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SDQR migrations in *Caenorhabditis elegans* are controlled by multiple guidance cues and changing responses to netrin UNC-6

Seonhee Kim, Xing-Cong Ren, Eileen Fox and William G. Wadsworth*

Department of Pathology, Robert Wood Johnson Medical School, Piscataway, NJ 08854, USA
*Author for correspondence (e-mail: wadswort@umdnj.edu)

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SUMMARY

The netrin guidance cue, UNC-6, and the netrin receptors, UNC-5 and UNC-40, guide SDQR cell and axon migrations in *C. elegans*. In wild-type larvae, SDQR migrations are away from ventral UNC-6-expressing cells, suggesting that UNC-6 repels SDQR. In *unc-6* null larvae, SDQR migrations are towards the ventral midline, indicating a response to other guidance cues that directs the migrations ventrally. Although ectopic UNC-6 expression dorsal to the SDQR cell body would be predicted to cause ventral SDQR migrations in *unc-6* null larvae, in fact, more migrations are directed dorsally, suggesting that SDQR is not always repelled from the dorsal source of UNC-6. UNC-5 is required for dorsal SDQR migrations, but not for the ventral migrations in *unc-6* null larvae. UNC-40 appears to moderate both the response to UNC-6 and to the other cues. Our results show that SDQR responds to multiple guidance cues and they suggest that, besides UNC-6, other factors influence whether an UNC-6 responsive cell migrates toward or away from an UNC-6 source in vivo. We propose that multiple signals elicited by the guidance cues are integrated and interpreted by SDQR and that the response to UNC-6 can change depending on the combination of cues encountered during migration. These responses determine the final dorsoventral position of the SDQR cell and axon.

Key words: *unc-6*, Netrin, *Caenorhabditis elegans*, Cell migration, Axon guidance, SDQR, UNC-6

INTRODUCTION

The patterning of the embryonic axon scaffold positions neurons and axon tracts at defined locations. During development, axonal growth cones migrate through their environment in response to molecular guidance cues. Studies suggest that individual pioneer axons are directed to specific locations by the actions of repulsive and attractive guidance cues. (Colamarino and Tessier-Lavigne, 1995b; Keynes and Cook, 1995; Tessier-Lavigne and Goodman, 1996; Winberg et al., 1998). Axons appear to have the ability to make pathfinding and target selection decisions based on the simultaneous assessment of multiple guidance cues (Tessier-Lavigne and Goodman, 1996; Winberg et al., 1998). In this combinatorial model, the guidance cues function together as antagonists or collaborators and axons assess the equilibrium between the signals to determine a directional response.

From the genetic and molecular analyses of a guidance cue, UNC-6, and its receptors, UNC-5 and UNC-40, a model of cell and axon migrations in *C. elegans* has emerged. In its simplest form, the model predicts that cells or axons expressing UNC-5 together with UNC-40 are repelled from UNC-6 sources, and cells and axons expressing UNC-40 (and perhaps another co-receptor) are attracted towards UNC-6 sources. This model is supported by several pieces of evidence discussed below.

Circumferential migrations in *C. elegans* require the gene *unc-6* (Hedgecock et al., 1990). In mutants, dorsal and ventral migrations of pioneer axons and mesodermal cells are disrupted. The UNC-6 protein is a member of the phylogenetically conserved netrin family (Serafini et al., 1994; Harris et al., 1996; Mitchell et al., 1996). The vertebrate netrins have been shown to have chemoattractant and chemorepellent activities for developing axons in the embryonic nervous system (Kennedy et al., 1994; Colamarino and Tessier-Lavigne, 1995a; Serafini et al., 1996) and have been implicated as a guidance cue in many different regions of the nervous system (Kennedy et al., 1994; Serafini et al., 1996; de la Torre et al., 1997; Deiner et al., 1997; Lauderdale et al., 1997; Livesey and Hunt, 1997; MacLennan et al., 1997; Metin et al., 1997; Richards et al., 1997; Strahle et al., 1997; Shirasaki et al., 1998). Netrin ectopically expressed throughout the developing fly CNS and nematode nervous system causes widespread guidance defects, indicating that the location of the netrin source is critical (Harris et al., 1996; Mitchell et al., 1996; Ren et al., 1999).

We have proposed that UNC-6 is a component of an extracellular matrix cue and that it is arranged within basement membranes or along cellular surfaces in a manner that allows it to interact with cell surface receptors on migrating axons or cells (Wadsworth and Hedgecock, 1992; Wadsworth et al., 1996). The direction- and tissue-specific guidance activities are mediated by distinct domains of UNC-6, suggesting that receptors interact at unique sites within the protein (Ishii et al., 1992; Wadsworth et al., 1996). UNC-6 is expressed by

neuroglia and pioneer neurons to provide global and local netrin cues. Each cue has a characteristic role depending on the cell type in which it is expressed, its location and the developmental stage of the animal (Wadsworth et al., 1996; Wadsworth and Hedgecock, 1996). An UNC-6 cue arising from ventral epidermoblasts and their descendants creates a stable global netrin cue peaking near the ventral midline of the body wall. It is thought that each migrating cell or axon is either attracted (ventral direction) or repelled (dorsal direction) from the UNC-6 source.

One aspect of the guidance activities that is poorly understood is how mechanistically UNC-6 guides both dorsal and ventral migrations. Two receptors are known to be important for mediating responses to the UNC-6 guidance cue. UNC-5, a receptor belonging to the immunoglobulin superfamily, is expressed in motile cells and is both necessary and sufficient to direct migrations dorsally, away from ventral UNC-6 sources (Hedgecock et al., 1990; Leung-Hagesteijn et al., 1992; Hamelin et al., 1993). UNC-40, a *C. elegans* homolog of the vertebrate protein encoded by *Deleted in Colorectal Cancer* (DCC), is also a member of the immunoglobulin superfamily and is required in migrating cells that respond to UNC-6 (Chan et al., 1996). DCC and the vertebrate homologues of UNC-5 have been implicated as receptors for vertebrate netrin (Keino-Masu et al., 1996; Leonardo et al., 1997). The precise role that netrin receptors play in mediating migrations in vivo is only partially understood. At one extreme, different classes of axons could have distinct receptor complexes and signaling pathways to mediate either attraction or repulsion by UNC-6. However, recent data indicates that the pathways involved in netrin-mediated attraction and repulsion are interconnected (Ming et al., 1997). In culture, growth cones of embryonic *Xenopus* spinal neurons normally turn towards a source of netrin-1. However, with the addition of a competitive analog of cAMP or an inhibitor of protein kinase A, the axons can be switched into being repulsed from the netrin-1 source. These experiments raise the possibility that in vivo different guidance signals could be simultaneously interpreted by neurons to generate different guidance behaviors in response to netrin (Ming et al., 1997).

