Cell, Vol. 92, 785–795, March 20, 1998, Copyright ©1998 by Cell Press

UNC-73 Activates the Rac GTPase and Is Required for Cell and Growth Cone Migrations in *C. elegans*

Robert Steven,*† Terrance J. Kubiseski,* Hong Zheng,* Sarang Kulkarni,* Jorge Mancillas,‡ Alberto Ruiz Morales,‡ Chris W. V. Hogue,* Tony Pawson,*† and Joe Culotti*†§

*Samuel Lunenfeld Research Institute of Mt. Sinai Hospital
Toronto, Ontario M5G 1X5
Canada
†Department of Molecular and Medical Genetics
University of Toronto
Toronto, Ontario M5S 1A8
Canada
‡Department of Anatomy and Cell Biology
University of California
Los Angeles, California 90024

Summary

***unc-73* is required for cell migrations and axon guidance in *C. elegans* and encodes overlapping isoforms of 283 and 189 kDa that are closely related to the vertebrate Trio and Kalirin proteins, respectively. UNC-73A contains, in order, eight spectrin-like repeats, a Dbl/Pleckstrin homology (DH/PH) element, an SH3-like domain, a second DH/PH element, an immunoglobulin domain, and a fibronectin type III domain. UNC-73B terminates just downstream of the SH3-like domain. The first DH/PH element specifically activates the Rac GTPase in vitro and stimulates actin polymerization when expressed in Rat2 cells. Both functions are eliminated by introducing the S1216F mutation of *unc-73(rh40)* into this DH domain. Our results suggest that UNC-73 acts cell autonomously in a protein complex to regulate actin dynamics during cell and growth cone migrations.**

Introduction

Cell and growth cone migrations in animals are guided by extracellular molecules that provide polarity information to the cell in the form of substratum bound or diffusible guidance cues (Tessier-Lavigne and Goodman, 1996). A major question in developmental biology is how this extracellular polarity information is transduced by the cell into the coordinated cytoskeletal and molecular motor activities that affect guided cell and growth cone migrations.

There is increasing evidence that the small Ras-related GTPases of the Rho family (i.e., Rho, Rac, and Cdc42) function in the receptor-mediated signaling cascades that regulate actin dynamics in a way that provides the cell with polarity and the force for motility (Hall, 1998). For example, Cdc42 induces formation of filopodia in cultured Swiss 3T3 fibroblasts, while Rac stimulates formation of lamellipodia and membrane ruffling (Ridley et al., 1992; Nobes and Hall, 1995). Each of these small G proteins can also independently regulate the formation of cell-substratum attachments associated with actin fibers. Consistent with a role in the coordinated cellular activities that occur during cell movement (Lin et al., 1994), it has been found that alterations in Rac function can cause axon guidance and cell migration defects in *Drosophila* (Luo et al., 1994; Murphy and Montell, 1996), mouse (Luo et al., 1996), and *C. elegans* (Zipkin et al., 1997).

While existing evidence strongly suggests that Rho, Rac, and Cdc42 are intimately involved in regulating cytoskeletal dynamics required for cell movements, it is not clear how the GTPase activities of these proteins are spatially and temporally regulated to carry out these functions in vivo. Three types of regulatory proteins have been described that influence the levels of functionally active GTP-bound versus inactive GDP-bound GTPases. These include guanine nucleotide exchange factors (GEFs), which activate GTPases by stimulating exchange of GDP for GTP; GTPase-activating proteins (GAPs), which stimulate endogenous GTPase activity; and guanine nucleotide dissociation inhibitors (GDIs), which associate with GDP- and GTP-bound forms of GTPases to effectively inhibit or activate their functions, respectively (Van Aelst and D'Souza-Schorey, 1997).

Nearly all proteins with GEF activity for Rho, Rac, or Cdc42 contain a conserved, approximately 200 residue Dbl homology (DH) domain, which on its own can catalyze the exchange of GDP for GTP. The prototype Dbl proto-oncoprotein has GEF activity for Cdc42, Rac1, and RhoA (Hart et al., 1994). Approximately 30 proteins with Rho family GEF domains are known, the classic example being CDC24 and its GTPase target CDC42, which affect cell polarity and budding in yeast (Zheng et al., 1994).

Here we describe the *unc-73* gene product from *C. elegans*, which provides insights into actin microfilament regulation as it relates to guided cell and growth cone migrations. Mutants of *unc-73* have a variety of defects in axon guidance and cell motility (see Results and Discussion). The predicted UNC-73A protein has two DH domains that have the potential to activate Rho family GTPases and additional protein motifs that implicate UNC-73 in protein–protein interactions. We show that one of the DH domains of UNC-73 specifically activates Rac in vitro and can stimulate actin polymerization at the plasma membrane when expressed in Rat2 cells growing in culture. UNC-73 is highly related to Trio and Kalirin, two recently discovered vertebrate proteins that exhibit interactions of possible relevance to the function of UNC-73 (Debant et al., 1996; Alam et al., 1997).

Results

unc-73 Encodes Proteins with Dbl and Pleckstrin Homology Domains

unc-73 was cloned by transposon insertion mutagenesis using previously described techniques (Leung-Hagesteijn et al., 1992). Two *unc-73* open reading frames of

§To whom correspondence should be addressed.

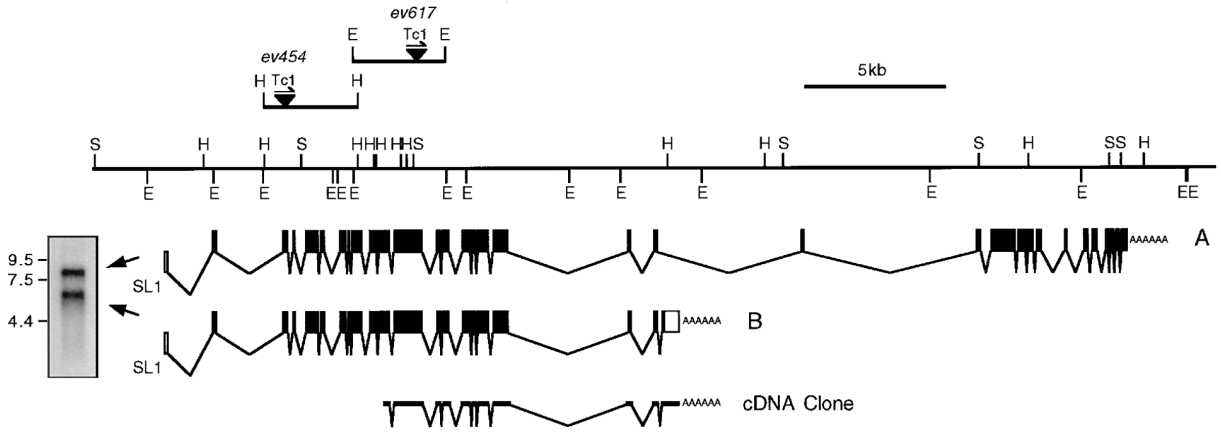

Figure 1. Schematic Structure of the *unc-73* Gene and Predicted mRNAs

Restriction map and exon structures of the *unc-73* gene, a cDNA, and predicted mRNAs. Restriction sites for Sal I (S), Hind III (H), and EcoR I (E) of cosmid C11B5 are indicated. Exons of the two predicted *unc-73* transcripts (A and B) are shown as filled (coding) or unfilled (noncoding) boxes. SL1 is a 22 nt *trans*-spliced 5′ leader sequence. The polyadenylation signals AAUAAA and UAUAAA are located 14 nts upstream of the polyA tail in the A and B transcripts, respectively. The Northern blot of 3 μg polyA RNA from mixed stage wild-type animals probed with a segment of the cDNA identifies two *unc-73* mRNAs of sizes predicted by cDNA sequencing. The orientation shown is opposite to the orientation of *unc-73* on the genetic map. Two Tc1-containing fragments used to identify *unc-73* are represented at the top of the figure.

