The C. elegans LAR-like receptor tyrosine phosphatase PTP-3 and the VAB-1 Eph receptor tyrosine kinase have partly redundant functions in morphogenesis

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SUMMARY

Receptor-like protein-tyrosine phosphatases (RPTPs) form a diverse family of cell surface molecules whose functions remain poorly understood. The LAR subfamily of RPTPs has been implicated in axon guidance and neural development. Here we report the molecular and genetic analysis of the C. elegans LAR subfamily member PTP-3. PTP-3 isoforms are expressed in many tissues in early embryogenesis, and later become localized to neuronal processes and to epithelial adherens junctions. Loss of function in ptp-3 causes low-penetrance defects in gastrulation and epidermal development similar to those of VAB-1 Eph receptor tyrosine kinase mutants. Loss of function in ptp-3 synergistically enhances phenotypes of mutations in the C. elegans Eph receptor VAB-1 and a subset of its ephrin ligands, but does not show specific interactions with several other RTKs or morphogenetic mutants. The genetic interaction of vab-1 and ptp-3 suggests that LAR-like RPTPs and Eph receptors have related and partly redundant functions in C. elegans morphogenesis.

Key words: Eph receptor, Phosphatase, RPTP, LAR, Morphogenesis, C. elegans

INTRODUCTION

Receptor-like protein-tyrosine phosphatases (RPTPs) form a diverse family of transmembrane enzymes that play roles in cell adhesion and cell signaling (Brady-Kalnay and Tonks, 1995; den Hertog et al., 1999). The LAR (Leukocyte Common Antigen Related) protein is the founding member of a subfamily of RPTPs known as type IIb RPTPs, defined by extracellular domains composed of immunoglobulin-like and fibronectin type III (FNIII) domains. The extracellular domain of LAR thus resembles those of cell adhesion proteins such as N-CAM, implying that it links cell adhesion and intracellular tyrosine phosphorylation.

Vertebrate genomes contain at least three LAR-like RPTP genes: LAR, PTPβ, and PTPσ. All three generate multiple protein isoforms by tissue-specific alternative splicing, and are expressed in complex patterns in many ectodermal and endodermal epithelia and in neural tissues (Pulido et al., 1995; Stoker and Dutta, 1998). In non-neuronal cells LAR family members localize to focal adhesions (Serra-Pagès et al., 1995), adherens junctions (Aicher et al., 1997) and regions in contact with basal laminae. In neurons, LAR family members are found on cell bodies, processes and growth cones, suggesting a role in modulating cell adhesion during axon outgrowth (Zhang et al., 1998; Zhang and Longo, 1995). The Drosophila LAR ortholog Lar (previously known as Dlar), is mostly expressed in the nervous system (Krueger et al., 1996), although expression in oogenesis has also been observed (Fitzpatrick et al., 1995).

The most detailed analysis of RPTP function in vivo has been in Drosophila. In mutants lacking Lar some motor axons bypass their correct target area, reflecting a failure in defasciculation at the point where the axons choose to extend into the muscle (Krueger et al., 1996). Lar is also required for normal target recognition by axons from retinal photoreceptors; in Lar mutants, these axons retract from their normal target layer, suggesting a role for Lar in recognition or adhesion to target layer cells (Clandinin et al., 2001; Maurel-Zaffran et al., 2001). Different defasciculation or outgrowth defects are seen in fly mutants lacking other RPTPs (Desai et al., 1996; Garrity et al., 1999; Sun et al., 2000a). The axonal phenotypes observed in Lar mutants are incompletely penetrant, likely because other RPTPs can substitute for loss of Lar function (Desai et al., 1997). Thus, in Drosophila, Lar functions to modulate cell adhesion during axon growth; several likely components of the Lar pathway have recently been identified based on their interactions with Lar in growth cone guidance (Bateman et al., 2000; Wills et al., 1999). Lar
has also recently been found to play roles in early embryonic morphogenesis in Drosophila, where it functions in polarization of somatic follicle cells (Bateman et al., 2001; Frydman and Spradling, 2001).

Mice lacking Lar have defects in mammary gland development (Schaapveld et al., 1997) and in glucose homeostasis (Ren et al., 1998), and have mild defects in the CNS (Yeo et al., 1997). Mice lacking PTPσ display mild neural and epithelial defects, including a slight decrease in brain size and reduction in the size of the posterior pituitary (Elchebly et al., 1999; Wallace et al., 1999); the cellular basis of these defects is unknown. PTPβ mutant mice display defects in spatial learning, yet show no neuroanatomical defects (Uetani et al., 2000).

The C. elegans genome contains 26 receptor protein-tyrosine phosphatases, including orthologs of most major vertebrate RPTP classes (Plowman et al., 1999; Wälchli et al., 2000). We report here the characterization of PTP-3, the C. elegans ortholog of the LAR subfamily. We identify a loss-offunction mutation in ptp-3, and show that this mutation causes defects in epidermal and early neural morphogenesis, although axon morphology in selected neurons appears normal. Epidermal and neural morphogenesis also require signaling via the C. elegans Eph receptor VAB-1 and its ephrin ligands (Chin-Sang et al., 1999; George et al., 1998; Wang et al., 1999). We find that ptp-3 and Eph signaling mutations show specific synergistic effects on morphogenesis. Our results suggest that in C. elegans PTP-3/LAR and VAB-1/Eph RTK pathways play partly redundant roles in morphogenesis, and raise the possibility that LAR type RPTPs and Eph RTKs play redundant roles in other animals.

**MATERIALS AND METHODS**

**C. elegans genetics**

C. elegans worms were cultured as described by Brenner (Brenner, 1974), at 20°C unless stated otherwise. Mutations used were: LGII: vab-1(e2, e116, e118, e699, dx31, e2027, m2), clr-1(e1745ts), tra-2(q122dm), unc-4(e120); LGIII: sqt-1(sc11), ina-1(gm119), daf-2(e1370); LGIV: efn-1/vab-2(ju1), efn-2(ev658); LGX: egl-15(n848), efn-3(ev969). Rearrangements used were: LGII: mDf57, mDf68, mDf89, mDf90, mCl1, mnl1 (previously known as mc6). Mutations not referenced in the text have been previously described (Riddle et al., 1997).

We constructed vab-1 ptp-3 double mutants by recombination. Typically, progeny of vab-1 unc-4/ptp-3 heterozygotes were screened for Vab Non-Unc recombinants. Recombinants were homozygosed, if possible, and presence of the op147 Tc1 insertion confirmed by PCR. Inviable double mutants were maintained heterozygous to the balancer chromosomes mnl1 or mnl1 (Edgeley and Riddle, 2001). mnl1 balances the vab-1 to ptp-3 interval; a version of mnl1 containing the GFP transgene mls14 were also used in later genetic constructions.