To study the attractive and repulsive activities of a guidance cue, we used genetic analysis in the nematode *C. elegans* to examine the roles that netrin UNC-6 has in the cell and axon migrations of SDQR, a neuron positioned along the right lateral side of the midbody. We altered the expression levels of UNC-6 and the position of the UNC-6-expressing cells using *unc-6* null mutants and ectopic UNC-6 expression. The postembryonic migrations of the SDQR cell body and axon were then observed in larvae. We report that, while normally the migrations are away from ventral UNC-6-expressing cells, the cell body and axon will migrate towards a dorsal cell that ectopically expresses UNC-6 in the embryo and larva. This change indicates that the response of SDQR to UNC-6 is influenced by other factors. We show that the dorsal migrations require the UNC-5 receptor and are probably moderated by UNC-40. Finally, we show that the SDQR cell body and the axon migrates ventrally in the absence of UNC-6, indicating that there are ventral-directing guidance cues that can work independently of UNC-6. The response to these cues is partially mediated by UNC-40 but does not require UNC-5. To explain the cell and axon migrations of SDQR, we propose that these cues and UNC-6 are simultaneously interpreted by SDQR and that coincident guidance cues encountered by SDQR in vivo can elicit a response that is specific to the combination of cues.

MATERIALS AND METHODS

General methods

Preparation of plasmid DNA, restriction enzyme digestions, agarose gel electrophoresis of DNA and other molecular biology methods were performed by standard methods (Sambrook et al., 1989). *C. elegans* cultures were maintained by standard methods (Brenner, 1974). For microscopy of living animals, the animals were mounted on a slide in a small drop of M9 buffer on a 5% agar pad (Sulston and Hodgkin, 1988). The buffer was sometimes supplemented with 25% ethanol to anaesthetize the animals.

Expression constructs

Plasmid IM#171, an *unc-6* expression vector carrying the *mec-7* promoter, was constructed by subcloning the *mec-7* promoter region (containing one synthetic intron) from the expression vector pPD96.41 (kindly provided by A. Fire) immediately upstream of the predicted translational start site of *unc-6*. The fusion was generated by using the polymerase chain reaction (PCR) to introduce unique *Hin*dIII and *Eco*RI sites upstream and downstream, respectively, of the *mec-7* promoter sequence. A unique *Eco*RI site was introduced by PCR into the genomic *unc-6* containing plasmid IM#58 at the predicted start site. Finally, the *Hin*dIII-*Eco*RI fragment of plasmid IM#58 containing the *unc-6* promoter sequences was replaced with the *Hin*dIII-*Eco*RI *mec-7* promoter fragment. Plasmid IM#175, a *gfp* expression vector using the *unc-119* promoter, was constructed by ligating the DP#mm045 (provided by M. Maduro and D. Pilgrim) *Hin*dIII-*Pst*I fragment of *unc-119* promoter sequence into the *Pst*I-*Eco*RI sites of the *gfp* vector pPD95.77 (provided by A. Fire).

Transgenic animals

Transgenic strains were obtained by microinjecting *unc-6* expression and GFP reporter plasmid DNA (10 μg/ml) together with the plasmid pRF4 into the ovaries of *unc-6* (+) hermaphrodites (Fire, 1986; Mello et al., 1991; Mello and Fire, 1995). Plasmid pRF4 was used as a coinjection marker to identify transgenic animals, it carries the dominant allele *rol-6 (su1006)*, which causes a twist in the body wall. Progeny inheriting and expressing *rol-6 (su1006)* and *GFP* were identified by their rolling phenotype and GFP expression. The *unc-119::gfp* transgene enables most neurons and some structures in the head to be visualized by fluorescence (Maduro and Pilgrim, 1995). In some lines, slight fluorescence of the body wall muscles and excretory canal was observed. At least three independent strains were established for each expression construct. From these strains, which carry the constructs as an extrachromosomal array, an integrated transgene was obtained by γ-irradiation (Mello and Fire, 1995). The resulting integrated lines were out-crossed repeatedly to N2 wild type. The strains generated and reported in this paper are: IM19[*urIs13 [IM#175, pRF4]];* IM30[*urIs23 [IM#171, IM#175, pRF4]];* IM26[*urIs19 [IM#171, IM#175, pRF4]];* IM29[*urIs22 [IM#171, IM#175, pRF4]]*.

By standard genetic procedure, transgenes were crossed into the *unc-6(ev400)* background to create: IM39[*urIs13; unc-6(ev400)];* IM46[*urIs23; unc-6(ev400)]*. Transgenes were also crossed into different *unc-5* and *unc-40* backgrounds to create: IM55[*unc-40(e271); urIs13];* IM57[*unc-40(e271); urIs23];* IM62[*unc-40(e1430); urIs13];* IM64[*unc-40(e1430); urIs23];* IM65[*unc-5(e53); urIs13];* IM67[*unc-5(e53); urIs23];* IM106[*unc-40(e1430); urIs13; unc-6(ev400)]*. The transgenes were also crossed into the *mec-4(d)* background to create:

IM225[*urIs23 [IM#171, IM#175, pRF4]; unc-6(ev400), mec-4(u231)*]; IM227[*urIs13 [IM#175, pRF4]; unc-6(ev400), mec-4(u231)*] and into the *mec-3* background to create IM294[*urIs23 [IM#171, IM#175, pRF4]; mec-3(e1338),unc-6(ev400)*].

Individual cell and nerve positions were recorded in fourth larval stage and young adult animals by epifluorescence microscopy. The cell body positions include ALMR, AVM, BDUR, CAN and SDQR (Fig. 1). Images were obtained using a Zeiss LSM 410 Invert Laser Scan microscope. Multiple nerve tracts were imaged using an extended depth of field. Because of the cylindrical nature of the animals, distances between individual tracts are distorted in the confocal micrographs.