7464 and 4914 nts that encode proteins of 2488 and 1638 amino acids, named UNC-73A and UNC-73B, respectively, were identified (Figures 1 and 2; see Experimental Procedures). 5′ and 3′ RACE plus cDNA analysis revealed a 5′ UTR of 112 nts fused to the 22 nt SL1 sequence (Krause and Hirsh, 1987) and 3′ UTRs of 35 and 592 nts for the A and B transcripts, respectively. The sizes of the two predicted *unc-73* transcripts correspond closely to the sizes of *unc-73* mRNAs detected on Northern blots (Figure 1). These likely represent the products of alternative splicing and polyadenylation of a single transcript. The cosmid C11B5 (Figure 1) and a minigene encoding the *unc-73B* transcript were shown to rescue the defects of *unc-73* hypomorphic mutations (Experimental Procedures, and see below).

From amino to C terminus, the UNC-73A isoform (Figure 3A) comprises a region with eight spectrin-like repeats (residues 254–1142), a Dbl homology domain (DH-1, residues 1204–1398; Figure 3C), a pleckstrin homology domain (PH-1, residues 1404–1517), an SH3-like domain (residues 1575–1634; Figure 3D), a putative PEST sequence (residues 1702–1734; Rechsteiner and Rogers, 1996), a second DH domain (DH-2, residues 1808–2000; Figure 3C), a second PH domain (PH-2, residues 2006–2119), an immunoglobulin-related (Ig) domain (residues 2302–2392), and a fibronectin type III (FnIII) domain (residues 2393–2488). The smaller UNC-73B isoform comprises the amino-terminal two-thirds of UNC-73A, up to and including the SH3-like sequence (Figure 2).

There are low-scoring BLAST similarities (Altschul et al., 1997) between the SH3-like domain of UNC-73 and SH3 domains with known structures: SEM-5 (Lim et al., 1994) and Fyn (Noble et al., 1993). However, the UNC-73 SH3-like domain is lacking the conserved tryptophan found near the middle of known SH3 domains. To test the significance of the BLAST similarities, threading was used (Bryant, 1996). The resulting alignments included 77% and 68% of residues to SEM-5 and Fyn (Z scores of 5.0 and 5.1), respectively (Figure 3D). These results indicate that the SH3-like domain of UNC-73 closely resembles a real SH3 fold.

Except for its C-terminal FnIII domain, the UNC-73A isoform is related in overall amino acid sequence and modular structure to Trio (Figure 3A), a vertebrate protein that interacts with the LAR receptor tyrosine phosphatase (Debant et al., 1996). In place of the UNC-73 FnIII domain, Trio has a protein serine/threonine kinase (PSK) domain at its C terminus. The amino terminus of UNC-73 (residues 10 to 228) is also related to the amino-terminal domains of Trio, another UNC-73-related protein called Kalirin (see below), a chicken expressed sequence tag (H60pc), a predicted *C. elegans* protein (F55D12.2), and three other DH-containing proteins: Dbs, Ost, and Dbl (Figure 3B). A function has not yet been attributed to this N-terminal region in any of these proteins.

A second protein related to UNC-73B, called Kalirin (Figure 3A), was identified in rat based on its ability to interact with an integral membrane neuropeptide-processing enzyme found on secretory vesicles in neurons and endocrine cells (Alam et al., 1997). Kalirin has the same overall modular structure as UNC-73B, including a previously unrecognized SH3-like domain (residues 1622–1681). It remains to be determined whether both short and long forms of Trio and Kalirin exist as predicted for UNC-73.

unc-73/reporter Fusions Are Expressed in Growing Neurons and Migrating Cells

We examined the temporal and spatial pattern of *unc-73* expression using *gfp* (Figure 4) and *lacZ* (data not shown) reporters driven by *unc-73* 5′ regulatory sequences (Experimental Procedures). All of the reporter constructs produced the same cell-specific expression pattern as transgenes.

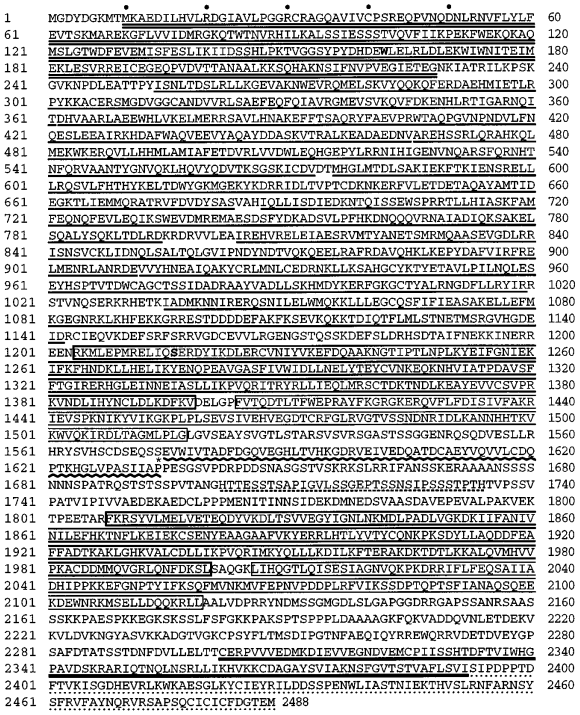

Figure 2. Sequence of the Two Predicted UNC-73 Proteins

UNC-73A, encoded by exons 1–19 and exons 21–33 of transcript A, is shown. UNC-73B, encoded by exons 1–20 of transcript B, is identical to the first 1634 amino acids of UNC-73A (asterisk), with the four additional amino acids (QNSE) encoded by exon 20 appended to its C terminus. The N-terminal (double underline), spectrin-like repeat (thin underlines), Dbl homology (thick boxes), Pleckstrin homology (thin boxes), SH3-like (wavy line), PEST (dashed underline), immunoglobulin (thick underline), and fibronectin type III (dotted underline) domains are indicated. The residues altered in *gm40* and *rh40* (162 and 1216, respectively) are indicated in bold.

The reporter transgenes express ubiquitously in the early embryo starting at about the 100 cell stage during gastrulation. In late embryogenesis and posthatching, expression is more limited. Strongest expression is observed in migrating cells and growing neurons as these cells undergo movements on the epidermis (Figure 4A). At hatching, the reporters express in many neurons throughout the animal, in several cells of the pharynx including some pharyngeal neurons, in the elongated processes of the excretory cells, in the amphid and phasmid sheath and socket cells, in the tail hypodermis, and at later stages in intestine, muscles, vulva, and somatic gonad including the gonad sheath and hermaphrodite distal tip cells (Figures 4B and 4F). We were unable to catalog all of the neuron types that express the *unc-73/reporter* fusions in hatchees (particularly the approximately 50 expressing neurons in the lateral and ventral ganglia behind the nerve ring), but in later stages of development we were able to identify most neuron types known to have axon guidance defects in *unc-73* mutants. These include the PLM, ALM, PDE, HSN, CAN, PHC, and PVN neurons and the ventral cord motorneurons.

The compactness of the embryo and the large number of cells that express the reporters obscure the precise timing of expression in individual cells during embryogenesis. The HSN neurons, however, are easy to visualize and are among the last neurons to extend axons postembryonically. Expression in the HSNs (Figure 4F) is absent in early larval stages, but begins late in the second larval stage (L2), precisely when axon outgrowth is initiated from the HSN cell bodies (Garriga et al., 1993).

The Q neuroblasts, Pn neuroectoblasts, sex myoblasts (SMs), and canal associated neurons (CANs) are defective in their migrations in *unc-73* mutants (Hedgecock et al., 1987; Chen et al., 1997; Forrester and Garriga, 1997) and express *unc-73* reporters (Figure 4). The left and right Q cells begin to express the GFP reporter as they initiate their migrations along the longitudinal axis of the epidermis during the early first larval (L1) stage, and expression in these cells continues beyond the completion of their first division (Figures 4C and 4D).

The Pn cells migrate from dorsal to ventral during embryogenesis and are aligned on either side of the ventral midline at hatching. In the L1 stage they undergo a second phase of movement in which they intercalate to form a single row of ventral midline cells. The *unc-73/reporters* express in the Pn cells just before this second phase of movement, which also involves a nuclear migration (Figure 4B). The distal tip cells also express the *unc-73/reporters* during their migration (Figure 4E).

The UNC-73 Protein Is Concentrated in Axons

To characterize the distribution of the UNC-73 protein in wild-type animals, we raised rabbit antisera to three different regions of UNC-73, each encoded as a fusion with the glutathione-S-transferase (GST) gene (Experimental Procedures). Affinity purified antisera recognized two proteins of approximately 280 and 190 kDa on Western blots that were present in soluble extracts of the wild type but greatly reduced in *unc-73(e936)* and *unc-73(ev509)* (Figure 5). These proteins are of the sizes predicted for UNC-73A and UNC-73B.

