, AND ZIPURSKY SL. Retinal axon target selection in *Drosophila* is regulated by a receptor protein tyrosine phosphatase. *Neuron* 22: 707–717, 1999.
52. GEBBINK MF, ZONDAG GC, WUBBOLTS RW, BEIJERSBERGEN RL, VAN ETTEN I, AND MOOLENAAR WH. Cell-cell adhesion mediated by a receptor-like protein tyrosine phosphatase. *J Biol Chem* 268: 16101–16104, 1993.
53. GERSHON TR, BAKER MW, NITABACH M, AND MACAGNO ER. The leech receptor protein tyrosine phosphatase HmLAR2 is concentrated in growth cones and is involved in process outgrowth. *Development* 125: 1183–1190, 1998.
54. GERSHON TR, BAKER MW, NITABACH M, WU P, AND MACAGNO ER. Two receptor tyrosine phosphatases of the LAR family are expressed in the developing leech by specific central neurons as well as select peripheral neurons, muscles, and other cells. *J Neurosci* 18: 2991–3002, 1998.
55. GERTLER FB, COMER AR, JUANG JL, AHERN SM, CLARK MJ, LIEBL EC, AND HOFFMANN FM. Enabled, a dosage-sensitive suppressor of mutations in the *Drosophila* Abl tyrosine kinase, encodes an Abl substrate with SH3 domain-binding properties. *Genes Dev* 9: 521–533, 1995.
56. GERTLER FB, NIEBUHR K, REINHARD M, WEHLAND J, AND SORIANO P. Mena, a relative of VASP and *Drosophila* Enabled, is implicated in the control of microfilament dynamics. *Cell* 87: 227–239, 1996.
57. HAJ F, MCKINNELL I, AND STOKER A. Retinotectal ligands for the receptor tyrosine phosphatase CRYPalpha. *Mol Cell Neurosci* 14: 225–240, 1999.
58. HAKEDA-SUZUKI S, NG J, TZU J, DIETZL G, SUN Y, HARMS M, NARDINE T, LUO L, AND DICKSON BJ. Rac function and regulation during *Drosophila* development. *Nature* 416: 438–442, 2002.
59. HALL A. The cellular functions of small GTP-binding proteins. *Science* 249: 635–640, 1990.
60. HAMANAKA H, MAEDA N, AND NODA M. Spatially and temporally regulated modification of the receptor-like protein tyrosine phosphatase zeta/beta isoforms with keratan sulphate in the developing chick brain. *Eur J Neurosci* 9: 2297–2308, 1997.
61. HARRINGTON RJ, GUTCH MJ, HENGARTNER MO, TONKS NK, AND CHISHOLM AD. The *C. elegans* LAR-like receptor tyrosine phosphatase PTP-3 and the VAB-1 Eph receptor tyrosine kinase have partly redundant functions in morphogenesis. *Development* 129: 2141–2153, 2002.
62. HAWORTH K, SHU KK, STOKES A, MORRIS R, AND STOKER A. The expression of receptor tyrosine phosphatases is responsive to sciatic nerve crush. *Mol Cell Neurosci* 12: 93–104, 1998.
63. HOFFMANN FM. *Drosophila* abl and genetic redundancy in signal transduction. *Trends Genet* 7: 351–355, 1991.
64. HOFFMANN KM, TONKS NK, AND BARFORD D. The crystal structure of domain 1 of receptor protein-tyrosine phosphatase mu. *J Biol Chem* 272: 27505–27508, 1997.
65. HOLT MR AND KOFFER A. Cell motility: proline-rich proteins promote protrusions. *Trends Cell Biol* 11: 38–46, 2001.
66. HOOFT VAN HUIJSDUIJNEN R. Protein tyrosine phosphatases: counting the trees in the forest. *Gene* 225: 1–8, 1998.
67. HOPKER VH, SHEWAN D, TESSIER-LAVIGNE M, POO M, AND HOLT C. Growth-cone attraction to netrin-1 is converted to repulsion by laminin- 1. *Nature* 401: 69–73, 1999.
68. HUNTER T AND SEFTON BM. Transforming gene product of Rous sarcoma virus phosphorylates tyrosine. *Proc Natl Acad Sci USA* 77: 1311–1315, 1980.
69. IWAI Y, USUI T, HIRANO S, STEWARD R, TAKEICHI M, AND UEMURA T. Axon patterning requires DN-cadherin, a novel neuronal adhesion receptor, in the *Drosophila* embryonic CNS. *Neuron* 19: 77–89, 1997.
