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 IIA 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 (Cladnin 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 (Schapaev 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-of-function 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 synergetic 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), sqt-1(sc143d); LGIII: ina-1(gm119), daf-2(e1370); LGV: efn-1(lm193), efn-2(ev658); LGX: egl-15(n848), efn-3(ev965). Rearrangements used were: LGII: mnDf57, mnDf83, mnDf89, mnDf90, mnCl, mlhl (previously known as mlc6). 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 transgene insertion confirmed by PCR. Inviable double mutants were maintained heterozygous to the balancer chromosomes mnl1 or mlh1 (Edgley and Riddle, 2001). mnl1 balances the vab-1 to ptp-3 interval; a version of mlhl 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 balancer chromosomes. For example, mC1, mC6, mIn1, mIs14 were also used in later genetic constructions.

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/ml) was injected into the gonad or gut of N2 hermaphrodites. The broods of injected animals were scored for embryonic lethal and larval morphology defects; embryonic lethality in such broods averaged 18 injected animals were scored for embryonic lethal and larval morphology defects; embryonic lethality in such broods averaged 18%.

To analyze transcripts from ptp-3 we made poly(A)+ mRNA from approximately 8 µg of total RNA isolated from mixed stage N2 animals. This RNA was electrophoresed in a 1.5% formaldehyde agarose gel. The gel was run at 60 V and the RNA was transferred to nylon membranes using a vacuum manifold. Blots were probed with a [32P]-labeled cDNA insert from the C. elegans PTP-3/LAR gene. Hybridizations were performed at 65°C in 5xSSC/1% SDS/0.5% sodium pyrophosphate for 12 to 24 hours. The membranes were exposed to X-Omat AR films at −80°C.
agarose gel and blotted using standard procedures (Sambrook et al., 1989). The blot was hybridized with a 32P-labeled *ppp-3B* cDNA. 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 PTP1L-5 (5′-GCCGAATTCCTCCAGGAGTT-3′) and PTP1L-6 (5′-CGTTTCTGCTGACTGTTCTGC-3′) were used in PCRs using cosmid F38A3 as template, yielding the expected 183 bp *ppp-3B* PCR fragment. This fragment was labeled with [32P]dATP and [α-32P]dCTP, and used to screen approx. 100,000 plagues using standard procedures (Sambrook et al., 1989). Three positives were identified and their inserts isolated. The longest *ppp-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-B18 (exon 18) (5′CTCTAGACTACATAATGCTTC3′) 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 OH-B18 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 OH-B18 primer in combination with primer OH-B19 (5′AGTACGACAGAGATATGACGG3′). 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: OH-B13 (exon 6) (5′ACTTGACAGATCTTCTACTTG3′) and OH-B31 (exon 13) (5′ATCTCCATCTTTGACGGTGC3′). This fragment was used as the anchor primer, and supplements I and II were introduced in the PCR. We found no evidence for alternative splicing of the *ppp-3* locus, although our analysis was not exhaustive. To test whether the genomic cosmid clone F38A3 could rescue *ppp-3* mutant phenotypes, we generated transgenic arrays by transforming wild-type animals with F38A3 (2 ng/μl) and the transforming wild-type animals with F38A3 (2 ng/μl). To test whether the genomic cosmid clone F38A3 could rescue *ppp-3* mutant phenotypes, we generated transgenic arrays by transforming wild-type animals with F38A3 (2 ng/μl). We identified Tc1 insertion between nucleotides 9486 and 9487 of the F38A3 clone, the flanking sequence being 5′ GAAGCTG[bp147]: nucleotide 3′ ACATTC GTGAC CGG3′.

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

**Construction of PTP-3B::GFP** To create the *ppp-3B::GFP* minigene, a unique *Prt1* site in the *ppp-3B* cDNA immediately 3′ to the coding region for the second phosphatase domain was used to insert 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 NcoI-NolI fragment from cosmid F38A3, corresponding to the *ppp-3B* promoter and exons 14-20 (Fig. 1A) was cloned into pSL1190 (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-3B::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, *juEx188* through *juEx191*, gave robust GFP expression and caused low-penetrance morphogenetic defects in wild-type genetic backgrounds and sickness and inviability in vab-1 *ppp-3* mutant backgrounds. A second series of transgenic arrays was therefore generated by injection of the PTP-3B::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 (*juEx222*-*juEx224*) did not cause lethality in wild-type or *vab-1* *ppp-3* mutant backgrounds and were used to assay rescuing activity of the transgenes.