To construct double mutants with unlinked mutations of similar phenotypes, and to obtain balanced strains in the event of a synthetic-lethal interaction, we used the mnl1 mls14 balancer, or other dominant markers on LGII to follow the ptp-3(+) chromosome. For example, to make ptp-3; ina-1 double mutants we crossed tra-2(q122gf) males with ptp-3 hermaphrodites and obtained ptp-3/tra-2 male cross progeny, which we mated with sqt-1; ina-1 animals. The non-male F1 progeny of this cross are either Roller females, of genotype tra-2(q122)/sqt-1; ina-1(+), or are Roller hermaphrodites, of genotype ptp-3/sqt-1; ina-1(+). Such Rol F1 hermaphrodites were selfed and Rol Egl F2 animals selected, putatively homozygous for ina-1 and heterozygous for ptp-3 (balanced over sqt-1). Non-Dpy Non-Rol F3 progeny were picked, putatively ptp-3; ina-1, and their progeny checked by PCR for homozygosity of the op147 insertion.

Quantitation of lethality and statistical analysis of differences in lethality between strains was performed as described previously (Chin-Sang et al., 1999). For viable strains shown in Fig. 7, at least three complete broods (>500 animals) were quantitated with the aid of a dissecting microscope. For balanced synthetic-lethal strains, estimations of lethality were based on counts of eggs spot-checked using Nomarski microscopy; heterozygous animals and balancer homozygotes were viable and excluded from counts based on expression of the mls14 transgene. The vab-1 alleles dx31, e2027, ju8, m2, e856, e118 and e116 caused 100% lethality in combination with ptp-3(op147), as determined by counts of strains of genotype vab-1 ptp-3/mnl1 mls14.

**ptp-3 deficiency heterozygote analysis**

The following chromosomal deficiencies failed to complement op147, based on the presence of the Ptp-3 morphology defects in cross-progeny resulting from crosses of op147 or op147+/+ males with deficiency heterozygotes: mDf83, mDf89, mDf90. The deficiency mDf57 complemented op147. To estimate the penetrance of lethal and morphological defects in op147/df heterozygotes, we first generated strains in which the deficiencies were balanced in trans to mnl1 mls14. Animals of genotype Df/mln1 mls14 were feminized by feeding them bacteria expressing RNA for the fem-1 gene, resulting in dsRNA-mediated interference (RNAi) of the endogenous fem-1 gene (Timmons and Fire, 1998). Bacterial strain HT115, harboring the fem-1 RNAi plasmid, was grown on NGM agar plates containing 1-10 mM IPTG and 25 μg/ml carbenicillin. One to two days later, L4 parental animals of genotype Df/mln1 mls14 were placed on the plate. Feminized progeny were crossed with op147 males; all non-GFP-expressing progeny from such a cross are of genotype op147/df. The penetrance of lethal phenotypes in such animals was quantitated at 20°C as described above. Animals of genotype op147/mDf90 displayed 6.9% embryonic lethality and 3.3% larval morphology defects (n=360).

**RNA interference of ptp-3**

A 1.3 kb AccI-SacI fragment from the ptp-3 cDNA, corresponding to the C terminus of the intracellular domain, was subcloned into the L4440 RNAi vector. This construct was linearized to allow in vitro synthesis of the plus and minus strands in separate reactions (Promega Riboprobe Combination System-T7/T3). The reactions were combined and the resulting dsRNA (approx. 0.75-1 μg/ul) was injected into the gonad or gut of N2 hermaphrodites. The broods of 18 injected animals were scored for embryonic lethal and larval morphological defects; embryonic lethality in such broods averaged 4.6% (range 0 to 20.6%); larval abnormalities averaged 1.1% (range 0 to 9.1%).

**Four-dimensional microscopy**

Timelapse microscopy in multiple focal planes (four-dimensional, 4-D) was performed as described previously (Chin-Sang et al., 1999). Movies were recorded at room temperature (approx. 22-23°C). We recorded movies from 37 op147 embryos and 30 vab-1(e2) op147 embryos derived from homozygous strains grown at 20°C. From balanced strains of genotype vab-1 ptp-3(op147)/mnl1 we recorded movies from eleven vab-1(dx31) ptp-3(op147) embryos and six vab-1(e2027) ptp-3(op147) embryos.

**Cloning and molecular analysis of ptp-3**

To analyze transcripts from ptp-3 we made poly(A)+ mRNA from approximately 80 μg of total RNA isolated from mixed stage N2 animals. This RNA was electrophoresed in a 1.5% formaldehyde agarose gel.
We isolated a cDNA encoding the PTP-3B isoform by screening a λZAP library (kindly provided by R. Barstead) with a PTP-3 PCR clone. Primers PTP-3 primers (5'-CGCCATGGTGCCTGGCGACGCGGTC-3') and PTP-3 (5'-CGCGTCCGCTAGCTTCTCGGC-3') were used in PCRs using cosmids F38A3 as template, yielding the expected 183 bp ptp-3 PCR fragment. This fragment was labeled with [α-32P]dATP and [α-32P]dCTP, and used to screen approx. 100,000 plaques using standard procedures (Sambrook et al., 1989). Three positives were identified and their inserts isolated. The longest ptp-3 cDNA contained a 5' UTR of 80 bp, a coding region of 4461 bp, and a 3' UTR of 467 bp.

We used RT-PCR to generate cDNA clones of the PTP-3A transcript. For cDNA synthesis, we used primer OH-18 (exon 18) (5'-TGCTAGCTAAATCATCCTTGACG-3') as the anchor primer. We carried out the RT reaction at 37°C for 1 hour using 6 μg of total N2 RNA, the anchor primer, and Superscript II Reverse Transcriptase (Gibco BRL). To amplify the fragments we used Vent DNA Polymerase (New England Biolabs) and the OOB-18 cDNA template in PCR reactions with the following combinations of primers:

To make the 847 bp fragment corresponding to exons 13-18, excluding 14, we used the OOB-18 primer in combination with primer OOB-19 (5'-GATACGAGAGATGGACGAGG-3') as the anchor primer. We used the following primers: OOB-33 (exon 6) (5'-ACTGTGACGATCTTTCTACTG-3') and OOB-31 (exon 13) (5'-ATTCATCTACATTGGGAGC-3'). This fragment was cloned into the EcoRV site of pBluescript. For the 1328 bp fragment that corresponds to exons 3-13, we used the following primers: OOB-6 (exon 6) (5'-ACTTGACGATCTTTCTACTG-3') and OOB-32 (exon 6) (5'-TGTTTCCCCAGGACCATCAG-3'). Both of these products were cloned (using an NcoI site in exon 6) into pSL1190, using the EcoRV site as a blunt end site for the end opposite the NcoI site. RT-PCR clones were sequenced to confirm that no mutations were present. We identified and worms homozygous for the insertion allele isolated. We determined that Tc1 had inserted between nucleotides 9486 and 9487 of the F38A3 clone, the flanking sequence being 5'-GAAGCTCT[147]:Tc1]ACATTG 3'.