70. JIANG G, DEN HERTOG J, SU J, NOEL J, SAP J, AND HUNTER T. Dimerization inhibits the activity of receptor-like protein-tyrosine phosphatase-alpha. *Nature* 401: 606–610, 1999.
71. JIANG YP, WANG H, D'EUSTACHIO P, MUSACCHIO JM, SCHLESSINGER J, AND SAP J. Cloning and characterization of R-PTP-kappa, a new member of the receptor protein tyrosine phosphatase family with a proteolytically cleaved cellular adhesion molecule-like extracellular region. *Mol Cell Biol* 13: 2942–2951, 1993.
72. JOHNSON KG AND HOLT CE. Expression of CRYP-alpha, LAR, PTP-delta, and PTP-rho in the developing *Xenopus* visual system. *Mech Dev* 92: 291–294, 2000.
73. JOHNSON KG, MCKINNELL IW, STOKER AW, AND HOLT CE. Receptor protein tyrosine phosphatases regulate retinal ganglion cell axon outgrowth in the developing *Xenopus* visual system. *J Neurobiol* 49: 99–117, 2001.
74. KAPLAN R, MORSE B, HUEBNER K, CROCE C, HOWK R, RAVERA M, RICCA G, JAYE M, AND SCHLESSINGER J. Cloning of three human tyrosine phosphatases reveals a multigene family of receptor-linked protein-tyrosine-phosphatases expressed in brain. *Proc Natl Acad Sci USA* 87: 7000–7004, 1990.
75. KAUFMANN N, DEPROTO J, RANJAN R, WAN H, AND VAN VACTOR D. *Drosophila* Liprin-alpha and the receptor phosphatase Dlar control synapse morphogenesis. *Neuron*. In press.
76. KAUFMANN N, WILLS ZP, AND VAN VACTOR D. *Drosophila* Rac1 controls motor axon guidance. *Development* 125: 453–461, 1998.
77. KAWACHI H, FUJIKAWA A, MAEDA N, AND NODA M. Identification of GIT1/Cat-1 as a substrate molecule of protein tyrosine phosphatase zeta/beta by the yeast substrate-trapping system. *Proc Natl Acad Sci USA* 98: 6593–6598, 2001.
78. KAWACHI H, TAMURA H, WATAKABE I, SHINTANI T, MAEDA N, AND NODA M. Protein tyrosine phosphatase zeta/RPTPbeta interacts with PSD-95/SAP90 family. *Brain Res* 72: 47–54, 1999.
79. KIDD T, BLAND KS, AND GOODMAN CS. Slit is the midline repellent for the robo receptor in *Drosophila*. *Cell* 96: 785–794, 1999.
80. KIDD T, BROSE K, MITCHELL KJ, FETTER RD, TESSIER-LAVIGNE M, GOODMAN CS, AND TEAR G. Roundabout controls axon crossing of the CNS midline and defines a novel subfamily of evolutionarily conserved guidance receptors. *Cell* 92: 205–215, 1998.

81. KOKEL M, BORLAND CZ, DELONG L, HORVITZ HR, AND STERN MJ. Clr-1 encodes a receptor tyrosine phosphatase that negatively regulates an FGF receptor signaling pathway in *Caenorhabditis elegans*. *Genes Dev* 12: 1425–1437, 1998.
82. KOLESKE AJ, GIFFORD AM, SCOTT ML, NEE M, BRONSON RT, MICZEK KA, AND BALTIMORE D. Essential roles for the Abl and Arg tyrosine kinases in neurulation. *Neuron* 21: 1259–1272, 1998.
83. KREBS EG. Historical perspectives on protein phosphorylation and a classification system for protein kinases. *Philos Trans R Soc Lond B Biol Sci* 302: 3–11, 1983.
84. KRUEGER NX AND SAITO H. A human transmembrane protein-tyrosine-phosphatase, PTP zeta, is expressed in brain and has an N-terminal receptor domain homologous to carbonic anhydrases. *Proc Natl Acad Sci USA* 89: 7417–7421, 1992.
85. KRUEGER NX, VAN VACTOR D, WAN HI, GELBART WM, GOODMAN CS, AND SAITO H. The transmembrane tyrosine phosphatase DLAR controls motor axon guidance in *Drosophila*. *Cell* 84: 611–622, 1996.