All of the antibodies preferentially localized to the nerve ring (a large, toroidally shaped neuropil located in the anterior of the animal) and to the ventral nerve cord (VNC) of wild-type animals from early larval to adult stages (Figure 4G). Staining of the nerve ring but not the VNC was also observed in late embryos. The nerve ring and VNC staining was evidently specific for UNC-73, since it could be blocked with an excess of the purified UNC-73 antigen and was absent in *unc-73(gm40)*-null mutant animals.

UNC-73 Is Required in Neurons for Axon Guidance

To test whether UNC-73 expressed in pioneering neurons can orient their growth cones in the general absence of UNC-73 on potential path and target cells, we used the *mec-7* promoter (Hamelin et al., 1992) to direct UNC-73B expression in mechanosensory neurons. Strains carrying the *mec-7::unc-73* transgene and/or the *mec-4::gfp* reporter gene in a heritable, extrachromosomal array were isolated using a dominant comarker (Experimental Procedures). In wild-type strains with the *mec-4::gfp* transgene, the PLM neurons (which are the

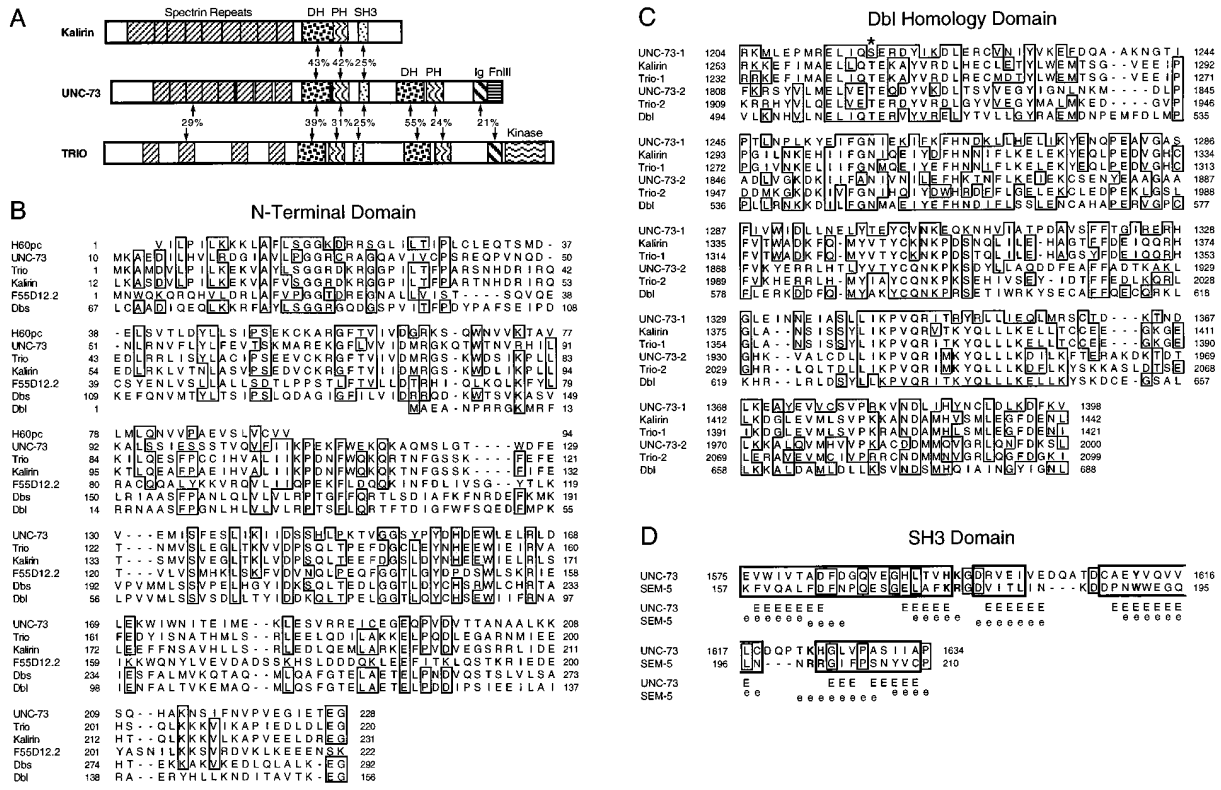

Figure 3. Sequence Comparison of UNC-73 with Other Proteins

(A) Comparison of the modular organization of UNC-73 with the vertebrate Trio and Kalirin proteins showing percent identity (if greater than 20%) of amino acid sequence between the known domains of the proteins.

(B and C) Multiple sequence alignments for the N-terminal and Dbl homology domains. Residues present in 50% or more of the sequences are highlighted. Sequences used are chicken H60pc (Z29355), human Trio (U42390), rat Kalirin (U70373), *C. elegans* F55D12.2 (Z75542), mouse Dbs (S76838), and human Dbl (X12556). The position of the S1216F alteration in *unc-73(rh40)* is indicated (asterisk in C).

(D) Alignment of the SH3 domain from UNC-73 and the C-terminal SH3 domain of *C. elegans* SEM-5 (S88446). Thick boxes represent the optimal alignments found by threading (Bryant, 1996). Conserved aromatic or charged residues are bold. All but two of the identical residues cluster near the poly-prolyl binding site in a 3-D model made with SCWRL (Bower et al., 1997). Known and PHD-predicted beta strands (Rost, 1996) are denoted by "e" and "E," respectively.

most severely affected of the touch receptors in *unc-73* mutants) looked normal: they extended a lateral axon anterior to the midbody and sent a branch to the ventral nerve cord in the region of the vulva (White et al., 1986). In *unc-73(e936)* mutants with the *mec-4::gfp* reporter alone, few of the lateral PLM axons grew anterior to the midbody (14/53) or extended a branch to the VNC in the region of the vulva (7/53). By contrast, in mutants also carrying the *mec-7::unc-73* transgene, most PLM axons grew anterior to the midbody (39/48) and extended a branch to the VNC in the region of the vulva (29/48). Nonautonomous rescue by expression of UNC-73 in a cell type other than the touch receptor neurons is unlikely in these experiments, since other *unc-73* mutant defects (e.g., locomotion, egg-laying) are not rescued by the *mec-7::unc-73* transgene. We conclude that UNC-73 acts cell autonomously to guide the PLM axons.

unc-73(gm40) Is a Putative Null Mutation

Seven independently derived *unc-73* mutations were separated into categories based on the severity of their uncoordinated phenotypes. The *gm40* and *gm33* mutations cause near paralysis; *e936*, *rh40*, and the two Tc1 alleles cause moderate uncoordination; and the *ev509* allele causes weak uncoordination. In addition to being paralyzed, the *gm40* and *gm33* animals often have withered tails, ectopic subventral vulvae, and egg-laying defects (see Hedgecock et al., 1990). *gm40* animals also exhibit maternally rescued embryonic and larval lethality (unpublished data).

Five *unc-73* mutations, including the ^{32}P-induced *e936* allele and the EMS-induced alleles *rh40*, *ev509*, *gm33*, and *gm40*, were located as single-stranded DNA conformational polymorphisms and sequenced (Experimental Procedures). *gm40* changes the tryptophan 162 UGG codon to a UAG stop. The *gm33* mutation (5′ . . . TTTTA **A**AACAG/ . . . 3′ to 5′ . . . TTTTA**G**AACAG . . . 3′) in the splice acceptor site of intron 6 creates a new consensus-matching splice acceptor site (5′ . . . TTTTAG/ . . . 3′), which, if utilized, would cause a frame shift and encode a protein that terminates prematurely. The weak *ev509* allele changes the splice donor of intron 2 from 5′ . . .