### Identification of op147::Tc1

We identified a Tc1 insertion in the PTP-3 coding region by screening the mutator strain MT3126 with primers specific to Tc1 and to the phosphatase domain of ptp-3, essentially as described previously (Gut et al., 1998). Two sets of ptp-3 primer pairs (sequences available on request) were used with Tc1-specific primers to screen 2880 cultures. A single Tc1 insertion mutation was identified and worms homozygous for the insertion allele isolated. The op147::Tc1 insertion allele was outcrossed 10 times to N2 prior to phenotypic and genetic analyses. By sequencing the insertion site we determined that Tc1 had inserted between nucleotides 9486 and 9487 of the F38A3 clone, the flanking sequence being 5'-GAAGCTCT[147]:Tc1]ACATTG 3'.

### Construction of PTP-3 GFP reporter genes and transgenic strains

#### Construction of PTP-3::GFP

To create the ptp-3::GFP minigene, a unique Prl-1 site in the ptp-3 cDNA immediately 3' to the coding region for the second phosphatase domain was inserted to GFP coding sequence (GFP variant F64L, S65T, derived from vectors generously provided by A. Fire) in frame with the ptp-3B protein (clone pBH8). A 14651 bp Ncol-Not fragment from cosmids F38A3, corresponding to the ptp-3B promoter and exons 14-20 (Fig. 1A) was cloned into pSL1190 (pBH1). From pBH8 a 5 kb Nol fragment was cloned into the Nol site of pBH1. The resulting clone, pCZ406, contains genomic sequence for the promoter and the first approx. 6.5 exons of PTP-3B; the second half of exon 19 and subsequent exons are present as cDNA, tagged with GFP.

We made two series of transgenic arrays containing the PTP-3::GFP minigene. The first series was formed by injection of pCZ406 at high concentrations (50 ng/μl), together with the marker plasmid pRF4 (30 ng/μl). Four such 'high concentration' arrays, juxEx188 through juxEx191, gave robust GFP expression and caused low-penetrance morphogenetic defects in wild-type genetic backgrounds and sickness and inviability in val-1 ptp-3 mutant backgrounds. A second series of transgenes was therefore generated by injection of the PTP-3::GFP minigene at low concentrations (5 ng/μl), together with pRF4 (30 ng/μl). Such 'low concentration' arrays gave weak GFP fluorescence; GFP expression in a pattern similar to that of the high-concentration arrays could be detected by immunostaining with anti-GFP antibodies (not shown). The low concentration arrays (juxEx222-juxEx224) did not cause lethality in wild-type or val-1 ptp-3 mutant backgrounds and were used to assay rescuing activity of the transgenes.

#### Construction of Ptp-3A::GFP

A 5090 bp DNA fragment corresponding to the putative ptp-3A promoter and ATG was amplified from N2 genomic DNA using Long PCR (Boehringer Mannheim) and cloned into the Prl-1 and BamHI sites of pPD122.34 using sites engineered into the primers; sequences of primers used are available on request. This construct was injected into N2 hermaphrodites 50 ng/μl with the pRF4 marker plasmid (30 ng/μl).

### Tissue-specific expression of PTP-3B

To make constructs expressing PTP-3B under the control of the unc-119 or jam-1 promoters we amplified appropriate promoter regions using PCR and cloned them into constructs containing the PTP-3B cDNA. Details of primer sequences and plasmid constructions are available on request. Transgenic arrays were established by coinjection of the appropriate promoter construct (5 ng/μl) and a SUR-5-GFP marker plasmid (30 ng/μl) into N2 wild-type animals. Arrays were introduced into a val-1 e2 ptp-3(op147) background by crossing and genome penetranec of morphogenetic phenotypes quantitated as described above.

### Anti-PTP-3 antibodies and immunostaining

A 2.1 kb PCR product corresponding to the entire PTP-3 intracellular domain (residues 1509-2190) was amplified from the ptp-3 cDNA using primers introducing Not1 and Sac1 sites. The PCR product was digested with Notl and Sac1 and cloned into pGEX4T-3 (Amersham), yielding a clone that fuses the PTP-3 intracellular domain with GST. The fusion protein antigen was purified from 6 liters of induced E. coli DH5α using a protocol developed by Doug Kellogg (Carroll et al., 1998), and used to immunize rabbits (Animal Pharm). Bleeds were tested for immunoreactivity against purified GST-PTP-3. 10 ml of serum from the most immunoreactive bleed was purified by
The genomic region contained in cosmid clone F38A3 is indicated. (B) Northern blot of total C. elegans mRNA extracted from mixed-stage populations, probed with the PTP-3B cDNA (see Materials and Methods). Messages of approx. 8 kb and approx. 5 kb are detected. (C) Partial genetic map of the center of linkage group II, showing map location of ptp-3, vab-1, and markers used in strain constructions.

**RESULTS**

The ptp-3 locus encodes two isoforms of a C. elegans LAR-like RPTP

We identified the ptp-3 gene in a PCR-based screen for C. elegans genes encoding protein tyrosine phosphatases (Gutch et al., 1998). The genomic sequence of the ptp-3 locus was determined by the C. elegans genome consortium, and is contained in cosmid clones C09D8 and F38A3 (Fig. 1A). Using northern blots of C. elegans mRNA we found that the ptp-3 locus generates two major transcripts, of approximately 8 kb and 5 kb (Fig. 1B). We determined the sequences of cDNAs corresponding to these two transcripts as described in Materials and Methods. Comparison of ptp-3 cDNA and genomic sequences revealed that the two transcripts have common 3' sequences and differ in their 5' ends. This arises from the use of alternative promoters and sets of 5' exons, as shown in Fig. 1A. The long transcript encodes a 2190 amino acid polypeptide designated PTP-3A, and the shorter ptp-3 transcript encodes a 1487 residue polypeptide designated PTP-3B. Exons 15 through 29 are common to both transcripts and encode the C-terminal 1452 residues common to both isoforms.