86. KYPTA RM, SU H, AND REICHARDT LF. Association between a transmembrane protein tyrosine phosphatase and the cadherin-catenin complex. *J Cell Biol* 134: 1519–1529, 1996.
87. LANDGRAF M, BOSSING T, TECHNAU GM, AND BATE M. The origin, location, and projections of the embryonic abdominal motorneurons of *Drosophila*. *J Neurosci* 17: 9642–9655, 1997.
88. LANIER LM, GATES MA, WITKE W, MENZIES AS, WEHMAN AM, MACKLIS JD, KWIATKOWSKI D, SORIANO P, AND GERTLER FB. Mena is required for neurulation and commissure formation. *Neuron* 22: 313–325, 1999.
89. LANIER LM AND GERTLER FB. From Abl to actin: Abl tyrosine kinase and associated proteins in growth cone motility. *Curr Opin Neurobiol* 10: 80–87, 2000.
90. LEDIG MM, HAJ F, BIXBY JL, STOKER AW, AND MUELLER BK. The receptor tyrosine phosphatase CRYPalpha promotes intraretinal axon growth. *J Cell Biol* 147: 375–388, 1999.
91. LEDIG MM, MCKINNELL IW, MRSIC-FLOGEL T, WANG J, ALVARES C, MASON I, BIXBY JL, MUELLER BK, AND STOKER AW. Expression of receptor tyrosine phosphatases during development of the retinotectal projection of the chick. *J Neurobiol* 39: 81–96, 1999.
92. LEE CH, HERMAN T, CLANDININ TR, LEE R, AND ZIPURSKY SL. N-cadherin regulates target specificity in the *Drosophila* visual system. *Neuron* 30: 437–450, 2001.
93. LIEBL EC, FORSTHOEFEL DJ, FRANCO LS, SAMPLE SH, HESS JE, COWGER JA, CHANDLER MP, SHUPERT AM, AND SEEGER MA. Dosage-sensitive, reciprocal genetic interactions between the Abl tyrosine kinase and the putative GEF trio reveal trio's role in axon pathfinding. *Neuron* 26: 107–118, 2000.
94. LOUREIRO J AND PEIFER M. Roles of Armadillo, a *Drosophila* catenin, during central nervous system development. *Curr Biol* 8: 622–632, 1998.
95. LUO L, LIAO YJ, JAN LY, AND JAN YN. Distinct morphogenetic functions of similar small GTPases: *Drosophila* Drac1 is involved in axonal outgrowth and myoblast fusion. *Genes Dev* 8: 1787–1802, 1994.
96. MAEDA N, NISHIWAKI T, SHINTANI T, HAMANAKA H, AND NODA M. 6b4 proteoglycan/phosphacan, an extracellular variant of receptor-like protein-tyrosine phosphatase zeta/RPTPbeta, binds pleiotrophin/heparin-binding growth-associated molecule (HB-GAM). *J Biol Chem* 271: 21446–21452, 1996.
97. MAEDA N AND NODA M. Involvement of receptor-like protein tyrosine phosphatase zeta/RPTPbeta and its ligand pleiotrophin/heparin-binding growth-associated molecule (HB-GAM) in neuronal migration. *J Cell Biol* 142: 203–216, 1998.
98. MAJETI R, XU Z, PARSLOW TG, OLSON JL, DAIKH DI, KILLEEN N, AND WEISS A. An inactivating point mutation in the inhibitory wedge of CD45 causes lymphoproliferation and autoimmunity. *Cell* 103: 1059–1070, 2000.
99. MATSUNAGA M, HATTA K, NAGAFUCHI A, AND TAKEICHI M. Guidance of optic nerve fibres by N-cadherin adhesion molecules. *Nature* 334: 62–64, 1988.
100. MAUREL P, RAUCH U, FLAD M, MARGOLIS RK, AND MARGOLIS RU. Phosphacan, a chondroitin sulfate proteoglycan of brain that interacts with neurons and neural cell-adhesion molecules, is an extracellular variant of a receptor-type protein tyrosine phosphatase. *Proc Natl Acad Sci USA* 91: 2512–2516, 1994.
101. MAUREL-ZAFFRAN C, SUZUKI T, GAHMON G, TREISMAN JE, AND DICKSON BJ. Cell-autonomous and -nonautonomous functions of LAR in R7 photoreceptor axon targeting. *Neuron* 32: 225–235, 2001.