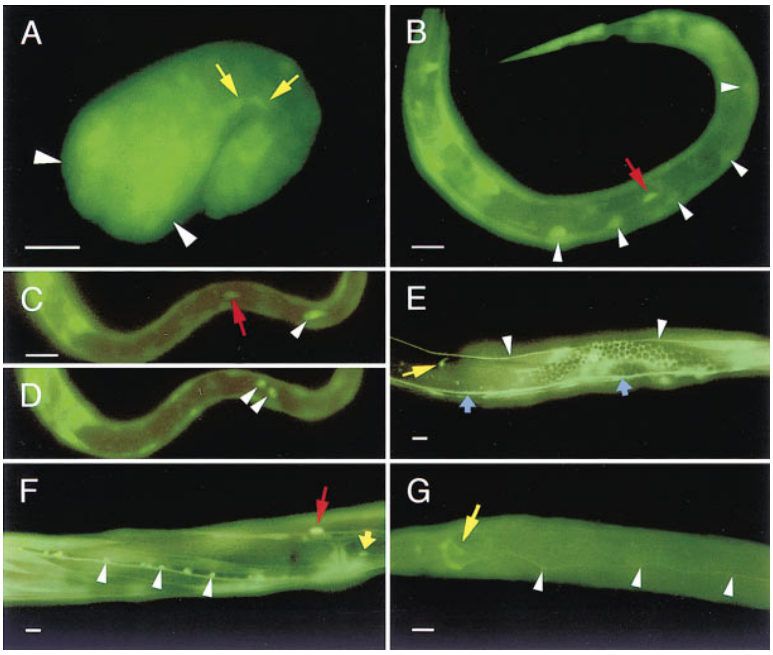

Figure 4. UNC-73 Expression Pattern

(A) At the beginning of neurulation (comma stage embryo), expression of *unc-73/gfp* is strongest in the region of the developing nervous system in the head (white arrowheads) and in the ventral nerve cord (yellow arrows).

(B) The P cells (white arrowheads) begin to express in early L1 just before they intercalate on the ventral midline. The process of intercalation proceeds from anterior to posterior (left to right) and is paralleled by a progressive anterior to posterior increase in expression of the *unc-73/gfp* reporter.

(C and D) In mid-L1, expression comes on in the Q neuroblasts on the left and right (white arrowheads in C and D, respectively). In this animal, QL has migrated posteriorly and has yet to divide, and QR has migrated anteriorly and divided once.

(E) The honeycomb expression in this L4 animal is most likely the somatic gonad. The distal tip cell (yellow arrow) at the leading edge of the growing somatic gonad and the excretory canal (thick blue arrows) also express *unc-73/gfp*. The dorsal nerve cord (white arrowheads) is also visible. Red arrows in (B), (C), and (F) point to CAN neurons. The transgenes in the animals of (A) and (F) were extrachromosomal and may have been lost in some cells.

(F) The motorneurons in the ventral nerve cord (e.g., white arrowheads) express throughout postembryonic development. The HSNL neuron (thick yellow arrow) and spindle-shaped body muscles are also visible.

(G) A wild-type L3 animal stained with an affinity purified polyclonal antibody to UNC-73. Note intense staining of nerve ring (yellow arrow) and ventral nerve cord (white arrowheads).

Scale bars = 10 μm.

/GTAA**G**TT . . . 3′ to 5′ . . . GTAA**A**TT . . . 3′. The *e936* allele changes the splice donor of intron 16 from 5′ . . . /**G**TAGGGC . . . 3′ to 5′ . . . **T**TAGGGC . . . 3′. The possibility of alternative splicing has not been carefully examined in any of the *unc-73* mutant alleles.

An *unc-73* Minigene Containing the First Dbl and Pleckstrin Homology Domains Rescues the Putative Null Phenotype

An *unc-73* minigene was constructed that encodes the UNC-73B protein of 1638 amino acids (Experimental Procedures). The minigene was found to rescue the defects of the *unc-73(e936)*, *unc-73(gm33)*, and *unc-73(gm40)* mutants by germline transformation (Experimental Procedures). The rescued mutants grow, reproduce, and move as well as the wild type.

An UNC-73B Dbl Homology Domain Has Guanine Nucleotide Release Activity on Rac, and Induces Membrane Ruffling

A fusion protein of GST with an UNC-73B peptide containing the DH-1, PH-1, and SH3-like domains (residues 1179 to 1638) was produced in baculovirus, purified, and then tested in a guanine nucleotide release assay with mammalian ^{3}H-GDP-Rac, ^{3}H-GDP-Rho, or ^{3}H-GDP-Cdc42 proteins as substrates. The results indicate that this portion of the UNC-73 protein has GEF activity for mammalian Rac, but not for Rho or Cdc42 (Figure 6).

The *unc-73(rh40)* allele causes a serine 1216 to phenylalanine alteration in the 13th residue of DH-1 (Figure 3C). This residue is conserved as a serine or threonine in DH domains that have demonstrable GEF activity on Rho-type GTPases, consistent with an important functional role for this site (see Discussion). When the S1216F mutation was introduced into the UNC-73/GST fusion described above, it reduced the in vitro guanine nucleotide release activity on Rac to background levels (Figure 6).

When injected into the nuclei of Rat2 cells, the wild-type UNC-73(DH-1/PH-1/SH3) coding sequence induced high levels of polymerized actin at the plasma membrane in the injected cells compared to controls (Figure 7). Although the DH-1/PH-1/SH3 peptide appeared localized around the nucleus in most injected cells, there were many examples of submembranous UNC-73 immunostaining that coincided with regions of actin reorganization at the plasma membrane (Figure 7). Expression of the S1216F mutant DH-1/PH-1/SH3 peptide in Rat2 cells failed to induce actin polymerization.

Discussion

UNC-73 Affects Many Cell and Growth Cone Migrations in *C. elegans*

Mutants of *unc-73* in *C. elegans* have many reported defects in cell and axon growth cone migrations. For example, previous results demonstrated that the moderately uncoordinated canonical *unc-73(e936)* allele has axon guidance defects in the ALM and PLM touch receptor neurons (Hedgecock et al., 1987; Siddiqui, 1990), the

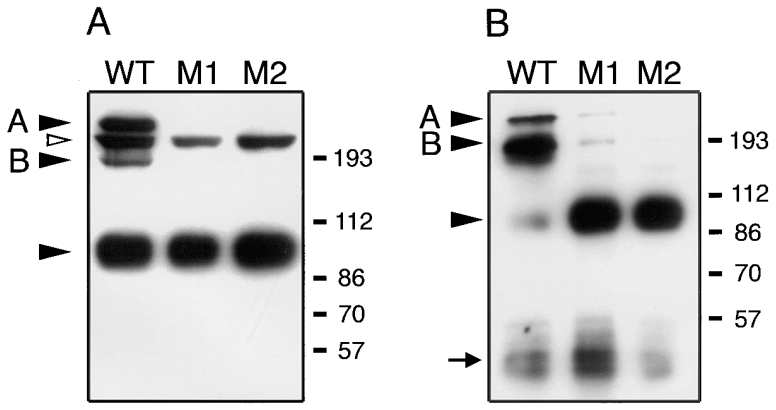

Figure 5. Western Blot Analysis of UNC-73

(A) Soluble protein extracts of *C. elegans* wild type (WT), *unc-73(e936)* (M1), and *unc-73(ev509)* (M2) were separated by SDS-PAGE and subjected to Western blotting. The predicted 283 and 189 kDa forms of UNC-73 (arrowheads in [A] and [B], respectively) are visible in the wild type but are absent in both mutants and are competed away by an excess of the UNC-73 antigen used to induce the antibodies (data not shown). The antibodies detect an unknown protein (arrowhead) that is present in the wild-type and mutant lanes and is also competed away by the UNC-73 antigen. The high molecular weight protein (open arrowhead) located between UNC-73A and UNC-73B is not competed away by the UNC-73 antigen.

(B) Soluble lysates of wild-type (WT), *unc-73(e936)* (M1), and *unc-73(ev509)* (M2) mutants were immunoprecipitated with affinity purified anti-UNC-73 antibodies and then examined by Western blotting using the same antibodies. Low levels of UNC-73A and UNC-73B are detected in the mutant extracts relative to the wild-type extracts in these preparations. The low molecular weight protein seen in (A) is also immunoprecipitated (arrowhead). Anti-UNC-73 antibodies from the immunoprecipitation are visible (arrow).