PTP-3A, like other LAR-like (type IIa) RPTPs, has an extracellular domain consisting of three N-terminal immunoglobulin-like (Ig) domains and eight fibronectin type III (FNIII) repeats, and an intracellular domain containing two protein-tyrosine phosphatase (PTP) domains. Within all these domains, the closest relative to PTP-3A is DLAR; vertebrate LAR-like proteins are slightly less similar (Fig. 2A,B). The smaller isoform, PTP-3B, lacks the N-terminal Ig domains and the four N-terminal FNIII repeats (Fig. 2A). Other LAR family genes generate multiple isoforms by alternative splicing, but none appears to use internal promoters to generate isoforms of the same domain structure as PTP-3B.

PTP-3 isoforms are widely expressed in early development and are later predominantly expressed in the nervous system

To learn where PTP-3 was expressed during development we generated animals expressing PTP-3::GFP transgenes (see Materials and Methods). PTP-3B::GFP transgenes partly rescued the defects of ptp-3 mutants (see below; Fig. 5A), suggesting that it reproduces part of the endogenous PTP-3B expression pattern. To identify the cells in which PTP-3A was expressed we used transcriptional fusion constructs that expressed GFP under the control of ptp-3A upstream sequences.

PTP-3B::GFP transgenes showed widespread expression in embryos. The earliest stage at which we detected GFP fluorescence in these embryos was during late gastrulation (approximately 250-300 minutes after first cleavage at 20°C). PTP-3B::GFP expression was observed uniformly on the surface of most, possibly all, cells in the embryo during gastrulation cleft closure and epidermal enclosure (Fig. 3A,D). In later embryos, larvae and adults PTP-3B::GFP expression became highest in the nervous system, including the nerve ring, dorsal cord, and ventral cord (Fig. 3G-I). PTP-3B::GFP was expressed in many but not all neurons, within which it was localized to neurites. In later embryos and larvae PTP-3B-GFP became localized within epithelial cells, apparently to adherens junctions (data not shown). The Ptp-3::A construct expressed GFP from the comma stage (380 minutes) onwards in many neurons that also expressed PTP-3B::GFP (data not shown). Thus, PTP-3 isoforms are expressed in many tissues during early development, and later become restricted to the nervous system and epithelial tissues. To determine the expression of endogenous PTP-3 proteins we raised antibodies against the intracellular domain of PTP-3; these antisera are...
**Fig. 2.** PTP-3 is the *C. elegans* ortholog of the LAR subfamily. (A) Percentage identity and similarity of PTP-3A Immunoglobulin-like (Ig-like) domains, Fibronectin Type III (FNIII) repeats, and phosphatase domains to those of its closest relative (DLAR), and a representative vertebrate LAR family member (rat: RnLAR). The PTP-3B isoform is also shown for comparison; cartoons are not to scale. DLAR contains a ninth FNIII repeat; the FNIII domains of PTP-3 align with the first eight repeats of DLAR. PTP-3A is the largest member of the LAR family; the extra size is mostly due to a larger ‘spacer’ region between the last FNIII repeat and the predicted transmembrane domain. Percentages are calculated from alignments using ClustalW. (B) Alignment of the N-terminal (D1) phosphatase domain of PTP-3 with those of other LAR family members (DLAR, HmLAR2, and rat LAR), using ClustalW. Within the phosphatase domains, DLAR is the most similar protein to PTP-3; within the vertebrate LAR family, PTP8 proteins are slightly more similar to PTP-3 than are LAR or PTP6. The op147 insertion disrupts codon Y1705 in the first phosphatase domain, between the conserved residues YINAN and FWRM. The predicted catalytic cysteine residue C1833 is marked (asterisk). Accession numbers are M27700 (DLAR), AF017083 (HmLAR2), and S46216 (rat LAR). The sequences of PTP-3 cDNAs have been deposited in GenGank, with accession numbers AF316539 (PTP-3A) and AF316540 (PTP-3B).

expected to recognize both PTP-3 isoforms. The staining of anti-PTP-3 antisera in wild-type animals (Fig. 3G) was weaker, but otherwise identical in pattern to the expression pattern of the PTP-3B::GFP transgenes.

**Loss of ptp-3 function results in defects in epidermal morphogenesis**

We generated a Tc1 transposon insertion mutation, op147, that disrupts the first phosphatase domain of PTP-3. This mutation is therefore predicted to disrupt catalytic activity of both PTP-3 isoforms (see Materials and Methods). We show below that by genetic criteria the op147 mutation behaves as a strong loss-of-function mutation in ptp-3.

Most ptp-3(op147) mutant animals appeared wild-type in morphology and behavior. The most obvious phenotype of ptp-3(op147) mutants was a variable and incompletely penetrant
defect in epidermal morphogenesis (Table 1; Fig. 4). At 20°C, 85% of ptp-3 animals appeared morphologically wild type. The remaining 15% displayed variable defects in epidermal morphology; about one third of these animals arrested in embryogenesis (see below); one third arrested during larval development, and the remainder developed to adulthood. A common morphological defect in ptp-3 larvae and adults was a swelling or blunting of the posterior epidermis (Fig. 4A). This ‘blunt posterior’ phenotype is similar to that observed in some animals mutant for the VAB-1 Eph receptor tyrosine kinase or the VAB-2/EFN-1 ephrin ligand (Fig. 4F). However, the distinctive anterior morphology defects (‘Notched head’) of vab-1 or vab-2 mutants were not seen in ptp-3 mutants, which only occasionally displayed a swollen or pinched head region (Fig. 4B). The op147 mutation is slightly temperature sensitive (Table 1).

We asked whether op147 behaved genetically as a loss-of-function mutation by examining the phenotypes of animals heterozygous for op147 in trans to chromosomal deficiencies spanning ptp-3 (Fig. 1C). Such op147/Δf animals displayed a similar range and penetrance of defects to those of op147 homozygotes (see Materials and Methods). dsRNA-mediated interference of ptp-3 caused morphogenetic defects similar to those of ptp-3(op147) mutants (see Materials and Methods).