In situ hybridization

To confirm the ectopic expression of the transgenes, in situ hybridizations was used to detect *unc*-6 RNA. A protocol developed for detection of RNA in whole-mount *C. elegans* embryos was used (Seydoux and Fire, 1994, 1995). AP-anti-Dig antibody (Boehringer Mannheim) was used for alkaline phosphatase (AP)-mediated detection. 1 mg/ml of DAPI was included in the staining solution to allow nuclei to be identified by epifluorescence microscopy.

RESULTS

The cell and axon migrations of the SDQR neuron

To study in detail the in vivo responses of a neuron to guidance cues, we examined the cell and axon migrations of SDQR. This neuron is part of the sublateral nerves that develop from axons of embryonic neurons in the ring ganglia that run either anteriorly or posteriorly along the epidermis beneath the four body wall muscles. The posteriorly directed sublateral nerves, in particular, comprise the axons from neurons SIA, SIB and SMB in the ventral ganglion, plus SMD in the lateral ganglia. During the first larval stage, the axons of postembryonic neurons SDQ and PLN join the dorsal anterior and ventral posterior sublateral nerves, respectively. Shortly after its birth, the SDQR neuron migrates dorsally to the lateral margin of the body wall muscle alongside the ALMR associated nerve (Figs 1, 2A). Insinuating beneath the body wall muscle and epidermis, the SDQR axon grows dorsally and then anteriorly along the dorsal epidermis toward the nerve ring. SDQR has several advantages for study. First, the cell and axon are relatively isolated from surrounding neurons. Second, the cell is born in the first larval stage, after the final expression pattern of UNC-6 has occurred (Wadsworth et al., 1996). Third, the sister neuron of SDQR, AVM, migrates ventrally to a position that is slightly posterior to SDQR (Figs 1A, 2B). This neuron serves as an easily recognizable positional reference and as a control for SDQR precursor cell migration and cell lineage.

UNC-6 directs migrations dorsally, but other cues direct migrations ventrally in the absence of UNC-6

We first examined the SDQR cell and axon migrations in *unc-6* null larvae. We used an *unc-119::gfp* reporter which expresses green fluorescent protein (GFP) throughout the nervous system (Chalfie et al., 1994; Maduro and Pilgrim, 1995) to observe migrations. The integrated transgene itself causes no abnormalities of axonal outgrowth and guidance when homozygous. While, in wild-type larvae, the SDQR cell and axon migrate to dorsal positions, away from ventral UNC-6-expressing cells (Figs 2A, 3A), in 72% of the *unc-6* null larvae, the cell body migrates ventrally (*n*=200, Figs 2B, 3A) and, in 78% of these cases, the cell's axon migrates ventrally (*n*=143, Figs 2B, 3C). This bias towards ventral migrations contrasts with the wandering migration pattern of most axons in *unc-6* null larvae (Hedgecock et al., 1990) and it suggests that SDQR responds to ventral-directing guidance cues in the absence of UNC-6.

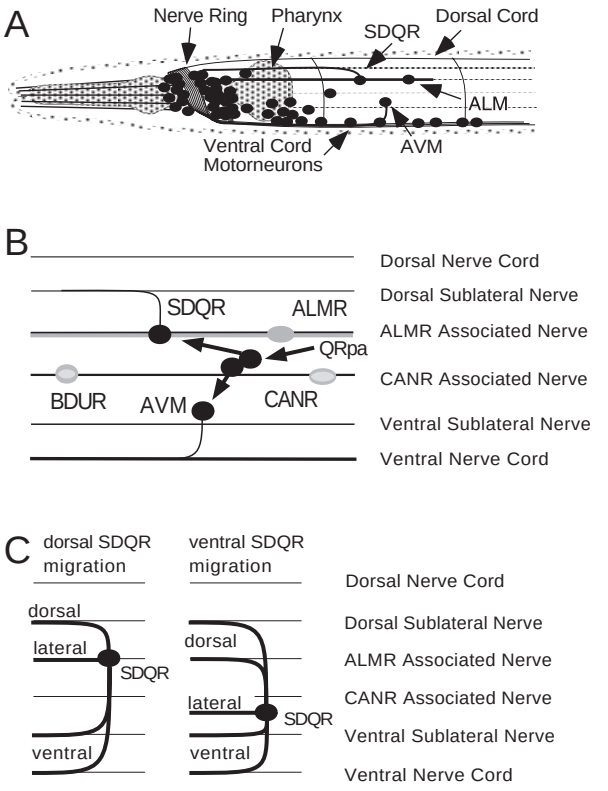

Fig. 1. Schematics of the *C. elegans* axon scaffold showing the positions of longitudinal nerves and neuron cell bodies along the right anterior body wall. (A) The relative positions of the SDQR and AVM neurons and the longitudinal nerves in the mature animal. (B) The SDQR cell body migration. Shortly after hatching, neuroblast QR undergoes an anterior migration, dividing to eventually give rise to QR.pa. The cell QR.pa divides to generate the two lateral neurons, SDQR and AVM. SDQR migrates dorsally and AVM ventrally to their final positions late in the first larval stage (Sulston and Horvitz, 1977). The SDQR axon then extends to the dorsal sublateral nerve and the AVM axon extends to the ventral nerve cord. (C) Summary of the SDQR cell and axon positions as observed in different genetic backgrounds. The SDQR cell migrates either dorsally to the dorsal sublateral nerve or ventrally below the CANR associated nerve. For each case, the dorsal, lateral or ventral trajectory of the axon is illustrated.