VD and DD motorneurons (Siddiqui, 1990; McIntire et al., 1992), and the AVK, HSN (Desai et al., 1988; Wightman et al., 1997), AVL (McIntire et al., 1992), PDE (Hedgecock et al., 1987), PHC, PVN (Siddiqui and Culotti, 1991), and PVP neurons (H. Bhatt and E. Hedgecock, personal communication). These neurons still extend axons in *unc-73* mutants, but the axons are often misdirected, abnormally branched, or foreshortened. The affected neurons include pioneers and nonpioneers, which together with the fasciculation defects reported for *unc-73(e936)* (McIntire et al., 1992) indicate that *unc-73* functions are not restricted to pioneer axon guidance.

unc-73 also affects several cell migrations in *C. elegans* (Hedgecock et al., 1987) including the longitudinal migrations of the HSN neurons (Desai et al., 1988), the Q neuroblasts (Hedgecock et al., 1987), and the sex myoblasts (Chen et al., 1997). Defects in the intercalation of the P neuroectoblasts into the ventral midline similar to those observed in mutants of *unc-40* (Chan et al., 1996) can account for the presence of subventral vulvae induced by lateral Pn cells that fail to intercalate in *unc-73* mutants (e.g., see Hedgecock et al., 1990). Finally, the severe alleles also have a withered tail, which most likely results from the observed failure of the CAN neurons to migrate posteriorly (Manser and Wood, 1990; Forrester and Garriga, 1997).

UNC-73 Acts Cell Autonomously

That *unc-73* has widespread effects on pioneer and nonpioneer growth cone and cell migrations indicates that it is required to guide many kinds of cell movements in *C. elegans*. In principle, the observed mutant defects could result from nonautonomous actions of *unc-73* on intercellular signaling mechanism(s) that guide migrations or from the autonomous action of *unc-73* within motile cells or growth cones. Consistent with cell autonomy, *unc-73::gfp* expression was observed in most neurons and migrating cells known to be affected by *unc-73* mutations (e.g., see Figure 4). Furthermore, expression in the Q, Pn, distal tip cells, and HSN neurons is coincident with the movements of these cells or their growth cones.

The prominent expression of *unc-73/gfp* in the nervous system is consistent with the expression of the UNC-73 protein as determined using immunodetection methods. The protein is most concentrated in the two major neuropils, the nerve ring and the VNC, from late embryogenesis to the adult stage. The lack of perfect correlation between the antibody and reporter results could reflect a lack of antibody sensitivity, enhanced turnover of the UNC-73 protein relative to the reporters, or inaccurate reflection of endogenous expression by the reporter transgenes.

The strongest evidence that UNC-73 acts cell autonomously in axon guidance is provided by the finding that expression of *unc-73* under the control of the *mec-7* promoter is sufficient to rescue PLM axon guidance defects in an *unc-73* mutant. By implication, UNC-73

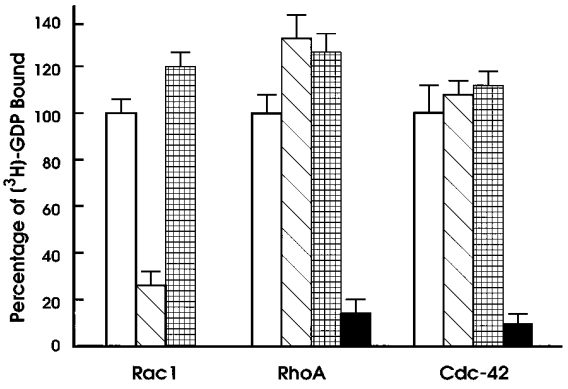

Figure 6. UNC-73(DH-1/PH-1/SH3) Has GEF Activity Specific for the Rho Family GTPase Rac1

Effects of the purified GST/UNC-73(DH-1/PH-1/SH3) on ^{3}H-GDP release from GST/Rac1, GST/RhoA, and GST/Cdc42. Two micrograms of each GTP-binding protein was preloaded with ^{3}H-GDP, then mixed in reaction buffer for 0 min (open bar) or 20 min with 0.5 μg GST/UNC-73(DH-1/PH-1/SH3) (striped bar), mutant S1216F GST/UNC-73(DH-1/PH-1/SH3) (crosshatched bar), or Sf9 lysates expressing the proto-oncogene Dbl (kindly provided by R. Cerione) (black bar).

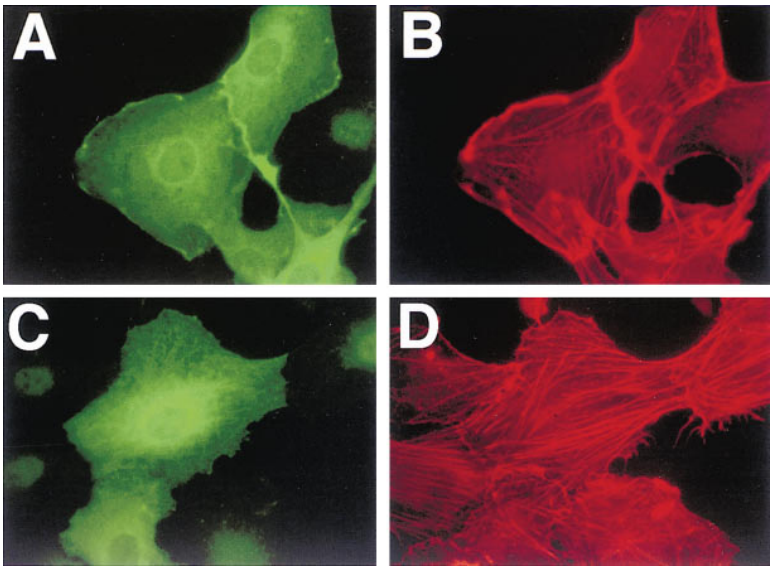

Figure 7. Distribution of Actin in Cells Expressing Wild-Type or S1216F Mutant UNC-73(DH-1/PH-1/SH3) Peptide

Serum-starved Rat2 fibroblasts were fixed 4 hr after injection with UNC-73 (DH-1/PH-1/SH3) (A and B) or with mutant S1216F UNC-73(DH-1/PH-1/SH3) (C and D) coding sequences. Injected cells were identified using rabbit anti-UNC-73 antibodies and FITC-conjugated goat anti-rabbit IgG (A and C). Actin filaments were visualized with fluorescently tagged phalloidin (B and D).

functions cell autonomously to guide other migrating growth cones and cells in *C. elegans*.

UNC-73 Dbl Homology Domains Link Guidance Cues to Axon Growth and Cell Migrations

Molecular analyses revealed two *unc-73* transcripts, A and B. Transcript A encodes a predicted protein (UNC-73A) of 2488 amino acids comprising (in order) eight spectrin-like repeats, a DH/PH element, a SH3-like domain, a second DH/PH element, an Ig domain, and a FnIII domain. The smaller transcript B encodes what is essentially a C-terminal truncated form of UNC-73A that extends to and includes the SH3-like domain. The biochemical analysis of several proteins that contain DH domains has revealed that they have guanine nucleotide exchange factor activity that activates GTPases of the Rho family (Cerione and Zheng, 1996). For example, the corresponding DH-1 and DH-2 domains of the UNC-73-related Trio protein have GEF activity for Rac and Rho, respectively (Debant et al., 1996).

Our finding that an UNC-73 peptide containing the first DH domain (DH-1) is capable of activating Rac and inducing actin reorganization characteristic of membrane ruffling when injected into mammalian cells favors the idea that UNC-73 regulates the actin cytoskeleton to affect migrations in *C. elegans*. Membrane ruffling is often associated with cell motility and can be induced in fibroblasts by injecting mammalian Rac but not Rho GTPases (Ridley et al., 1992).

The substitution of serine 1216 with phenylalanine, as found in the *unc-73(rh40)* mutant protein, inactivated both the GEF and actin reorganization functions of the UNC-73 DH-1 domain (Figures 6 and 7). This finding provides direct evidence for the importance of DH domain function in an intact organism. The residue affected by the *unc-73(rh40)* mutation is a serine or threonine in the great majority of DH domains, consistent with an important role in catalyzing or regulating Rho family GEF activity. Interestingly, only the DH domains of RasGRF and Sos isoforms as well as Net1 have distinct amino acids at this position, which might influence their ability to activate Rho family GTPases. Indeed, Rho family GEF activity has not been directly demonstrated for DH domains from Sos and RasGRF proteins using conventional in vitro assays, although recent data raise the possibility that the Sos DH domain may stimulate the Rac GTPase in vivo (Nimnual et al., 1998). The GEF activity of Net1 has not yet been examined. It will be of interest to investigate whether the residue corresponding to Ser1216 of UNC-73 is of general importance for DH domain function.