### Table 1. Penetrance of ptp-3(op147) visible and lethal phenotypes

<table>
<thead>
<tr>
<th>Genotype, temperature (°C)</th>
<th>Embryonic lethality</th>
<th>Larval lethality</th>
<th>Deformed adult</th>
<th>Wild-type adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>ptp-3(op147) 15°C (746)</td>
<td>4.1%</td>
<td>5.8%</td>
<td>2.3%</td>
<td>87.8%</td>
</tr>
<tr>
<td>ptp-3(op147) 20°C (945)</td>
<td>5.3%</td>
<td>5.0%</td>
<td>3.7%</td>
<td>85.8%</td>
</tr>
<tr>
<td>ptp-3(op147) 22.5°C (669)</td>
<td>5.1%</td>
<td>1.3%</td>
<td>3.3%</td>
<td>90.2%</td>
</tr>
<tr>
<td>ptp-3(op147) 25°C (462)</td>
<td>11.6%</td>
<td>8.2%</td>
<td>3.8%</td>
<td>76.1%</td>
</tr>
</tbody>
</table>

Taken together, these data suggest that ptp-3(op147) causes a strong loss of ptp-3 function. However, the nature of the op147 lesion suggests that it may not be a complete molecular null mutation in PTP-3, as Tc1 insertions in genes can be removed by splicing (Rushforth and Anderson, 1996) or somatic excision (Eide and Anderson, 1988). Consistent with this possibility, extremely weak anti-PTP-3 staining was observed in approx. 70% of ptp-3(op147) mutant animals; in the remaining 30%, anti-PTP-3 staining was completely absent. Finally, if PTP-3 has phosphatase-independent functions then these may be unaffected by op147.

To confirm that the mutant defects in op147 strains were due to the op147 mutation, we asked whether transgenic arrays containing ptp-3(+) genomic DNA could rescue op147 mutant phenotypes. Transgenes containing cosmid F38A3 rescued ptp-3 mutant phenotypes (Fig. 5A); because the ptp-3(op147) phenotype is weak, we assayed rescue of vab-1 ptp-3 synthetic lethal phenotypes (see Materials and Methods). The F38A3 clone does not contain ptp-3 exons 1-4 (see Fig. 1A) and thus cannot encode full-length PTP-3A. Thus, overexpression of PTP-3B can partly rescue the defects of ptp-3(op147) mutants. PTP-3B:GFp transgenes also displayed partial rescuing activity, consistent with these genomic rescue experiments (Fig. 5).

### ptp-3 mutants are defective in embryonic neuroblast and epidermal movements, but display normal axon guidance in selected neurons

The epidermal morphogenetic defects of ptp-3 mutants arise during embryogenesis, and are reminiscent of those seen in vab-1 and efn-1 mutants. vab-1 (Eph receptor) and efn-1 (ephrin, previously vab-2) mutant embryos display defects in two phases of embryonic cell movements: closure of the ventral gastrulation cleft by short-range neuroblast movements, and enclosure of the embryo by epidermal cell shape changes (Chin-Sang et al., 1999; George et al., 1998). We therefore asked whether ptp-3 mutant embryos were also defective in these embryonic morphogenetic processes.

Using four-dimensional (4-D) microscopy we found that ptp-3 mutant embryos were defective in both closure of the gastrulation cleft and in epidermal enclosure (Fig. 6, second and third rows). Of 37 ptp-3 mutant embryos recorded we observed defects in gastrulation cleft closure in seven; six of these seven subsequently failed to enclose the epidermis and arrested at the early enclosure stage of morphogenesis. The remaining 30 displayed normal gastrulation cleft closure and

![Fig. 4. Morphological phenotypes of ptp-3 mutants.](Image)

(A-D) Representative ptp-3(op147) mutant L1 stage larvae (grown at 25°C) showing defects in morphogenesis. The most common defect is a bulging or pinching of the posterior body (A); however, pinched or notched heads are also occasionally seen (B). Some inviable ptp-3(op147) larvae are starved, apparently a result of defects in pharyngeal morphogenesis (arrow in C). Some ptp-3(op147) larvae are deformed along the entire body (D). A wild-type L1 larva (E) and vab-1(null) mutant larva (F) showing the head morphology defect are shown for comparison. Scale bar, 13 μm (A-D), 20 μm (E,F).

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epidermal morphogenesis. The defects in ptp-3 mutants could be classified using the same criteria as used to classify vab-1 or efn-1 embryonic phenotypes (see Fig. 6 legend). Thus, ptp-3 mutant embryos display low-penetration defects in gastrulation and epidermal enclosure, similar to those observed in vab-1 or efn-1 mutants.

Genetic analysis in Drosophila has implicated RPTPs in axon guidance and fasciculation. We examined the process morphology of selected neurons (mechanosensory neurons and GABAergic motor neurons) in ptp-3 mutants using GFP markers and found no significant defects in axon guidance (data not shown). ptp-3(op147) mutants displayed normal locomotion, feeding, defecation and egg-laying behavior.

With the caveat that ptp-3(op147) may not abolish PTP-3 function, these observations suggest that if ptp-3 functions in axon guidance in C. elegans its functions are subtle or redundant.

vab-1 and ptp-3 mutations have synergistic effects on morphogenesis

Our data show that PTP-3 signaling and VAB-1 Eph RTK signaling function in the same processes of embryonic morphogenesis. To ask whether ptp-3 signaling interacted with Eph receptor signaling we constructed strains containing ptp-3(op147) and vab-1 null [vab-1(0)] mutations. The vab-1(p0) ptp-3 double mutant strains displayed strongly enhanced morphogenetic defects compared to vab-1 null mutants. vab-1(0) mutations alone result in approx. 50% embryonic lethality and approx. 80% total lethality (George et al., 1998); despite this high level of lethality, some animals are viable and fertile, and vab-1 null mutant strains can be propagated as homozygotes. In contrast, vab-1(p0) ptp-3 double mutant animals were completely inviable and always arrested during embryogenesis. We analyzed the embryogenesis of such double mutants using 4-D microscopy, and found that all vab-1(p0) ptp-3 double mutant animals displayed severe defects in neuroblast movement during closure of the gastrulation cleft, and consistently arrested during early epidermal enclosure (Fig. 6, fourth row). These phenotypes correspond to the severe Class I phenotype, observed in a small fraction of vab-1(0) mutants (George et al., 1998). We did not observe any new morphogenetic phenotypes in the double mutants. Because the double mutants are more strongly affected than expected from additivity of mutant phenotypes these data indicate that VAB-1 and PTP-3 play related and partly redundant roles in morphogenesis.