SDQR is directed towards a dorsal ectopic UNC-6 source in *unc-6* null larvae

The influence of UNC-6 expression on the SDQR cell and axon migrations was examined by observing the migrations in larvae where UNC-6 is ectopically expressed. To express ectopic netrin cues from a specific neuronal source, we fused the *unc-6*-coding region to upstream regulatory sequences from *mec-7*. The *mec-7* gene encodes a β-tubulin that is expressed in touch receptor neurons (Savage et al., 1989; Hamelin et al.,

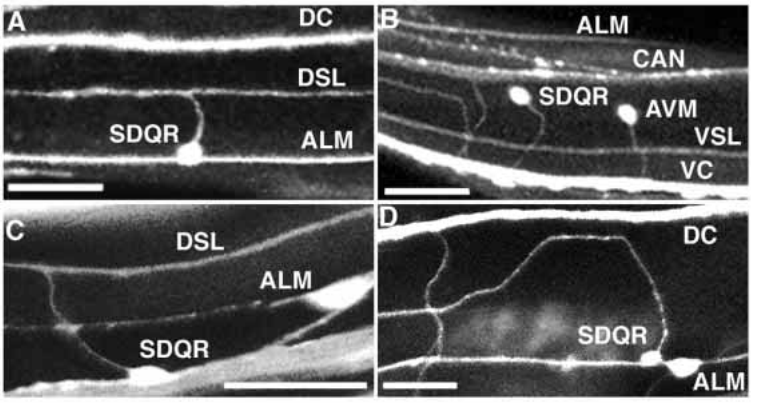

Fig. 2. Confocal micrographs of SDQR axon migrations in the larva. Anterior is shown to the left. Scale bars, 25 μm. The neurons were visualized by GFP expression. (A) SDQR axon migration in an *unc-6 (+)* animal. The axon migrates dorsally from the cell body at the ALM associated nerve to the dorsal sublateral nerve where it turns and migrates anteriorly. (B) SDQR axon migration in an *unc-6 (–)* animal. The SDQR cell body is ventrally mispositioned and the axon migrates to the ventral nerve cord. (C) SDQR axon migration in an *mec-7::unc-6* transgenic, *unc-6 (–)* animal. The SDQR cell body is ventrally mispositioned and the axon migrates across the ALM UNC-6 source to the dorsal sublateral nerve. (D) In the transgenic larvae, the dorsal sublateral nerve is sometimes absent. The SDQR axon always migrates to its normal dorsal sublateral position. Abbreviations: ALM, ALM associated nerve; CAN, CAN associated nerve; DC, dorsal nerve cord; DSL, dorsal sublateral nerve; VC, ventral nerve cord; VSL, ventral sublateral nerve.

1992). Neuronal ectopic expression was chosen because UNC-6 is normally expressed by some neurons, the temporal order of nervous system development is well characterized, and both the source and target are at the same interface, between the epidermis and epidermal basement membrane. For each experiment, we compared three independent chromosomal integrates, obtaining similar results. The *mec-7* regulatory sequence causes sustained, high-level gene expression in the embryonic ALM/PLM and postembryonic AVM/PVM mechanosensory neurons (Savage et al., 1989, 1994; Hamelin et al., 1992, 1993; Chalfie et al., 1994; Birchall et al., 1995; Chan et al., 1996). Using an *unc-6* cDNA probe for in situ hybridization, we confirmed these integrated transgenes express in the predicted patterns (data not shown). For all strains reported, the morphology of the neurons appears normal and the animals are sensitive to touch.

Although ALM and AVM are both in the vicinity of SDQR in the mature animal (Fig. 1), only ALM is born and expresses *mec-7* before SDQR migrations. The ALM neuron is born in the embryo and extends a pioneering axon during early formation of the axon scaffold (Durbin, 1987). Expression of *mec-7* in ALM occurs during axon outgrowth and it continues throughout development. Ectopic UNC-6 expression from ALM is effective; in *mec-7::unc-6* embryos, the migrations of nearby pioneer axons are disrupted (Fig. 2D, Ren et al., 1999). On the contrary, AVM is not born until the first larval stage and *mec-7* expression does not occur until axon outgrowth in subsequent developmental stages. This expression is too late to influence pioneering axons. AVM and SDQR are sister neurons, arising from an anterior-posterior division. While initially they are lateral to one another, both cells migrate so that SDQR becomes positioned anterior and dorsal to AVM (Fig. 1B). In *unc-6* null animals, when SDQR migrates ventrally (Fig. 1C; discussed below), the position of AVM remains posterior to SDQR. In this case, AVM can be slightly dorsal, slightly ventral or directly lateral to SDQR because the dorsoventral position of the ventrally mispositioned SDQR cell body varies between the CANR-associated nerve and the ventral sublateral nerve (Fig. 1C). Reflecting the timing of axon outgrowth, the MEC-7 β-tubulin is first detected in AVM at the second larval stage by MEC-7 antibody, *mec-7::gfp*, and *mec-7::lacZ* (Walthall and Chalfie, 1988; Hamelin et al., 1992; Mitani et al., 1993; Chalfie et al., 1994). Compared to ALM, *mec-7* expression by AVM is weaker and is in proportion to the fewer microtubules found in AVM (Chalfie and Thomson, 1979; Hamelin et al., 1992). UNC-6 expression driven by the *mec-7* promoter in AVM is too late to influence the SDQR cell migration. For the initial SDQR axon migration, AVM could be a source of UNC-6 although, when axon outgrowth begins, the SDQR cell body is positioned either at the ALM source of UNC-6 or is ventral to the ALM source and lateral to the AVM cell body.

We expressed the *mec-7::unc-6* transgene in *unc-6* null larvae to examine how the distribution of UNC-6 influences the migrations. We expected that, if UNC-6 always acts as a repulsive guidance cue for SDQR, then the migrations should be directed ventrally, away from the dorsal UNC-6-expressing ALM neuron. Moreover, if signals from the ventral-directing cues and ectopic UNC-6 collaborate with each other then we expect that the frequency of ventral migrations should be greater than in *unc-6* null larvae. We observe, however, an increase in the number of larvae with dorsal SDQR cell and axon migrations (Fig. 2C). Compared to 28% of *unc-6* null larvae, the SDQR cell body migrates dorsally in 50% of *unc-6* null larvae that express the *mec-7::unc-6* transgene (n=200 for each; P<0.00001, one-tailed Fishers Exact Test; Fig. 3A). Even when the cell migrates to the ventral position, the axon migrates dorsally towards the UNC-6 source in 20% of the larvae with the transgene, compared to 5% in *unc-6* null larvae (n=143 and 101, respectively; P<0.0006; Figs 2C, 3C). Finally, the dorsal migration of SDQR is not due to ectopic UNC-6 overexpression that obscures the pattern and directional information since, when the *mec-7::unc-6* transgene is expressed in the *unc-6(+)* background, the endogenous UNC-6 can be interpreted by SDQR to direct wild-type migrations (Fig. 3).