102. MCANDREW PE, FROSTHOLM A, EVANS JE, ZDILAR D, GOLDOWITZ D, CHIU IM, BURGHES AH, AND ROTTER A. Novel receptor protein tyrosine phosphatase (RPTPrho) and acidic fibroblast growth factor (FGF-1) transcripts delineate a rostrocaudal boundary in the granule cell layer of the murine cerebellar cortex. *J Comp Neurol* 391: 444–455, 1998.
103. MENG K, RODRIGUEZ-PENA A, DIMITROV T, CHEN W, YAMIN M, NODA M, AND DEUEL TF. Pleiotrophin signals increased tyrosine phosphorylation of beta beta-catenin through inactivation of the intrinsic catalytic activity of the receptor-type protein tyrosine phosphatase beta/zeta. *Proc Natl Acad Sci USA* 97: 2603–2608, 2000.
104. MENG TC, FUKADA T, AND TONKS NK. Reversible oxidation and inactivation of protein tyrosine phosphatases in vivo. *Mol Cell* 9: 387–399, 2002.
105. MILEV P, FRIEDLANDER DR, SAKURAI T, KARTHIKEYAN L, FLAD M, MARGOLIS RK, GRUMET M, AND MARGOLIS RU. Interactions of the chondroitin sulfate proteoglycan phosphacan, the extracellular domain of a receptor-type protein tyrosine phosphatase, with neurons, glia, and neural cell adhesion molecules. *J Cell Biol* 127: 1703–1715, 1994.
106. MOURTON T, HELLBERG CB, BURDEN-GULLEY SM, HINMAN J, RHEE A, AND BRADY-KALNAY SM. The PTPmu protein-tyrosine phosphatase binds and recruits the scaffolding protein RACK1 to cell-cell contacts. *J Biol Chem* 276: 14896–14901, 2001.
107. MUELLER BK, LEDIG MM, AND WAHL S. The receptor tyrosine phosphatase CRYPalpha affects growth cone morphology. *J Neurobiol* 44: 204–218, 2000.
108. MULLER T, CHOIDAS A, REICHMANN E, AND ULLRICH A. Phosphorylation and free pool of beta-catenin are regulated by tyrosine kinases and tyrosine phosphatases during epithelial cell migration. *J Biol Chem* 274: 10173–10183, 1999.
109. MUSTELIN T AND ALTMAN A. Dephosphorylation and activation of the T cell tyrosine kinase pp56lck by the leukocyte common antigen (CD45). *Oncogene* 5: 809–813, 1990.
110. MUSTELIN T, COGGESHALL KM, AND ALTMAN A. Rapid activation of the T-cell tyrosine protein kinase pp56lck by the CD45 phosphotyrosine phosphatase. *Proc Natl Acad Sci USA* 86: 6302–6306, 1989.
111. NAM HJ, POY F, KRUEGER NX, SAITO H, AND FREDERICK CA. Crystal structure of the tandem phosphatase domains of RPTP LAR. *Cell* 97: 449–457, 1999.
112. NEWSOME TP, ASLING B, AND DICKSON BJ. Analysis of *Drosophila* photoreceptor axon guidance in eye-specific mosaics. *Development* 127: 851–860, 2000.
113. NEWSOME TP, SCHMIDT S, DIETZL G, KELEMAN K, ASLING B, DEBANT A, AND DICKSON BJ. Trio combines with dock to regulate Pak activity during photoreceptor axon pathfinding in *Drosophila*. *Cell* 101: 283–294, 2000.
114. NISHIWAKI T, MAEDA N, AND NODA M. Characterization and developmental regulation of proteoglycan-type protein tyrosine phosphatase zeta/RPTPbeta isoforms. *J Biochem* 123: 458–467, 1998.
115. O'BRIEN SP, SEIPEL K, MEDLEY QG, BRONSON R, SEGAL R, AND STREULI M. Skeletal muscle deformity and neuronal disorder in Trio exchange factor-deficient mouse embryos. *Proc Natl Acad Sci USA* 97: 12074–12078, 2000.
116. O'GRADY P, THAI TC, AND SAITO H. 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, 1998.
117. OON SH, HONG A, YANG X, AND CHIA W. Alternative splicing in a novel tyrosine phosphatase gene (DPTP4E) of *Drosophila melanogaster* generates two large receptor-like proteins which differ in their carboxyl termini. *J Biol Chem* 268: 23964–23971, 1993.
118. PAYNE HR, BURDEN SM, AND LEMMON V. Modulation of growth cone morphology by substrate-bound adhesion molecules. *Cell Motil Cytoskeleton* 21: 65–73, 1992.