The synergistic effects of vab-1 and ptp-3 mutations on morphogenesis could reflect their redundant function in the same set of cells or in different sets of cells. VAB-1 is expressed and required in neuroblasts and neurons during embryogenesis, whereas PTP-3 shows a more widespread distribution. We used tissue-specific promoters to ask whether PTP-3 function was required in neurons or in epidermal cells in a vab-1 ptp-3 mutant background. Only expression of PTP-3 under the control of the pan-neural unc-119 promoter caused a significant decrease in the lethality of vab-1 ptp-3 double mutants (Fig. 5B). Expression of PTP-3 using epidermal promoters gave partial rescue that was not statistically significant. We conclude that one focus for the synergistic effects of VAB-1 and PTP-3 on epidermal development is the developing nervous system, although the partial rescue observed might indicate that PTP-3 functions in both neuronal and epidermal cells.

PTP-3 may function redundantly with kinase-dependent and kinase-independent functions of VAB-1

Mutations predicted to eliminate VAB-1 kinase activity [vab-1(k) mutations] do not cause complete loss of VAB-1 function, leading to the model that VAB-1 has both kinase-dependent and kinase-independent functions (George et al., 1998). The kinase-independent function of VAB-1 may involve signaling via the ephrin VAB-2/EFN-1 (Chin-Sang et al., 1999; Wang et al., 1999). We used double mutant analysis to
determine whether PTP-3 function is redundant with one or both of these VAB-1 pathways.

We found that the phenotypes of vab-1 kinase mutants were dramatically enhanced by loss of ptp-3 function (Fig. 6, Fig. 7A). vab-1(k) mutations in a ptp-3(+ ) background cause approx. 10% embryonic lethality (George et al., 1998), whereas vab-1(k) ptp-3(op147) double mutants displayed 80-100% embryonic lethality, depending on the vab-1(k) allele used. We analyzed vab-1(k) ptp-3 double mutants using 4-D microscopy and confirmed that this enhancement was due to increased penetrance and severity of embryonic morphogenetic defects seen in the single mutants (Fig. 6, bottom row). We conclude that ptp-3 functions redundantly with the kinase-dependent function of VAB-1.

Because vab-1(0) ptp-3 double mutants are more severely affected than vab-1(k) ptp-3 double mutants, we reasoned that PTP-3 function must also be redundant with the VAB-1 kinase-independent pathway. To address this possibility we made double mutants between ptp-3(op147) and vab-1 missense mutations affecting the extracellular domain. Extracellular domain missense alleles of VAB-1 such as e699 cause stronger phenotypes than vab-1(k) alleles. However, vab-1(e699) showed weaker interactions with ptp-3 than did the vab-1(k) alleles, although the double mutants were more severely affected than expected from additivity (Fig. 7A). These data are consistent with PTP-3 also functioning redundantly with the kinase-independent function of VAB-1.

**ptp-3 mutations synergize with efn-1 ephrin mutations but not with efn-2 or efn-3 mutations**

From the synergistic genetic interactions of ptp-3 and vab-1 mutations we conclude that PTP-3 and VAB-1 play related and partly redundant roles in morphogenesis. We therefore investigated if PTP-3 displayed genetic interactions with the ephrin ligands for VAB-1. Mutations in three ephrins (EFN-1, EFN-2 and EFN-3) have been identified in *C. elegans*; genetic and biochemical analysis indicates that these ligands function in both the kinase-dependent and kinase-independent VAB-1 pathways (Chin-Sang et al., 1999; Wang et al., 1999). Mutations in the ephrin EFN-1 cause defects in gastrulation and epidermal enclosure similar to those of vab-1 mutants. We found that ptp-3; efn-1 double mutants displayed synergistic enhancement (Fig. 7B), although the double mutant strains displayed a different range of phenotypes from those of vab-1(k) ptp-3 double mutants, in that many animals arrested during larval as opposed to embryonic development. EFN-1 may function both in the kinase-dependent and kinase-independent pathways (Chin-Sang et al., 1999; Wang et al., 1999). The synergism of ptp-3 with vab-1(e699) and with efn-1 confirms that ptp-3 function is redundant with both VAB-1 pathways.

**Fig. 6.** Time-lapse analysis of morphogenesis in ptp-3 mutants and vab-1 ptp-3 double mutants. Time series from five different embryos are shown: the first row is wild type; the second and third rows depict two representative ptp-3[op147] embryos; the fourth row shows a vab-1(dx31) ptp-3[op147] embryo (vab-1(0) double mutant), and the fifth row shows a typical vab-1(e2) ptp-3[op147] embryo (vab-1(k) double mutant). All panels are ventral views, with anterior to the left. First column: beginning of closure of the ventral gastrulation cleft (approx. 230-250 minutes after first cleavage); note the enlarged gastrulation cleft (arrowheads) in all mutant genotypes. Second column: later closure of gastrulation cleft. Third column: early epidermal enclosure. Fourth column: mid-epidermal enclosure. Times for each series are relative to the frame in the first column. Of the two ptp-3 embryos shown, the upper series shows a severely affected embryo, with enlarged gastrulation cleft and early failure in epidermal enclosure; this animal arrested at the enclosure stage, corresponding to the Class I phenotype of vab-1 mutants (George et al., 1998). The lower ptp-3 series shows an embryo with slightly enlarged gastrulation cleft; this embryo underwent normal epidermal enclosure and hatched with normal morphology, corresponding to the Class V phenotype of vab-1 or vab-2 embryos. Both vab-1 ptp-3 embryos shown displayed defects in gastrulation cleft closure and arrested at epidermal enclosure.
Fig. 7. Synergism of ptp-3 with vab-1 and vab-2 and lack of synergism with intra-1 or clr-1.

(A) Lethality was quantitated as described in Materials and Methods; error bars show s.e.m.
Strains were raised at 20°C unless indicated. Data for vab-1 are from George et al. (George et al., 1998). Strains doubly mutant for ptp-3(op147) and weaker vab-1 kinase alleles (the missense alleles e2 and ju63) showed partially penetrant synergistic lethality yet were viable as homozygotes; strains containing stronger kinase alleles were completely inviable as double mutants with op147 (not shown). (B) Synergism of ptp-3 with efn mutations. Data for efn-1 from Chin-Sang et al. (Chin-Sang et al., 1999). Only efn-1 displays synergistic lethality with ptp-3. Over 90% of the lethality in vab-1(kenase) ptp-3 double mutants occurred during embryogenesis, whereas vab-1(e699) and efn-1 double mutants displayed approx. 60% embryonic lethality and approx. 30% larval lethality. (C) Synergism of the RPTP clr-1 with vab-1 or ptp-3 was tested using the temperature-sensitive clr-1(e1745), which is fully viable at 15°C and 20°C and is a fully penetrant larval lethal at 25°C. vab-1 clr-1 homzygotes were viable at 20°C; clr-1 ptp-3 strains were viable at 15°C but not at 20°C, possibly suggestive of a mild enhancement of the Clr-1 phenotype. Embryonic lethality was quantitated using balanced strains of genotype clr-1(mnl1 mls14, vab-1 clr-1(mnl1 mls14), and clr-1 ptp-3(mnl1 mls14). Strains were raised at 25°C and the embryonic lethality of non-GFP-expressing animals quantitated.