UNC-6 expression in the embryo, not in the larva, is required to guide SDQR migrations

How does the ectopic UNC-6 secreted by ALM establish a gradient that influences SDQR? As SDQR migrates, UNC-6 secreted by ALM into the flowing environment of the pseudocoelomic cavity would not be expected to form dorsoventral gradients. Rather, stable gradients must form by interactions with the basement membranes and cellular surfaces that SDQR will contact. In fact, in wild-type animals,

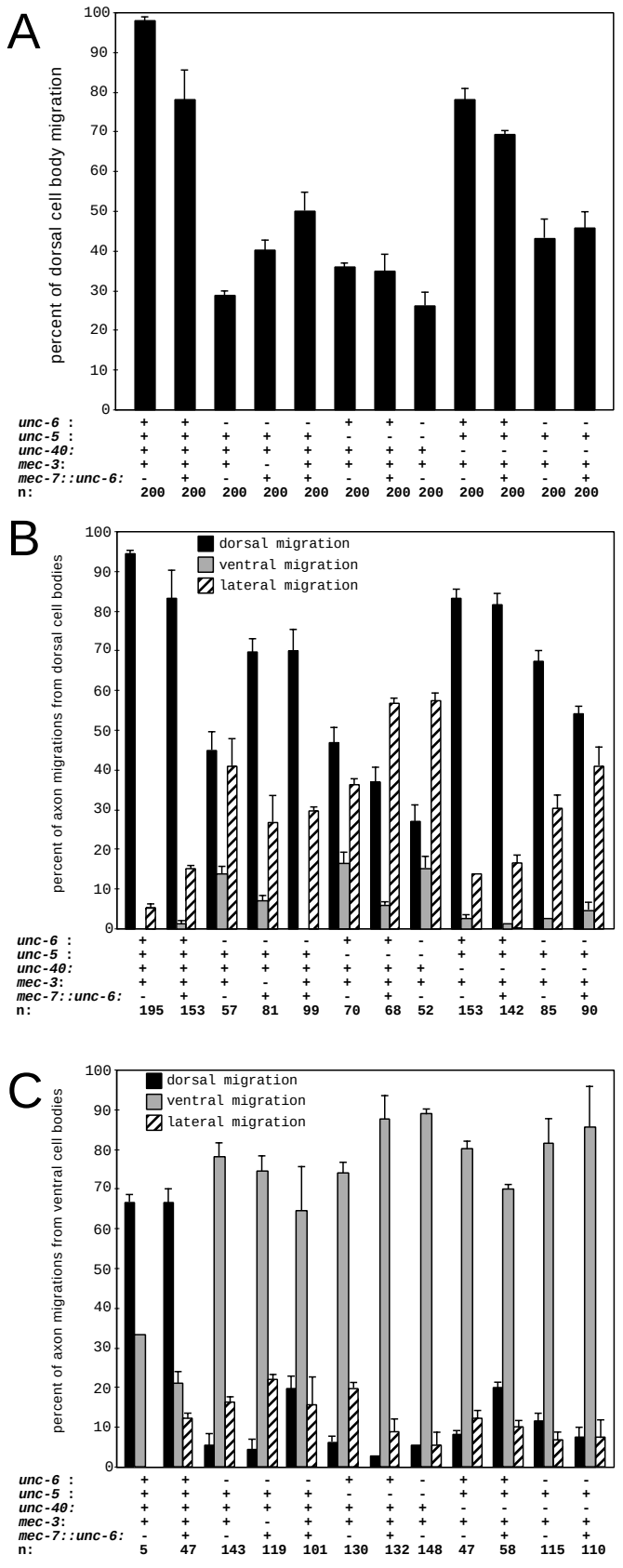

Fig. 3. Summary of the SDQR cell and axon migrations in different genetic backgrounds. The migrations are influenced in wild-type larvae by netrin UNC-6 that is expressed by ventral cells. In larvae expressing the *mec-7::unc-6* transgene, a UNC-6-expressing cell is dorsal to SDQR. Cell body positions and the direction of axon migrations in wild-type and mutant larvae are illustrated in Fig. 1. (A) Netrin UNC-6, UNC-5 and UNC-40-mediated dorsal cell migration. Genotypes: for *unc-5, unc-6, unc-40* and *mec-3* alleles; +, wild type; –, loss-of-function allele. For *mec-7::unc-6;* +, integrated transgene; –, no transgene. Mean ± s.e.m. The differences between larvae were compared by the one-tailed Fisher Exact Test and significant differences are reported in the text. (B) The migration pattern of axons from SDQR cell bodies that migrated dorsally. For each strain, the frequency of axons that migrate dorsally, laterally, or ventrally was scored. (C) The migration pattern of axon from SDQR cell bodies that migrated ventrally.

UNC-6 expression often anticipates migrations and is not necessarily concurrent to them, suggesting that UNC-6 expression with respect to basement membrane modeling and epidermal cell development is critical (Wadsworth and Hedgecock, 1992; Wadsworth et al., 1996). Because of these considerations, we postulate that embryonic expression of UNC-6 is required to guide SDQR migrations and that concurrent larval expression has little influence. To test this, we genetically ablated ALM in the late embryo. We took advantage of the induced degeneration of the mechanosensory neurons by dominant mutations of *mec-4* (*mec-4(d)*), a member of the degenerin gene family postulated to encode a subunit of a mechanotransducing channel (Chalfie and Au, 1989; Driscoll and Chalfie, 1991; Mitani et al., 1993; Lai et al., 1996). In *mec-4(d)* animals, cells develop and then degenerate. Degeneration is initiated shortly after *mec-4(d)* is expressed, although disrupted cells can persist for hours (Hall et al., 1997). In *unc-6(–), mec-4(d)* mutants that express the *mec-7::unc-6* transgene, ALM is missing in embryos by hatching, while AVM often, but not always, degenerates by the young adult stage. A sensitive marker of *mec-4(d)*-induced cell disruption is GFP fluorescence, which disappears early in a cell that undergoes the degenerative process. We scored SDQR migrations in young adults that had either no ALM or AVM neurons or had no ALM but normal AVM cell bodies and axons as measured by GFP fluorescence.