119. PELES E, NATIV M, CAMPBELL PL, SAKURAI T, MARTINEZ R, LEV S, CLARY DO, SCHILLING J, BARNEA G, AND PLOWMAN GD. The carbonic anhydrase domain of receptor tyrosine phosphatase beta is a functional ligand for the axonal cell recognition molecule contactin. *Cell* 82: 251–260, 1995.

120. Pot DA and Dixon JE. Active site labeling of a receptor-like protein tyrosine phosphatase. *J Biol Chem* 267: 140–143, 1992.
121. Pot DA, Woodford TA, Remboutsika E, Haun RS, and Dixon JE. Cloning, bacterial expression, purification, and characterization of the cytoplasmic domain of rat LAR, a receptor-like protein tyrosine phosphatase. *J Biol Chem* 266: 19688–19696, 1991.
122. Powell SK and Kleinman HK. Neuronal laminins and their cellular receptors. *Int J Biochem Cell Biol* 29: 401–414, 1997.
123. Pulido R, Krueger NX, Serra-Pages C, Saito H, and Streuli M. Molecular characterization of the human transmembrane protein-tyrosine phosphatase delta. Evidence for tissue-specific expression of alternative human transmembrane protein-tyrosine phosphatase delta isoforms. *J Biol Chem* 270: 6722–6728, 1995.
124. Pulido R, Serra-Pages C, Tang M, and Streuli M. 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, 1995.
125. Reinhard M, Giehl K, Abel K, Haffner C, Jarchau T, Hoppe V, Jockusch BM, and Walter U. The proline-rich focal adhesion and microfilament protein VASP is a ligand for profilins. *EMBO J* 14: 1583–1589, 1995.
126. Riehl R, Johnson K, Bradley R, Grunwald GB, Cornel E, Lilienbaum A, and Holt CE. Cadherin function is required for axon outgrowth in retinal ganglion cells in vivo. *Neuron* 17: 837–848, 1996.
127. Rosdahl JA, Mourton TL, and Brady-Kalnay SM. Protein kinase C delta (PKCdelta) is required for protein tyrosine phosphatase mu (PTPmu)-dependent neurite outgrowth. *Mol Cell Neurosci* 19: 292–306, 2002.
128. Saito H. Structural diversity of eukaryotic protein tyrosine phosphatases: functional and evolutionary implications. *Semin Cell Biol* 4: 379–387, 1993.
129. Sap J, D'eustachio P, Givol D, and Schlessinger J. Cloning and expression of a widely expressed receptor tyrosine phosphatase. *Proc Natl Acad Sci USA* 87: 6112–6116, 1990.
130. Schaapveld RQ, Schepens JT, Bachner D, Attema J, Wieringa B, Jap PH, and Hendriks WJ. Developmental expression of the cell adhesion molecule-like protein tyrosine phosphatases LAR, RPTPdelta and RPTPsigma in the mouse. *Mech Dev* 77: 59–62, 1998.
131. Schindelholz B, Knirr M, Warrior R, and Zinn K. Regulation of CNS and motor axon guidance in *Drosophila* by the receptor tyrosine phosphatase DPTP52F. *Development* 128: 4371–4382, 2001.
132. Seeger M, Tear G, Ferres-Marco D, and Goodman CS. Mutations affecting growth cone guidance in *Drosophila*: genes necessary for guidance toward or away from the midline. *Neuron* 10: 409–426, 1993.
133. Seimiya H, Sawabe T, Inazawa J, and Tsuruo T. Cloning, expression and chromosomal localization of a novel gene for protein tyrosine phosphatase (PTP-U2) induced by various differentiation-inducing agents. *Oncogene* 10: 1731–1738, 1995.
134. Serra-Pages C, Kedersha NL, Fazikas L, Medley Q, Debant A, and Streuli M. The LAR transmembrane protein tyrosine phosphatase and a coiled-coil LAR-interacting protein co-localize at focal adhesions. *EMBO J* 14: 2827–2838, 1995.
135. Serra-Pages C, Medley QG, Tang M, Hart A, and Streuli M. Liprins, a family of LAR transmembrane protein-tyrosine phosphatase-interacting proteins. *J Biol Chem* 273: 15611–15620, 1998.
136. Serra-Pages C, Saito H, and Streuli M. Mutational analysis of proprotein processing, subunit association, and shedding of the LAR transmembrane protein tyrosine phosphatase. *J Biol Chem* 269: 23632–23641, 1994.