The ephrins EFN-2 and EFN-3 have minor roles in embryogenesis, as loss of efn-2 or efn-3 function causes only mild defects in morphogenesis (Wang et al., 1999). ptp-3; efn-2 and ptp-3; efn-3 double mutants and a ptp-3; efn-2; efn-3 triple mutant did not show synergistic enhancement of morphogenetic defects (Fig. 7B). We conclude that PTP-3 functions redundantly with EFN-1, but not with EFN-2 or EFN-3, in regulating embryonic morphogenesis.

Specificity of the synergistic interaction of vab-1 and ptp-3 mutations

Does the synergistic enhancement of vab-1 or efn-1 phenotypes by ptp-3 reflect a specific interaction between the VAB-1 Eph RTK and PTP-3/LAR pathways? To address this question, we first asked whether ptp-3 displayed genetic interactions with another C. elegans RTK, the FGFR homolog EGL-15, which functions in cell migration (DeVore et al., 1995). We found that ptp-3; egl-15 double mutants displayed additive phenotypes, in that egl-15 egg-laying phenotypes were neither enhanced nor suppressed (data not shown), suggesting that PTP-3 does not function synergistically or antagonistically with EGL-15/FGFR signaling. Because LAR has been shown to negatively regulate insulin receptor signaling in cell culture (Kulas et al., 1996), we also asked whether ptp-3 displayed genetic interactions with the C. elegans insulin receptor homolog DAF-2 (Kimura et al., 1997). daf-2 mutations cause a dauer-constitutive phenotype at 25°C; a ptp-3; daf-2 double mutant strain also displayed a dauer-constitutive phenotype at the restrictive temperature (data not shown), suggesting that ptp-3 may not repress insulin receptor signaling in C. elegans.

We also asked whether mutations in another RPTP displayed genetic interactions with vab-1. The only other C. elegans RPTP for which mutations are known is the Type II RPTP CLR-1, which functions antagonistically with EGL-15 (Kokel et al., 1998). vab-1 clr-1 double mutants displayed at most a mild enhancement of the Vab-1 embryonic lethal phenotype; clr-1 ptp-3 double mutants similarly displayed a mild enhancement of Ptp-3 phenotypes (Fig. 7C). In Drosophila, DLAR and the CLR-1-like phosphatase DPTP69D function redundantly in some contexts; in contrast, our data suggest that the incomplete penetrance of ptp-3 or vab-1 phenotypes does not reflect redundancy with CLR-1.

While these data suggest that the interaction of PTP-3 and VAB-1 reflects redundant functions of these specific pathways, it remained possible that loss of function in ptp-3 might non-specifically enhance the defects of other epidermal...
morphogenesis mutants. To address this possibility we made animals doubly mutant for ptp-3 and a weak allele of the α-integrin INA-1 (Baum and Garriga, 1997). Like VAB-1, INA-1 is expressed in the nervous system, and loss of ina-1 function causes morphogenetic defects in the epidermis. We found that ptp-3; ina-1 double mutants displayed additive effects (Fig. 7A). These data suggest that PTP-3 and INA-1 have independent functions in morphogenesis, and that loss of PTP-3 function does not enhance all morphogenetic mutants.

DISCUSSION

The evolution of LAR-like RPTPs

The LAR-like RPTPs have been highly conserved in animal evolution. LAR-like RPTPs have been identified in vertebrates, Drosophila (DLar), leeches (HmLAR1, HmLAR2), protochordates (Matthews et al., 1991), and now in nematodes. In all vertebrate species examined, three LAR subfamily RPTPs are expressed: LAR itself, and the closely related proteins PTPσ and PTPβ. The Drosophila and C. elegans genomes each contain a single LAR-like gene, whereas the leech Hirudo medicinalis expresses two LAR-family genes, HmLAR1 and HmLAR2 (Gershon et al., 1998). Ancestral metazoans may thus have expressed a single LAR-like gene that became duplicated in the annelid and vertebrate lineages.

Vertebrate LAR subfamily genes are expressed in distinct but partly overlapping patterns, both in the developing and adult nervous systems and in a variety of non-neuronal tissues (Schaapveld et al., 1998). Like its vertebrate orthologs, PTP-3 displays widespread, almost ubiquitous expression in early C. elegans embryos. PTP-3 later becomes localized to neuronal processes, as found for other vertebrate and invertebrate LAR family members (e.g. Tian et al., 1991). Outside the nervous system LAR-like proteins are often found in proliferating epithelia, such as those of the lung and gut. In some mature C. elegans epidermal cells PTP3 appears to localize to adherens junctions; vertebrate LAR and PTPσ proteins are also found in adherens junctions (Aicher et al., 1997), where they interact with β-catenin (Kypta et al., 1996). The potential role of PTP-3 in epidermal adherens junctions is unclear, as ptp-3 mutant phenotypes do not resemble those resulting from loss of function in the catenin/cadherin complex (Costa et al., 1998); furthermore, loss of function in ptp-3 did not enhance or suppress the phenotypes of loss-of-function mutations in other adherens junction proteins such as HMP-1 (data not shown).

Most LAR family genes generate multiple transcripts and encode multiple protein isoforms, although the way in which these isoforms arise is apparently not conserved between animal phyla. Vertebrate LAR, PTPσ, and PTPβ genes all undergo alternative splicing involving exons encoding both extracellular and intracellular domains (O’Grady et al., 1994; Zhang and Longo, 1995). Many LAR family isoforms differ only within their extracellular domains, suggesting that the different isoforms could interact differently with ligands. For example, inclusion of a small exon within FNIII repeat 5 modulates the binding of LAR to laminin-nidogen (O’Grady et al., 1998).