If early embryonic expression from ALM is sufficient to direct SDQR migrations dorsally in the *mec-7::unc-6* transgenic animals, then the removal of ALM late in the embryo may not have much affect on SDQR migrations. However, if concurrent expression from ALM is required to direct the SDQR migrations dorsally, then SDQR migration should be like that of *unc-6* null larvae when ALM is missing. We observe that there is no significant difference in the frequency of dorsal SDQR cell migrations when ALM has degenerated in the late embryo. Compared to 50% dorsal cell migrations in *unc-6* null larvae that express the *mec-7::unc-6* transgene, the SDQR cell body migrates dorsally in 57% of the larvae when ALM degenerates (n=200 for each; P<0.9, one-tailed Fishers Exact Test; Fig. 4A). Also, embryonic degeneration of ALM does not significantly change the frequency of dorsal SDQR axon migrations when the SDQR cell body migrates ventrally. Compared to 20% dorsal axon migrations in *unc-6* null larvae that express the *mec-7::unc-6* transgene, the SDQR axon migrates dorsally in 27% of the larvae without ALM (n=143 and 86, respectively; P<0.2; Fig. 4C). Similar results were obtained when animals with ablated AVM were scored. These results support the observations made

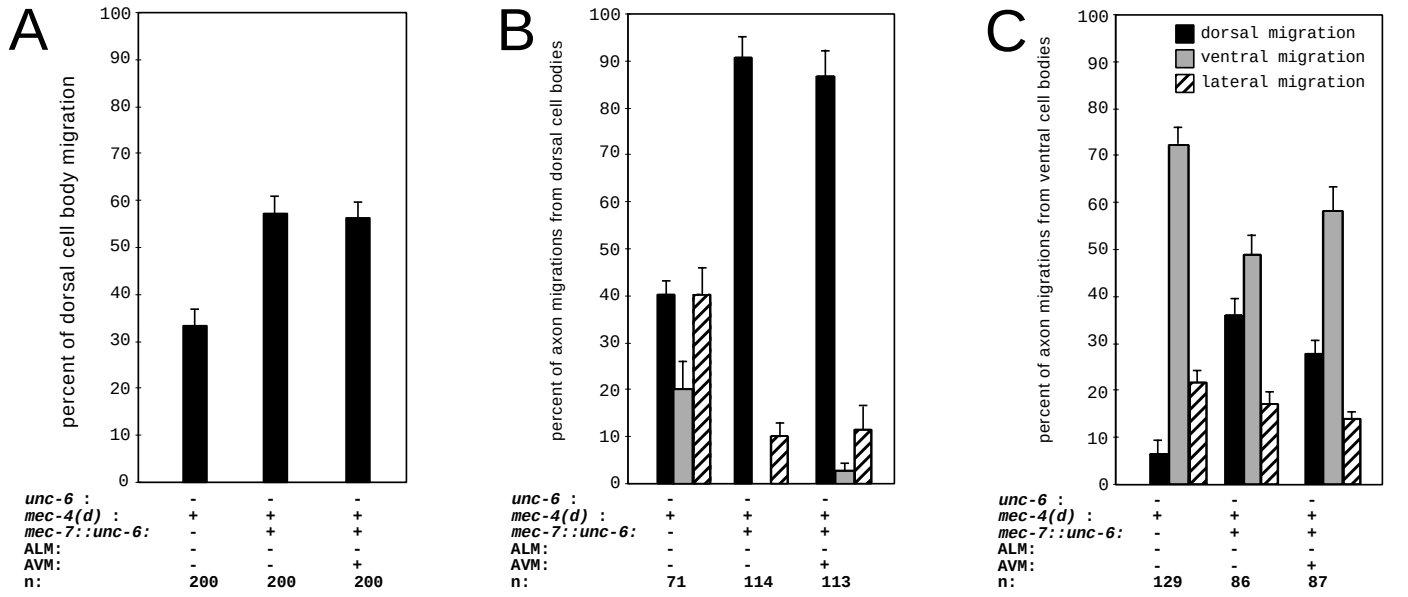

Fig. 4. Summary of the SDQR cell and axon migrations in *mec-4(d)* animals. The *mec-7::unc-6* transgene is expressed by ALM in the embryo and subsequent developmental stages and by AVM beginning in the L2 larval stage. In the *unc-6* null, *mec-4(d)* larvae that express the *mec-7::unc-6* transgene, ALM degenerates by hatching. AVM degenerate in late larval stages. (A) ALM is not required during the L1 stage for SDQR dorsal cell migrations. Embryonic netrin UNC-6 expression by ALM is sufficient to direct the SDQR cell migration. Genotypes: for *unc-6*; +, wild type; –, loss-of-function allele. For *mec-4(d)*; +, *mec-4(u231)* allele. For *mec-7::unc-6;* +, integrated transgene; –, no transgene. For ALM and AVM; +, positive fluorescence; –, no fluorescence. Mean ± s.e.m. (B) The migration pattern of axons from SDQR cell bodies that migrated dorsally. For each strain, the frequency of axons that migrate dorsally, laterally or ventrally was scored. (C) The migration pattern of axon from SDQR cell bodies that migrated ventrally. There are no significant differences in the frequency of SDQR migrations between *mec-4(d)* and *mec-4(+)* animals without the *mec-7::unc-6* transgene (Fig. 4).

in wild-type animals that indicate early UNC-6 expression influences later cell and axon migrations (Wadsworth et al., 1996).

The SDQR dorsal migrations are induced by *mec-7::unc-6* expression in *mec-3*-expressing cells

Current models predict that migrating axons follow concentration differences and require netrin gradients for directed outgrowth. If SDQR can only be repelled by UNC-6, then the dorsal SDQR migrations in the *mec-7::unc-6* animals require an UNC-6 gradient that peaks ventrally of ALM. The possible sources that could establish such a gradient before SDQR migrates are the ventral muscle cells, the ventral epidermal cells and ventral cord neurons. However, these cells are not known to express MEC-7, and we cannot detected any *mec-7::unc-6* transgene expression by these cells. Moreover, it is unlikely that undetectable levels of UNC-6 expression by these cells could direct SDQR dorsally since ALM is a major ectopic source and a competing ventral source would have to establish a steeper opposing gradient to drive SDQR towards and sometimes across ALM.