We were unable to produce a sufficient quantity of soluble UNC-73 DH-2 peptide to test its substrate specificity in vitro, but the corresponding domain in Trio has GEF activity for Rho but not Rac. A potential target for the putative DH-2 GEF activity is RhoA, which is enriched in the nerve ring in the same manner as UNC-73 (Chen and Lim, 1994).

Possible in vivo targets for the DH-1 GEF activity of UNC-73 include MIG-2 (a *C. elegans* Rac-related protein), CeRAC-1, CeRAC-2, and CeCDC-42 (Chen et al., 1996a, 1996b). CeRAC-1 and CeCDC-42 are expressed at hypodermal cell boundaries during embryo elongation, but CeCDC-42, like UNC-73 and RhoA, is also expressed in the nerve ring later in development (R. van Weeghel and J. C., unpublished data). The *mig-2* expression pattern overlaps with that of *unc-73*. Loss-of-function mutations in *mig-2* affect only Q cell and coelomocyte migrations, whereas gain-of-function mutations in *mig-2* produce many of the same defects as *unc-73* loss-of-function mutations, including defects in axon guidance and in other cell migrations (Zipkin et al., 1997). This suggests that there are additional Rho family targets for UNC-73 activity that mediate axon guidance and cell migrations.

An *unc-73* minigene that encodes the smaller UNC-73B protein is able to effectively rescue the *gm40* nonsense mutant phenotype. One possible explanation for this result is that the second DH/PH element, as well as the C-terminal Ig and FnIII-like domains of UNC-73, have no function in *C. elegans*. This would be surprising in

light of the higher evolutionary conservation of the second relative to the first DH/PH element in UNC-73A compared to the corresponding element in Trio (Figure 3A). UNC-73A may, therefore, have specific function(s) that have not been identified. Another possible explanation is that the *gm40* mutation does not totally eliminate expression of the more C-terminal domains of UNC-73A, in which case rescue by the minigene might represent a form of intragenic complementation. More experimentation is required to distinguish between these possibilities.

A Model for UNC-73 Activity

In addition to defects in axon guidance and cell migrations, *unc-73* mutants also have limited effects on cell fate determination and on the polarity of lineage programs in *C. elegans*. The defects in cell fate have been described as an inability of certain neuroblasts in the touch receptor lineages to segregate developmental potential at their terminal divisions exclusively into their most anterior daughters (which become the touch receptor neurons). In the *e936* mutant, both daughters occasionally inherit the ability to express the *mec-3* homeobox gene, which plays a large role in determining touch cell characteristics (Way et al., 1992). *unc-73* mutations also enhance defects in the polarity of vulval lineages observed in mutants of *lin-18* (P. Sternberg and W. Katz, personal communication). A common theme in the developmental processes affected by *unc-73*, including cell and growth cone migrations, segregation of putative developmental determinants, and cell lineages, is that these processes all require polarized activities of the actin cytoskeleton. Since UNC-73 can activate Rho family GTPases and actin polymerization typical of membrane ruffling, a unifying hypothesis is that UNC-73 is required in migrating and dividing cells for the spatial regulation of actin and cytoskeletal polarity that occurs in response to extracellular polarity cues. In principle, these requirements for *unc-73* could account for the lethality of the *gm40* allele.

Clues as to the mechanisms that UNC-73 might utilize to carry out its in vivo functions are provided by other aspects of its composition. UNC-73 is similar to Trio in its overall modular structure, except that the Ig domain present in both proteins is followed by a FnIII domain in UNC-73 and a protein serine/threonine kinase (PSK) domain in Trio. The Trio protein, however, does not show any in vitro kinase activity toward itself or several other tested substrates (Debant et al., 1996). Trio does, however, bind to the LAR receptor tyrosine phosphatase in both yeast two-hybrid and coimmunoprecipitation experiments, raising the possibility that UNC-73 binds to a similar PTPase in *C. elegans*. Interestingly, the gene for the *Drosophila* LAR PTPase is almost exclusively expressed by developing neurons, and mutations in the gene cause defects in motor axon pathfinding (Krueger et al., 1996).

The spectrin-like, Ig, and FnIII domains of UNC-73 are likely to mediate interactions with unidentified proteins, whereas the PH domains probably mediate the association of proteins with specific membrane phosphoinositides (Lemmon et al., 1997). Additional functions for UNC-73 are suggested by its relatedness to Kalirin. Kalirin was isolated for its ability to interact with the integral membrane enzyme PAM (peptidylglycine α-amidating monooxygenase) of large dense-core vesicles and has also been shown to enhance neuritic process formation in cultured rat corticotropic tumor cells (Alam et al., 1997). The domain structure of Kalirin, its ability to affect cell morphology, and its PAM-binding properties indicate that Kalirin is a component of a signal transduction pathway that links routing of large dense-core vesicles with changes in the submembranous actin cytoskeleton (Alam et al., 1997). This suggests a possible role for UNC-73 in vesicle routing and neuropeptide processing in addition to its proposed role in actin-based molecular motor and cell shaping activities.

In conclusion, we have found that UNC-73 is an activator of Rho family GTPases that affects actin polymerization, cell migrations, and axon guidance in *C. elegans*. These results are strong genetic evidence that activators of Rho family GTPases play an important role in the guided migrations of cells and growth cones. The specific GTPases involved should be detectable by biochemical and genetic screens for UNC-73-interacting proteins (Run et al., 1996). The fact that UNC-73 is highly related to two mammalian proteins, Trio and Kalirin, raises the possibility that the latter proteins may regulate cell and growth cone motility in mammals.

Experimental Procedures

C. elegans Strains

The *unc-73* alleles *gm33* and *gm40* were kindly provided by G. Garriga (U. C. Berkeley). *rh40* was provided by E. Hedgecock (Johns Hopkins U.). All other *C. elegans* strains were newly isolated or obtained from the *C. elegans* Genetics Stock Center (U. of Minnesota) in care of Theresa Stiernagle.

Mutagenesis and Cloning of *unc-73*

The EMS-induced *ev509* allele was isolated in a noncomplementation screen for new *unc-73* alleles. Two independently derived *unc-73* mutations, *ev454::Tc1* and *ev617::Tc1*, were isolated in mutator strain RW7097 (Mori et al., 1988). The Tc1 transposons responsible for both mutations and flanking sequences were identified and cloned by standard techniques (see Leung-Hagesteijn et al., 1992). Using Tc1-flanking DNA as a probe, genomic phage DNAs that span the region were isolated and then mapped by Alan Coulson (Cambridge, England) to cosmid C11B5, which was shown to rescue *unc-73* mutants by germline transformation (Mello et al., 1991). A similar probe was used to identify six copies of a single cDNA clone of 3.5 kb (10^6 plaques from the S. Kim library) that encodes a 2.9 kb open reading frame corresponding to exons 10–20 of *unc-73*.

5′ and 3′ RACE

The 5′ end of the *unc-73* mRNA was deduced by 5′ RACE (Frohman et al., 1988) using nested reverse primers RP3 (5′-CTACATCGTCCT CCAGG-3′) and RP4 (5′-GGTTGCTCTCGTGATGG-3′) specific to exon 2. This produced an approximately 200 nt product that ended in the sequence of SL1. The 5′ RACE product encodes a short open reading frame that contains a possible initiator methionine codon and is spliced in frame to exon 2. The 3′ RACE reaction was performed using the forward primer RS40 (5′-TGTGATGCCGGTGCTTA TTC-3′) specific to exon 31.

The *unc-73* Minigene

The *unc-73* minigene was constructed from three DNA fragments that together comprise the following: genomic DNA including 2.3 kb of 5′ regulatory sequence plus the first ten and one-half exons with intervening sequences, cDNA encoding the remainder of exon

11 plus exons 12–19 and part of exon 20, and genomic DNA that includes the remainder of exon 20 and extends 825 bp beyond the polyadenylation site of transcript B.

The minigene was injected into *unc-73(e936)* animals along with pRF4, a dominant *rol-6* cotransformation marker. The transgenic array carrying the minigene and the *rol-6* marker was passed genetically into balanced *unc-73(gm40)* and *unc-73(gm33)* strains.

Nucleic Acid Analyses

Basic manipulations of DNA and RNA including SSCP analysis were performed as described in Sambrook et al. (1989), Leung-Hagesteijn et al. (1992), and Chan et al. (1996).