We have shown that the C. elegans ptp-3 gene encodes at least two isoforms, PTP-3A and PTP-3B, by use of alternative promoters; this genomic organization has so far not been found in other LAR genes. Our anti-PTP-3 antibodies, which should recognize both isoforms, showed staining weaker than that of an isoform-specific PTP-3B::GFP transgene but otherwise indistinguishable, implying that both isoforms are expressed in similar patterns. The ptp-3(op147) insertion allele affects a phosphatase domain common to both isoforms and should decrease the function of both isoforms, although it may not affect phosphatase-independent functions of PTP-3, as proposed for Drosophila Lar (Mauren-Zaffran et al., 2001). Transgenes encoding only PTP-3B can rescue most or all of the defects observed in ptp-3 mutants, suggesting that PTP-3B function is necessary for morphogenesis. Consistent with this hypothesis, a deletion mutation that specifically disrupts the PTP-3A isoform (K. Gengyo-Ando and S. Mitani, personal communication) does not cause defects in embryonic morphogenesis and does not synergize with vab-1 mutations (data not shown). PTP-3A might have no function in embryonic morphogenesis, or its functions might be redundant with PTP-3B, such that only mutations disrupting both isoforms cause morphogenetic defects.

The functions of LAR-like RPTPs

Work on Drosophila has established that LAR-like RPTPs function in axon guidance and fasciculation. In Drosophila, some Lar mutant phenotypes are synergistically enhanced in double mutant combinations with two other RPTPs, DPTP69D and DPTP99A, showing that the partial penetrance of Lar null mutant defects reflects redundant functions of these RPTPs (Desai et al., 1997). Genetic interactions indicate that in Drosophila neural RPTPs also function as positive regulators of Robo/Slit-based growth cone repulsion from the midline (Sun et al., 2000a). Mutations in murine LAR-like RPTPs cause subtle defects in neural tissues, although it is unclear if these reflect defects in axon guidance. The three vertebrate LAR-like RPTPs have overlapping expression patterns, suggesting that the subtle phenotypes of LAR, PTPσ, and PTPβ mutants could reflect redundant functions of these RPTPs. We have found that in C. elegans Lar plays a subtle role in early neural and epidermal development, as reflected by the mild defects of ptp-3 mutants. The mild phenotypes of LAR mutants in Drosophila, mice and C. elegans suggest the possibility that these proteins function in highly redundant signaling processes.

Our analysis of ptp-3 mutant phenotypes has revealed that PTP-3 and Eph signaling are required in similar processes of embryogenesis. Loss of function in PTP-3, in the Eph receptor VAB-1, or in the ephrin ligand EFN1-1, causes incompletely penetrant defects in neuroblast movements during closure of the gastrulation cleft, and in later epidermal morphogenesis. In vab-1 ptp-3 double mutants the penetrance and severity of these defects are dramatically enhanced, although no new defects are seen in the double mutants. The simplest interpretation of this synergistic genetic interaction is that PTP-3 and VAB-1 function in closely related pathways, and that these pathways have partly redundant functions in controlling neuroblast movements. In contrast to the extremely variable defects of vab-1(0) or ptp-3 single mutants, vab-1(0) ptp-3 double mutants display a consistent arrest at early epidermal enclosure. This suggests that the variability of the single mutant phenotypes reflects compensation by the other
pathway; that is, the variability of the Vab-1 null phenotype reflects the ability of PTP-3 signaling to partly compensate for lack of VAB-1, and vice versa. The synergistic interaction of ptp-3 with efn-1 mutations is consistent with previous data showing that EFN-1 functions in the VAB-1 pathway in embryonic morphogenesis (Chin-Sang et al., 1999). ptp-3 does not show similar synergistic lethal interactions with ephrins efn-2 and efn-3, consistent with their relatively minor roles in embryogenesis (Wang et al., 1999).

Because vab-1 and ptp-3 mutants display defects in the movements of ventral neuroblasts during gastrulation cleft closure, we favor the hypothesis that VAB-1 and PTP-3 function redundantly within the same sets of neuronal precursors. This hypothesis is supported by our tissue-specific expression data. Significant rescue of vab-1 ptp-3 mutant phenotypes was only observed when PTP-3B was expressed using a pan-neural promoter and not when using epithelial or epidermal-specific promoters. While these experiments do not address whether VAB-1 and PTP-3 function in the same individual neurons, they are consistent with VAB-1 and PTP-3 functioning in the same tissue. The incomplete rescue observed in these experiments could reflect failure to accurately reproduce the endogenous PTP-3 expression pattern, or it could reflect an additional role for PTP-3 in epidermal cells.

Several PTPs, including LMW-PTP, FAP-1, and SHP-2, appear to function downstream of Eph receptors (Lin et al., 1999; Miao et al., 2000; Stein et al., 1998). Although PTP-3 is unlikely to function directly in the VAB-1/Eph receptor signaling pathway, this is to our knowledge the first indication of a specific genetic interaction between a receptor-like PTP and Eph pathways. Our proposal that a receptor PTP and receptor PTK function redundantly in promoting signaling through a common pathway raises the question of the mechanism by which this may be achieved by two apparently antagonistic enzymes. While there are abundant examples of PTPs antagonizing PTK-dependent signaling pathways, there are also many examples of PTPs that function positively to promote signaling. For example, CD45, the prototypic receptor PTP, plays an essential positive role in signaling through T and B cell receptors (Neel, 1997) and PTP-α promotes signaling events associated with cell growth (Zheng et al., 1992). In both cases, the PTPs appear to dephosphorylate an inhibitory site of tyrosine phosphorylation at the C terminus of Src family PTKs, activating the kinase. Thus, a PTP can function in concert with a PTK to promote tyrosine phosphorylation. The SH2-domain-containing phosphatase SHP-2, and its Drosophila homologue Csw, function positively in several RTK pathways (Allard et al., 1996; Perkins et al., 1996). In the case of the RTK Torso, Csw can promote signaling by dephosphorylation of inhibitory phosphotyrosines in the Torso cytoplasmic domain (Cleghon et al., 1998). We think it unlikely that PTP-3 acts via dephosphorylation of VAB-1, as PTP-3 mutations have dramatic effects in a VAB-1 null mutant background. PTP-3 might promote signaling in the VAB-1 pathway by dephosphorylation of a downstream substrate; elucidation of the substrates of PTP-3 will be required to test this possibility.

LAR-like RPTPs and Eph RTKs have been implicated in related aspects of cellular behavior in other organisms. Both Eph RTKs and LAR can cause axonal growth cone collapse (Baker and Macagno, 2000; Drescher et al., 1995), and thus can promote repulsive interactions between growth cones and their substrates (Orioli and Klein, 1997). However, in some situations LAR-like RPTPs may promote cell adhesion or growth-cone attraction, rather than repulsion (e.g. Sun et al., 2000b), suggesting that Eph signaling and LAR signaling could play antagonistic or synergistic roles depending on the specific cellular context. Our finding that Eph signaling and LAR play related and partly redundant roles in C. elegans morphogenesis suggests that these two pathways may also be intimately connected in other organisms.

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