137. Shintani T, Watanabe E, Maeda N, and Noda M. Neurons as well as astrocytes express proteoglycan-type protein tyrosine phosphatase zeta/RPTPbeta: analysis of mice in which the PTPzeta/RPTPbeta gene was replaced with the LacZ gene. *Neurosci Lett* 247: 135–138, 1998.
138. Shock LP, Bare DJ, Klinz SG, and Maness PF. Protein tyrosine phosphatases expressed in developing brain and retinal Muller glia. *Brain Res* 28: 110–116, 1995.
139. Skarnes WC, Moss JE, Hurtley SM, and Beddington RS. Capturing genes encoding membrane and secreted proteins important for mouse development. *Proc Natl Acad Sci USA* 92: 6592–6596, 1995.
140. Smith JM and Mayer BJ. Abl: mechanisms of regulation and activation. *Front Biosci* 7: D31–D42, 2002.
141. Sommer L, Rao M, and Anderson DJ. RPTP delta and the novel protein tyrosine phosphatase RPTP psi are expressed in restricted regions of the developing central nervous system. *Dev Dyn* 208: 48–61, 1997.
142. Stepanek L, Sun QL, Wang J, Wang C, and Bixby JL. Cryp-2/cPTPRO is a neurite inhibitory repulsive guidance cue for retinal neurons in vitro. *J Cell Biol* 154: 867–878, 2001.
143. Steven R, Kubiseski TJ, Zheng H, Kulkarni S, Mancillas J, Ruiz Morales A, Hogue CW, Pawson T, and Culotti J. Unc-73 activates the Rac GTPase and is required for cell and growth cone migrations in *C. elegans*. *Cell* 92: 785–795, 1998.
144. Stoker AW. Isoforms of a novel cell adhesion molecule-like protein tyrosine phosphatase are implicated in neural development. *Mech Dev* 46: 201–217, 1994.
145. Stoker AW. Receptor tyrosine phosphatases in axon growth and guidance. *Curr Opin Neurobiol* 11: 95–102, 2001.
146. Stoker AW, Gehrig B, Haj F, and Bay BH. Axonal localisation of the CAM-like tyrosine phosphatase CRYP alpha: a signalling molecule of embryonic growth cones. *Development* 121: 1833–1844, 1995.
147. Stoker AW, Gehrig B, Newton MR, and Bay BH. Comparative localisation of CRYP alpha, a CAM-like tyrosine phosphatase, and NgCAM in the developing chick visual system. *Dev Brain Res* 90: 129–140, 1995.
148. Stone KE and Sakaguchi DS. Perturbation of the developing *Xenopus* retinotectal projection following injections of antibodies against beta1 integrin receptors and N-cadherin. *Dev Biol* 180: 297–310, 1996.
149. Streuli M, Krueger NX, Ariniello PD, Tang M, Munro JM, Blattler WA, Adler DA, Disteche CM, and Saito H. Expression of the receptor-linked protein tyrosine phosphatase LAR: proteolytic cleavage and shedding of the CAM-like extracellular region. *EMBO J* 11: 897–907, 1992.
150. Streuli M, Krueger NX, Hall LR, Schlossman SF, and Saito H. A new member of the immunoglobulin superfamily that has a cytoplasmic region homologous to the leukocyte common antigen. *J Exp Med* 168: 1523–1530, 1988.
151. Streuli M, Krueger NX, Thai T, Tang M, and Saito H. Distinct functional roles of the two intracellular phosphatase like domains of the receptor-linked protein tyrosine phosphatases LCA and LAR. *EMBO J* 9: 2399–2407, 1990.
152. Streuli M, Krueger NX, Tsai AY, and Saito H. A family of receptor-linked protein tyrosine phosphatases in humans and *Drosophila*. *Proc Natl Acad Sci USA* 86: 8698–8702, 1989.
153. Stuckey JA, Schubert HL, Fauman EB, Zhang ZY, Dixon JE, and Saper MA. Crystal structure of *Yersinia* protein tyrosine phosphatase at 2.5 A and the complex with tungstate. *Nature* 370: 571–575, 1994.
154. Sun Q, Bahri S, Schmid A, Chia W, and Zinn K. Receptor tyrosine phosphatases regulate axon guidance across the midline of the *Drosophila* embryo. *Development* 127: 801–812, 2000.
155. Sun QL, Bookman RJ, and Bixby JL. Growth cones migrate up gradients of PTP-delta but not laminin. *Abstr Soc Neurosci* 25: 1282, 1999.