**Tissue-specific expression of PTP-3B** To test whether the genomic cosmid clone F38A3 could rescue *ppp-3* mutant phenotypes, we generated transgenic arrays by transforming wild-type animals with F38A3 (2 ng/μl) and the plasmid pRF4 (30 ng/μl), which confers a Roller phenotype. We obtained several such extrachromosomal arrays in wild-type background, then introduced these arrays into a *vab-1(e2)* *ppp-3*(*op147*) mutant background by crossing. We homogenized for the *vab-1* and *ppp-3* mutations and confirmed homozygosity by complementation tests for *vab-1* and by PCR to detect op147::Tc1. The e2 *op147* double mutant displays approx. 81% embryonic lethality. If the transgenic arrays completely rescued *ppp-3* mutant phenotypes the embryonic lethality in transgenic animals should be at most suppressed to the level found in a *vab-1(e2)* *ppp-3*(*+*) strain, which is approx. 10.2% (George et al., 1998). Of four such arrays, three (*juEx183, juEx184, juEx186*) showed significant rescue of the *vab-1* *ppp-3* synthetic lethality (Fig. 5); these arrays did not rescue the phenotypes of *vab-1(e2)* single mutants (not shown). Two integrated versions of *juEx183, juIs138* and *juIs139*, were generated by UV/methylpsoralen mutagenesis.

**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 *ppp-3*, essentially as described previously (Gutch et al., 1998). Two sets of *ppp-3* primer pairs (sequences available on request) were used for 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′ GAAGCTG[bp147]: nucleotide 3′ ACATTC GTGAC CGG3′.
The genomic sequence of the C. elegans genes encoding protein tyrosine phosphatases was identified in a PCR-based screen for ptp-3. We used a microscope or a Zeiss Axioplan 2.

Materials and Methods. Comparison of cDNAs corresponding to these two transcripts as described in 8 kb and 5 kb (Fig. 1B). We determined the sequences of the ptp-3 locus generates two major transcripts, of approximately 21 kb and 14 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-3 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 epidermal cells, apparently to adherens junctions (data not shown). The Ptp-3A::GFP 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 GenBank, 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. 3 G) 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

**Fig. 3.** Embryonic and neuronal expression of PTP-3. (A-F) Expression of PTP-3B::GFP in enclosure stage embryos. Animals are of genotype juEx189; GFP is visualized by anti-GFP immunostaining (green), animals are also immunostained with MH27 to visualize adherens junctions (red). (A-C) A lateral confocal projection of an embryo prior to epidermal enclosure. The dorsal sheet of epidermal cells is visualized by MH27 staining; PTP-3B::GFP expression is widespread in surface cells; expression in ventral neuroblasts is marked in A (arrowhead). (D-F) A post-gastrulation embryo; medial confocal section showing widespread expression of PTP-3B::GFP at cell surfaces in epidermal, neuronal, pharyngeal, muscle, and endodermal tissue layers. (G) Wild-type L1 larva stained with anti-PTP-3 antibodies and MH27. Nerve ring staining is marked by the arrowhead. (H,I) PTP-3B::GFP expression in L1 (H) and adult (I) (juEx189) stained with anti-GFP antibodies; note intense nerve ring expression (arrowhead in I) and staining in neuronal processes. Scale bar, 10 μm (A-F); 30 μm (G, H); 100 μm (I).
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/Df 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>
<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>

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

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...
and GABAergic motor neurons) in axon guidance and fasciculation. We examined the process of axon guidance via the ephrin VAB-2/EFN-1 (Chin-Sang et al., 1998). The kinase-independent function of VAB-1 may involve 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(+1) 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 VAB-1 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.
and mild defects in morphogenesis (Wang et al., 1999).

Specificity of the synergistic interaction of vab-1, EFN-3, in regulating embryonic morphogenesis. Functions redundantly with EFN-1, but not with EFN-2 or morphogenetic defects (Fig. 7B). We conclude that PTP-3 triple mutant did not show synergistic enhancement of lethality with ptp-3. Over 90% of the lethality in vab-1(kinase) 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 late larval lethal at 25°C. vab-1 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[mIn1 mls14], vab-1 clr-1[mIn1 mls14], and clr-1 ptp-3[mIn1 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 those 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 epithelia, such as those of the lung and gut. In some mature

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 EFN-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., 1998; 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 RTK 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 RTK-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 RTKs, activating the kinase. Thus, a PTP can function in concert with a RTK 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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