Although a single mutation was identified in each *unc-73* allele by SSCP, the size of the *unc-73* gene restricted us from analyzing the entire gene for every allele.

***lacZ* and *gfp* Fusion Constructs**

A 3.8 kb XbaI-ScaI genomic DNA fragment that contains approximately 1.3 kb of the promoter region, exon 1, and part of exon 2 encoding 25 N-terminal residues of UNC-73 was cloned in frame into the XbaI-SmaI digested *lacZ* or *gfp* reporter vectors pPD95.57, pPD95.69, pPD95.77, and pPD95.57 (kindly provided by A. Fire) that had the NLS removed by digesting with KpnI and religating. The size of the *unc-73* 5′ regulatory region was increased to 4.3 kb in a second set of constructs by cloning in the 3.0 kb SalI-XbaI DNA fragment found just 5′ to the 3.8 kb XbaI-ScaI fragment in the genomic sequence. All reporter constructs were injected into the distal gonad arms of either wild-type or *dpy-20(e1282)* animals with the addition of pRF4 (dominant *rol-6*) or pMH86 (wild-type *dpy-20*), respectively, as cotransformation markers. Transgenic animals containing the *lacZ* reporter constructs were stained with X-Gal using the protocol described by Xie et al. (1995).

***mec-7::unc-73* Constructs**

The *mec-7::unc-73* transgene was constructed by subcloning the 5′ regulatory region of *mec-7* (Chan et al., 1996) upstream of a sequenced *unc-73* cDNA encoding UNC-73B. This transgene was coinjected with *mec-4::gfp* (gift of Monica Driscoll) to visualize PLM neuron morphologies and the dominant *rol-6* marker to identify transformed progeny.

Expression of UNC-73/GST Fusion Proteins and Immunodetection

Three different regions of the *unc-73* cDNA, two EcoRI fragments from nucleotides 2097–3430 and 3647–5619, and a PCR-generated fragment corresponding to nucleotides 3737–4449 were cloned into the pGEX1 expression vector (numbers refer to the corresponding region of transcript B). Clone pNW181 encodes a portion of the spectrin-like repeats, clone pNW180 encodes the C-terminal 238 amino acids from transcript B including DH-1 and PH-1, and clone pNW186 encodes DH-1. All of the GST/UNC-73 fusion proteins expressed from these plasmids were insoluble and were therefore purified by isolating bacterial inclusion bodies (Sambrook et al., 1989).

Polyclonal antibodies were generated in rabbits as described by Hamelin et al. (1992). UNC-73 antibodies were affinity purified using a slightly modified version of the filter-binding protocol found in Sambrook et al. (1989). Mixed-stage *C. elegans* populations were stained with the affinity purified antibodies as described by Finney and Ruvkun (1990). Western blotting and immunoprecipitations were carried out as described by Olivier et al. (1993). Affinity purified antibodies were tested for their ability to recognize the corresponding GST fusion (and, in one case, a corresponding TrpE fusion) by Western blot analysis before being used for immunolocalization studies.

Protein Production and Guanine Nucleotide Releasing Assays

DNA fragments encoding the DH-1/PH-1/SH3 region (residues 1179 to 1638) of wild-type and S1216F mutant *unc-73* were subcloned into the pAcGHLT-A vector (Pharmingen) for baculovirus production of GST-fusion proteins that carry a His tag. Both the wild-type and mutant proteins were purified from Hi5 cells using nickel columns (Qiagen) and glutathione Sepharose beads (Pharmacia) by standard procedures. Low molecular mass GTPases were purified from *E. coli* as described in Self and Hall (1995). ^{3}H-GDP release assays of preloaded small mammalian GTPases were performed as described by Debant et al. (1996).

Cell Culture and Microinjection

Wild-type and S1216F mutant DH-1/PH-1/SH3 sequences were cloned into the eukaryotic expression vector pEFPLINK.2, which carries the *myc* epitope tag at the 5′ end (Marais et al., 1995), for nuclear microinjection experiments. Rat2 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 10 mM HEPES (pH 7.5). After seeding (2 days), the cells were serum-starved for 24 hr. Nuclear microinjections of DNA, immunodetection of proteins, and photography were as described in Bar-Sagi (1995). Cells were incubated for 4 hr following the microinjection. A purified anti-UNC-73(DH-1/PH-1/SH3) primary antibody and an FITC-conjugated goat anti-rabbit antibody and rhodamine-conjugated phalloidin were used to detect the UNC-73 peptide and actin, respectively, in the injected cells.

Acknowledgments

We thank Gian Garriga for providing mutant alleles *gm33* and *gm40* and sharing unpublished results; Andy Fire for providing reporter vectors; Alan Coulson for mapping phage clones and providing cosmids; Arthur Roach and Tracy Saxton for technical advice; Rachel Kindt, Rebecca Burdine, and David Greenstein for help with cell identifications; and David Merz for helpful discussions. Some strains were provided by the Caenorhabditis Genetics Center, which is funded by the NIH National Center for Research Resources. This work was supported by a grant from the Canadian MRC, a Howard Hughes Medical Research Scholar Award, and a Terry Fox Cancer Research Scientist Award to T. P., a grant from the Canadian NCIC (007215) to J. C., a postdoctoral fellowship from the Canadian NCIC to T. J. K., and a Canadian NSERC postgraduate scholarship to R. S.

Received October 12, 1997; revised February 9, 1998.

References

Alam, M.R., Johnson, R.C., Darlington, D.N., Hand, T.A., Mains, R.E., and Eipper, B.A. (1997). Kalirin, a cytosolic protein with spectrin-like and GDP/GTP exchange factor–like domains that interacts with peptidylglycine alpha-amidating monooxygenase, an integral membrane peptide-processing enzyme. J. Biol. Chem. *272*, 12667–12675.

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zheng, Z., Miller, W., and Lipman, D.J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. *25*, 3389–3402.

Bar-Sagi, D. (1995). Mammalian cell microinjection assay. Meth. Enzym. *255*, 436–442.

Bower, M.J., Cohen, F.E., and Dunbrack, R.L., Jr. (1997). Prediction of protein side-chain rotamers from a backbone dependent rotamer library: a new homology modeling tool. J. Mol. Biol. *267*, 1268–1282.

Bryant, S.H. (1996). Evaluation of threading specificity and accuracy. Proteins *26*, 172–185.

Cerione, R.A., and Zheng, Y. (1996). The Dbl family of oncogenes. Curr. Opin. Cell Biol. *8*, 216–222.

Chan, S.S., Zheng, H., Su, M.W., Wilk, R., Killeen, M.T., Hedgecock, E.M., and Culotti, J.G. (1996). UNC-40, a *C. elegans* homolog of DCC (Deleted in Colorectal Cancer), is required in motile cells responding to UNC-6 netrin cues. Cell *87*, 187–195.

Chen, E.B., Branda, C.S., and Stern, M.J. (1997). Genetic enhancers of sem-5 define components of the gonad-independent guidance mechanism controlling sex myoblast migration in Caenorhabditis elegans hermaphrodites. Dev. Biol. *182*, 88–100.

Chen, W., and Lim, L. (1994). The Caenorhabditis elegans small GTP-binding protein RhoA is enriched in the nerve ring and sensory

neurons during larval development. J. Biol. Chem. *269*, 32394–32404.

Chen, W.N., Yap, S.F., and Lim, L. (1996a). Isolation of a gene coding for the Caenorhabditis elegans Rac2 homologue, a Ras-related small GTP-binding protein. Gene *180*, 217–219.

Chen, W., Chen, S., Yap, S.F., and Lim, L. (1996b). The Caenorhabditis elegans p21-activated kinase (CePAK) colocalizes with CeRac1 and CDC42Ce at hypodermal cell boundaries during embryo elongation. J. Biol. Chem. *271*, 26362–26368.

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

Desai, C., Garriga, G., McIntire, S.L., and Horvitz, H.R. (1988). A genetic pathway for the development of the Caenorhabditis elegans HSN motor neurons. Nature *336*, 638–646.

Finney, M., and Ruvkun, G. (1990). The *unc-86* gene product couples cell lineage and cell identity in *C. elegans*. Cell *63*, 895–905.

Forrester, W.C., and Garriga, G. (1997). Genes necessary for C. elegans cell and growth cone migrations. Development *124*, 1831–1843.