156. Tagawa M, Shirasawa T, Yahagi Y, Tomoda T, Kuroyanagi H, Fujimura S, Sakiyama S, and Maruyama N. Identification of a receptor-type protein tyrosine phosphatase expressed in postmitotic maturing neurons: its structure and expression in the central nervous system. *Biochem J* 321: 865–871, 1997.
157. Tertoolen LG, Blanchetot C, Jiang G, Overvoorde J, Gadella TW Jr, Hunter T, and Hertog JDJ. Dimerization of receptor protein-tyrosine phosphatase alpha in living cells. *BMC Cell Biol* 2: 8, 2001.
158. Thomas PE, Wharram BL, Goyal M, Wiggins JE, Holzman LB, and Wiggins RC. Glepp1, a renal glomerular epithelial cell (podocyte) membrane protein-tyrosine phosphatase. Identification, molecular cloning, and characterization in rabbit. *J Biol Chem* 269: 19953–19962, 1994.
159. Tian SS, Tsoulfas P, and Zinn K. Three receptor-linked protein-tyrosine phosphatases are selectively expressed on central nervous system axons in the *Drosophila* embryo. *Cell* 67: 675–680, 1991.

160. TIAN SS AND ZINN K. An adhesion molecule-like protein that interacts with and is a substrate for a *Drosophila* receptor-linked protein tyrosine phosphatase. *J Biol Chem* 269: 28478–28486, 1994.
161. TOMEMORI T, SEKI N, SUZUKI Y, SHIMIZU T, NAGATA H, KONNO A, AND SHIRASAWA T. Isolation and characterization of murine orthologue of PTP-BK. *Biochem Biophys Res Commun* 276: 974–981, 2000.
162. TONKS NK, CHARBONNEAU H, DILTZ CD, FISCHER EH, AND WALSH KA. Demonstration that the leukocyte common antigen CD45 is a protein tyrosine phosphatase. *Biochemistry* 27: 8695–8701, 1988.
163. TONKS NK, DILTZ CD, AND FISCHER EH. Purification of the major protein-tyrosine-phosphatases of human placenta. *J Biol Chem* 263: 6722–6730, 1988.
164. UETANI N, KATO K, OGURA H, MIZUNO K, KAWANO K, MIKOSHIBA K, YAKURA H, ASANO M, AND IWAKURA Y. Impaired learning with enhanced hippocampal long-term potentiation in PTPdelta-deficient mice. *EMBO J* 19: 2775–2785, 2000.
165. USHIRO H AND COHEN S. Identification of phosphotyrosine as a product of epidermal growth factor-activated protein kinase in A-431 cell membranes. *J Biol Chem* 255: 8363–8365, 1980.
166. VAN DER SAR AM, ZIVKOVIC D, AND DEN HERTOG J. Eye defects in receptor protein-tyrosine phosphatase alpha knock-down zebrafish. *Dev Dyn* 223: 292–297, 2002.
167. VAN LIESHOUT E, VAN DER HEIJDEN I, HENDRIKS W, AND VAN DER ZEE C. Lar Ptp-deficient mice show impaired sensory and motor nerve regeneration following crush lesion. *Abstr Soc Neurosci* 25: 216, 1999.
168. VAN VACTOR D, O'REILLY AM, AND NEEL BG. Genetic analysis of protein tyrosine phosphatases. *Curr Opin Genet Dev* 8: 112–126, 1998.
169. VERHEYEN EM AND COOLEY L. Profilin mutations disrupt multiple actin-dependent processes during *Drosophila* development. *Development* 120: 717–728, 1994.
170. WALLACE MJ, BATT J, FLADD CA, HENDERSON JT, SKARNES W, AND ROTIN D. Neuronal defects and posterior pituitary hypoplasia in mice lacking the receptor tyrosine phosphatase PTPsigma. *Nat Genet* 21: 334–338, 1999.
171. WALLACE MJ, FLADD C, BATT J, AND ROTIN D. The second catalytic domain of protein tyrosine phosphatase delta (PTP delta) binds to and inhibits the first catalytic domain of PTP sigma. *Mol Cell Biol* 18: 2608–2616, 1998.
172. WANG J AND BIXBY JL. Receptor tyrosine phosphatase-delta is a homophilic, neurite-promoting cell adhesion molecule for CNS neurons. *Mol Cell Neurosci* 14: 370–384, 1999.