To experimentally test whether expression of the *mec-7::unc-6* transgene in touch cells is responsible for the dorsal migrations of SDQR, we scored SDQR migrations in *mec-3* mutant animals that have the *mec-7::unc-6* transgene. The *mec-3* gene encodes a LIM-type homeodomain protein required for the differentiation of the touch cells (Way and Chalfie, 1988; Duggan et al., 1998). The gene is expressed in the touch cells and the FLP and PVD cells. In *mec-3* mutants, the expression of *mec-7* is reduced (Hamelin et al., 1992; Mitani et al., 1993). For example, it has been reported that, in 45% of *mec-3(1338)* animals, there is no detectable staining of ALM using an anti-MEC-7 antibody and, in 55%, there is only weak staining (Mitani et al., 1993).

Reducing the expression level of the *mec-7::unc-6* transgene by *mec-3*, decreases the frequency of dorsal SDQR migrations. Compared to 50% dorsal cell migrations in *unc-6(–)* mutants that express the *mec-7::unc-6* transgene, the SDQR cell body migrates dorsally in 40% of the *mec-3(–), unc-6(–)* mutants that express the *mec-7::unc-6* transgene (n=200 for each; P<0.04; Fig. 3A). The cell migrates dorsally in 28% of *unc-6(–)* animals that do not have the *mec-7::unc-6* transgene. The *mec-3* mutation also reduces the frequency of dorsal SDQR axon migrations when the SDQR cell body migrates ventrally. Compared to 20% dorsal axon migrations in *unc-6(–)* mutants that express the *mec-7::unc-6* transgene, the SDQR axon migrates dorsally in 4% of the *mec-3(–), unc-6(–)* mutants that express the *mec-7::unc-6* transgene (n=101 and 119, respectively; P<0.0005; Fig. 3C). The axon migrates dorsally in 5% of *unc-6(–)* animals that do not have the *mec-7::unc-6* transgene. These results indicate that expression of the *mec-7::unc-6* transgene in *mec-3*-expressing cells induces the dorsal SDQR migrations in *mec-7::unc-6* animals. We conclude that the ALM touch receptor neuron is the source of UNC-6 responsible for directing the dorsal SDQR migrations.

UNC-5 is required for the dorsal, but not ventral migrations of SDQR

We determined the roles that the UNC-5 netrin receptor plays in SDQR migrations. The SDQR cell and axon migrations of

unc-5 null mutants, either with or without ectopic UNC-6 expression, are like those in *unc-6* null mutants (Fig. 3). Larvae with both the *unc-5* and *unc-6* mutations have the same SDQR migration phenotypes as larvae with either mutation alone. These results are consistent with other evidence suggesting that the UNC-5 receptor is required for the dorsal migrations of SDQR in response to ventral UNC-6 sources (Hedgecock et al., 1990; Hamelin et al., 1993). Furthermore, we have observed that the ectopic UNC-6 expression in *unc-5; unc-6* larvae causes severe abnormalities, most animals arrest during development and the rare adult hermaphrodites are sterile. In these animals, UNC-5 may be needed to prevent inappropriate responses by cells to ectopic UNC-6. Finally, ventral migrations are not affected in the *unc-5* larvae, suggesting that the response to ventral-directing guidance cues are not primarily mediated by UNC-5.

UNC-40 mediates SDQR ventral migrations

We next determined the roles that the UNC-40 netrin receptor plays in SDQR cell and axon migrations. Compared to wild type, the SDQR migrations in *unc-40* mutants are disrupted, however, the defects are less penetrant than those in *unc-6* or *unc-5* mutants. The cell body in 77% of the *unc-40* larvae migrates to the normal dorsal position, compared to 98% in wild type, 28% in *unc-6* null larvae and 35% in *unc-5* null larvae (n=200 for each; Fig. 3A). This intermediate phenotype suggests that the *unc-40* mutation may influence the cell migration by decreasing the response to one or more of the guidance cues. To address this, we examined the cell migrations in *unc-40; unc-6* double null mutants. If the *unc-40* mutation has no effect on the response to ventral-directing signals, the frequency of ventral migrations should be the same as in *unc-6* null mutants. We find, however, that more cell bodies migrate dorsally in these larvae (43%) than in *unc-6* null mutants (n=200 for each; P<0.002; Fig. 3A), suggesting that the *unc-40* mutation impairs the SDQR cell response to the ventrally directing cues.

The pattern of axon migrations further suggests that the influence of the ventral-directing cues is stronger towards the ventral midline. Comparing *unc-40; unc-6* larvae to *unc-6* larvae, the axon migrations vary at cell bodies that are dorsal, but not at cell bodies that are ventral. The axons from dorsally positioned cell bodies migrate dorsally more frequently in *unc-40; unc-6* larvae than in *unc-6* larvae (67% for *unc-40; unc-6* larvae, n=85, and 45% for *unc-6* null larvae, n=57; P<0.003; Fig. 3B), whereas axons from ventrally positioned cell bodies have similar migrations that are mostly ventral (81% for *unc-40; unc-6* larvae, n=115, and 78% for *unc-6* null larvae, n=143; P<0.3).

UNC-40 has been implicated as a component of a netrin receptor (Hedgecock et al., 1990; Chan et al., 1996; Keino-Masu et al., 1996; Kolodziej, 1996; Fazeli et al., 1997). Several experiments suggest that UNC-40 helps to mediate SDQR cell and axon responses to netrin UNC-6. First, the *unc-40* mutation disrupts the SDQR dorsal cell migration, a migration that requires UNC-6 (98% and 77% dorsal axon migration for wild-type and *unc-40* larvae, respectively; n=200 for each). Second, in contrast to ectopic UNC-6 expression in *unc-6* null mutants, ectopic expression in *unc-40; unc-6* mutants does not increase dorsal cell migrations (43% for *unc-40; unc-6* larvae and 45% for *unc-40; unc-6* larvae with ectopic UNC-6; n=200 for each; P<0.7; Fig. 3A) nor the frequency of axons from ventrally positioned cell bodies that migrate dorsally towards the ectopic UNC-6 source (12% for *unc-40; unc-6* larvae, n=115, and 7% for *unc-40; unc-6* larvae with ectopic UNC-6, n=110; P<0.9; Fig. 3C). These experiments, however, are not conclusive because of the possibility that the SDQR migration patterns are also influenced simultaneous by UNC-40-mediated responses to the ventral-directing cues. Currently, we cannot remove the influence of these cues. Finally, the *unc-40* larvae reveal a difference between the cell body and axon responses to the guidance cues. Whereas ectopic UNC-6 expression did not influence the dorsal cell migration (77% for *unc-40* larvae and 71% for *unc-40* larvae with ectopic UNC-6; n=200 for each; P<0.9; Fig. 3A), it did cause more axons from ventrally positioned cell bodies to migrate dorsally (8%, n=47, for *unc-40* larvae and 20%, n=58, for *unc-40* larvae with ectopic UNC-6; P<0.03; Fig. 3C).