Frohman, M.A., Dush, M.K., and Martin, G.R. (1988). Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligonucleotide primer. Proc. Natl. Acad. Sci. USA *85*, 8998–9002.

Garriga, G., Desai, C., and Horvitz, H.R. (1993). Cell interactions control the direction of outgrowth, branching and fasciculation of the HSN axons of Caenorhabditis elegans. Development *117*, 1071–1087.

Hall, A. (1998). Rho GTPases and the actin cytoskeleton. Science *279*, 509–514.

Hamelin, M., Scott, I.M., Way, J.C., and Culotti, J.G. (1992). The *mec-7* beta-tubulin gene of Caenorhabditis elegans is expressed primarily in the touch receptor neurons. EMBO J. *11*, 2885–2893.

Hart, M.J., Eva, A., Zangrilli, D., Aaronson, S., Evans, T., Cerione, R.A., and Zheng, Y. (1994). Cellular transformation and guanine nucleotide exchange activity are catalyzed by a common domain on the *dbl* oncogene product. J. Biol. Chem. *269*, 62–65.

Hedgecock, E.M., Culotti, J.G., Hall, D.H., and Stern, B.D. (1987). Genetics of cell and axon migrations in Caenorhabditis elegans. Development *100*, 365–382.

Hedgecock, E.M., Culotti, J.G., and Hall, D.H. (1990). The *unc-5*, *unc-6*, and *unc-40* genes guide circumferential migrations of pioneer axons and mesodermal cells on the epidermis in *C. elegans*. Neuron *4*, 61–85.

Krause, M., and Hirsh, D. (1987). A *trans*-spliced leader sequence on actin mRNA in *C. elegans*. Cell *49*, 753–761.

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

Lemmon, M.A., Falasca, M., Ferguson, K.M., and Schlessinger, J. (1997). Regulatory recruitment of signaling molecules to the cell membrane by pleckstrin-homology domains. Trends Cell Biol. *7*, 237–242.

Leung-Hagesteijn, C., Spence, A.M., Stern, B.D., Zhou, Y., Su, M.W., Hedgecock, E.M., and Culotti, J.G. (1992). UNC-5, a transmembrane protein with immunoglobulin and thrombospondin type 1 domains, guides cell and pioneer axon migrations in *C. elegans*. Cell *71*, 289–299.

Lim, W.A., Richards, F.M., and Fox, R.O. (1994). Structural determinants of peptide-binding orientation and of sequence specificity in SH3 domains. Nature *372*, 375–379.

Lin, C.H., Thompson, C.A., and Forscher, P. (1994). Cytoskeletal reorganization underlying growth cone motility. Curr. Opin. Neurobiol. *4*, 640–647.

Luo, L., Liao, Y.J., Jan, L.Y., and Jan, Y.N. (1994). Distinct morphogenetic functions of similar small GTPases: *Drosophila* Drac1 is involved in axonal outgrowth and myoblast fusion. Genes Dev. *8*, 1787–1802.

Luo, L., Hensch, T.K., Ackerman, L., Barbel, S., Jan, L.Y., and Jan, Y.N. (1996). Differential effects of the Rac GTPase on Purkinje cell axons and dendritic trunks and spines. Nature *379*, 837–840.

Manser, J., and Wood, W.B. (1990). Mutations affecting embryonic cell migrations in Caenorhabditis elegans. Dev. Genet. *11*, 49–64.

Marais, R., Light, Y., Paterson, H.F., and Marshall, C.J. (1995). Ras recruits Raf-1 to the protein membrane for activation by tyrosine phosphorylation. EMBO J. *14*, 3136–3145.

McIntire, S.L., Garriga, G., White, J., Jacobson, D., and Horvitz, H.R. (1992). Genes necessary for directed axonal elongation or fasciculation in *C. elegans*. Neuron *8*, 307–322.

Mello, C.C., Kramer, J.M., Stinchcomb, D., and Ambros, V. (1991). Efficient gene transfer in C. elegans: extrachromosomal maintenance and integration of transforming sequences. EMBO J. *10*, 3959–3970.

Mori, I., Moerman, D.G., and Waterston, R.H. (1988). Analysis of a mutator activity necessary for germline transposition and excision of Tc1 transposable elements in Caenorhabditis elegans. Genetics *120*, 397–407.

Murphy, A.M., and Montell, D.J. (1996). Cell type-specific roles for Cdc42, Rac, and RhoL in *Drosophila* oogenesis. J. Cell Biol. *133*, 617–630.

Nimnual, A.S., Yatsula, B.A., and Bar-Sagi, D. (1998). Coupling of Ras and Rac guanosine triphosphates through the Ras exchanger Sos. Science *279*, 560–563.

Nobes, C.D., and Hall, A. (1995). Rho, Rac, and CDC42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. Cell *81*, 53–62.

Noble, M.E., Musacchio, A., Saraste, M., Coutneidge, S.A., and Wierenga, R.K. (1993). Crystal structure of the SH3 domain in human Fyn; comparison of the three dimensional structures of SH3 domains in tyrosine kinases and spectrin. EMBO J. *12*, 2617–2624.

Olivier, J.P., Raake, T., Henkemeyer, M., Dickson, B., Mbamalu, G., Margolis, B., Schlessinger, J., Hafen, E., and Pawson, T. (1993). A *Drosophila* SH2-SH3 adaptor protein implicated in coupling the sevenless tyrosine kinase to an activator of Ras guanine nucleotide exchange, Sos. Cell *73*, 179–191.

Rechsteiner, M., and Rogers, S.W. (1996). PEST sequences and regulation by proteolysis. Trends Biol. Sci. *267*, 267–271.

Ridley, A.J., Paterson, H.F., Johnston, C.L., Diekmann, D., and Hall, A. (1992). The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. Cell *70*, 401–410.

Rost, B. (1996). PHD: predicting one-dimensional protein structure by profile based neural networks. Meth. Enzymol. *266*, 525–539.

Run, J.-Q., Steven, R., Hung, M.-S., van Weeghel, R., Culotti, J.G., and Way, J.C. (1996). Suppressors of the unc-73 gene of Caenorhabditis elegans. Genetics *143*, 225–236.

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

Self, A.J., and Hall, A. (1995). Purification of recombinant Rho/Rac/G25K from Escherichia coli. Meth. Enzymol. *256*, 3–10.

Siddiqui, S.S. (1990). Mutations affecting axonal growth and guidance of motor neurons and mechanosensory neurons in the nematode Caenorhabditis elegans. Neurosci. Res. Suppl. *13*, S171–S190.

Siddiqui, S.S., and Culotti, J.G. (1991). Examination of neurons in wild type and mutants of Caenorhabditis elegans using antibodies to horseradish peroxidase. J. Neurogenet. *7*, 193–211.

Tessier-Lavigne, M., and Goodman, C.S. (1996). The molecular biology of axon guidance. Science *274*, 1123–1133.

Van Aelst, L., and D'Souza-Schorey, C. (1997). Rho GTPases and signaling networks. Genes Dev. *11*, 2295–2322.

Way, J.C., Run, J.-Q., and Wang, A.Y. (1992). Regulation of anterior cell-specific mec-3 expression during asymmetric cell division in C. elegans. Dev. Dyn. *194*, 289–302.

White, J.G., Southgate, E., Thomson, J.N., and Brenner, S. (1986). The structure of the nervous system of the nematode *Caenorhabditis elegans*. Philos. Trans. R. Soc. Lond. *314*, 1–340.

Wightman, B., Baran, R., and Garriga, G. (1997). Genes that guide

growth cones along the C. elegans ventral nerve cord. Development *124*, 2571–2580.

Xie, G., Jia, Y., and Aamodt, E. (1995). A C. elegans mutant screen based on antibody or histochemical staining. Genet. Anal. *12*, 95–100.

Zheng, Y., Cerione, R., and Bender, A. (1994). Control of the yeast bud-site assembly GTPase Cdc42. Catalysis of guanine nucleotide exchange by Cdc24 and stimulation of GTPase activity by Bem3. J. Biol. Chem. *269*, 2369–2372.

Zipkin, I.D., Kindt, R.M., and Kenyon, C.J. (1997). Role of a new Rho family member in cell migration and axon guidance in *C. elegans*. Cell *90*, 883–894.

GenBank Accession Numbers

The accession numbers for the sequences reported in this paper are AF048834 (*unc-73* transcript A) and AF048835 (*unc-73* transcript B).