173. WANG R, ST. JOHN PL, KRETZLER M, WIGGINS RC, AND ABRAHAMSON DR. Molecular cloning, expression, and distribution of glomerular epithelial protein 1 in developing mouse kidney. *Kidney Int* 57: 1847–1859, 2000.
174. WIGGINS RC, WIGGINS JE, GOYAL M, WHARRAM BL, AND THOMAS PE. Molecular cloning of cDNAs encoding human GLEPP1, a membrane protein tyrosine phosphatase: characterization of the GLEPP1 protein distribution in human kidney and assignment of the GLEPP1 gene to human chromosome 12p12–p13. *Genomics* 27: 174–181, 1995.
175. WILLS Z, BATEMAN J, KOREY CA, COMER A, AND VAN VACTOR D. The tyrosine kinase Abl and its substrate enabled collaborate with the receptor phosphatase Dlar to control motor axon guidance. *Neuron* 22: 301–312, 1999.
176. WILLS Z, MARR L, ZINN K, GOODMAN CS, AND VAN VACTOR D. Profilin and the Abl tyrosine kinase are required for motor axon outgrowth in the *Drosophila* embryo. *Neuron* 22: 291–299, 1999.
177. WYSZYNSKI M, KIM E, DUNAH AW, PASSAFARO M, VALTSCHANOFF JG, SERRA-PAGES C, STREULI M, WEINBERG RJ, AND SHENG M. Interaction between GRIP and liprin-alpha/SYD2 is required for AMPA receptor targeting. *Neuron* 34: 39–52, 2002.
178. XIE Y, YEO TT, ZHANG C, YANG T, TISI MA, MASSA SM, AND LONGO FM. The leukocyte common antigen-related protein tyrosine phosphatase receptor regulates regenerative neurite outgrowth in vivo. *J Neurosci* 21: 5130–5138, 2001.
179. XIONG Q, GUO X, ZONG CS, JONG SMS, JIANG Y, CHAN J, AND WANG LH. Cloning and expression of chicken protein tyrosine phosphatase gamma. *J Biomed Sci* 3: 266–274, 1996.
180. YAN H, GROSSMAN A, WANG H, D'EUSTACHIO P, MOSSIE K, MUSACCHIO JM, SILVENNOINEN O, AND SCHLESSINGER J. A novel receptor tyrosine phosphatase-sigma that is highly expressed in the nervous system. *J Biol Chem* 268: 24880–24886, 1993.
181. YANG XH, SEOW KT, BAHRI SM, OON SH, AND CHIA W. 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, 1991.
182. YEO TT, YANG T, MASSA SM, ZHANG JS, HONKANIEMI J, BUTCHER LL, AND LONGO FM. Deficient LAR expression decreases basal forebrain cholinergic neuronal size and hippocampal cholinergic innervation. *J Neurosci Res* 47: 348–360, 1997.
183. YU Q, LENARDO T, AND WEINBERG RA. The N-terminal and C-terminal domains of a receptor tyrosine phosphatase are associated by non-covalent linkage. *Oncogene* 7: 1051–1057, 1992.
184. ZHANG JS, HONKANIEMI J, YANG T, YEO TT, AND LONGO FM. Lar tyrosine phosphatase receptor: a developmental isoform is present in neurites and growth cones and its expression is regional- and cell-specific. *Mol Cell Neurosci* 10: 271–286, 1998.
185. ZHANG JS AND LONGO FM. Lar tyrosine phosphatase receptor: alternative splicing is preferential to the nervous system, coordinated with cell growth and generates novel isoforms containing extensive CAG repeats. *J Cell Biol* 128: 415–431, 1995.
186. ZHANG ZY, WANG Y, WU L, FAUMAN EB, STUCKEY JA, SCHUBERT HL, SAPER MA, AND DIXON JE. The Cys(X)5Arg catalytic motif in phosphoester hydrolysis. *Biochemistry* 33: 15266–15270, 1994.
187. ZHEN M AND JIN Y. The liprin protein SYD-2 regulates the differentiation of presynaptic termini in *C. elegans*. *Nature* 401: 371–375, 1999.
188. ZIPKIN ID, KINDT RM, AND KENYON CJ. Role of a new Rho family member in cell migration and axon guidance in *C. elegans*. *Cell* 90: 883–894, 1997.
189. ZONDAG GC, KONINGSTEIN GM, JIANG YP, SAP J, MOOLENAAR WH, AND GEBBINK MF. 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, 1995.