The role of the embryonic dorsal sublateral nerve in SDQR pathfinding

As the SDQR axon migrates dorsally, selective fasciculation along the dorsal sublateral tract could be one means by which the SDQR axon finds the correct dorsoventral position to turn anteriorly. We observed that, in a few larvae (<8%), the embryonic dorsal sublateral tract is deflected from the region where SDQR joins the tract. However, the dorsally migrating SDQR axon always finds the correct sublateral position (Fig. 2D), indicating that whether the SDQR axon adopts the sublateral tract is independent of whether embryonic axons are present in that tract. Furthermore, the SDQR axon was never observed to migrate to the dorsal nerve cord. These results suggest that cues, not associated with the nerve, are involved in dorsoventrally positioning the SDQR axon.

The AVM cell

The sister of SDQR, the AVM neuron, migrates ventrally and extends a process to the ventral cord (Figs 1A, 2B). In the mutant and transgenic lines, AVM migrations are distinct from that of SDQR and we conclude that the neurons retain different responses to the guidance cues. First, the anteroposterior position of AVM is always posterior to SDQR, suggesting specific responses to anteroposterior cues. Second, AVM axon migrations are only rarely abnormal. In all the larvae scored, the AVM axon migrated ventrally in 96%, laterally in 4% and never dorsally. The ectopic UNC-6 expression had no effect on AVM migrations. Finally, ventrally migrating SDQR axons often turn anteriorly at the ventral sublateral nerve whereas the AVM axons nearly always migrate to the ventral nerve cord.

DISCUSSION

The combined actions of attractive and repulsive guidance cues are thought to direct particular guidance decisions (Goodman, 1996; Tessier-Lavigne and Goodman, 1996). A genetic analysis using *Drosophila* has shown that by manipulating the expression of genes encoding Netrin A, Netrin B, Semaphorin II and Fasciclin II, the ability of motor axons to select their muscle targets can be altered (Winberg et al., 1998). The results indicate that the signals elicited by these molecules are simultaneously assessed by the growth cones and that they

work in combination to amplify or antagonize one another. Current models of circumferential migration in *C. elegans*, put simply, predict that cells or axons expressing *unc-5* together with *unc-40* are repelled from UNC-6 sources, and axons expressing *unc-40* (and perhaps an as yet unidentified co-receptor) are attracted towards UNC-6 sources. It is thought that during migrations axons turn and migrate along pre-existing longitudinal tracts in response to selective fasciculation cues that cause stronger responses than those to UNC-6. In our study, we used genetics to examine the ability of UNC-6-expressing cells to attract or repulse the cell and axon migrations of SDQR. We have found that, like the motor axons in *Drosophila*, the SDQR migrations are simultaneously influenced by several guidance cues. Furthermore, our results suggest that the response to UNC-6 is regulated by the combination of cues SDQR encounters. Finally, we show that UNC-5 and UNC-40 help mediate the SDQR responses and that other receptors must also be involved.

A model for the migrations of SDQR

From the migrational patterns of SDQR when different gene functions are altered, we can infer how some molecular guidance cues help to guide SDQR migrations. The observation that SDQR migrates dorsally, away from UNC-6 sources, in wild-type larvae, but migrates ventrally in *unc-6* null larvae, indicates that UNC-6 helps to repel SDQR from the ventral midline and that SDQR can respond to other cues that will direct it ventrally in the absence of UNC-6. The activity of the ventral-directing cues may normally be masked by the stronger repellent activity of UNC-6. From these observations, it would be predicted that when UNC-6 is ectopically expressed dorsal to SDQR, the repellent activity would collaborate with the ventral-directing cues to direct SDQR migrations ventrally. In fact, we find more of the migrations are dorsal, towards the UNC-6 source. To explain these results, we propose that the signals elicited by UNC-6 and other guidance cues are integrated and interpreted by SDQR and that the response to UNC-6 is regulated by the combination of cues.

At the dorsal sublateral position, cues could cause the SDQR response to UNC-6 to be inhibited or modified from repulsive to attractive (Fig. 5). This is consistent with reports that axon responsiveness to netrin can be modified by extrinsic factors (Ming et al., 1997; Shirasaki et al., 1998). The longitudinal nerves are positioned at the centers or margins of the epidermis and muscle cells. Molecules at the surface of these cells, working in concert with guidance cues arranged in gradients, could molecularly define different dorsoventral positions (Ren et al., 1999). In this model, migrating axons adopt different dorsoventral positions depending on which combination of cues they interact with. For example, both SDQR and ventral motoneuron axons migrate dorsally in response to UNC-6. While motoneuron axons are repelled by UNC-6 to the dorsal midline, SDQR interacts with a second cue at the dorsal sublateral position that modifies its response to UNC-6 and prevents further dorsal migration. The affect on SDQR migrations at this position would be the same whether the source of UNC-6 was a ventral cell or was the ALM neuron. Possibly, some SDQR cells and growth cones explore dorsally in the *mec-7::unc-6* animals and are affected by dorsal sublateral cues.

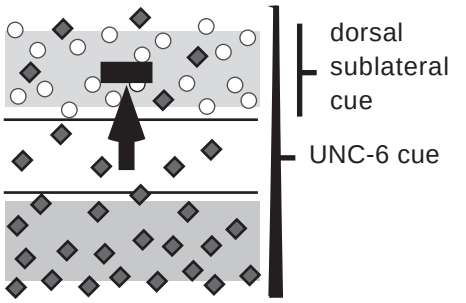

Fig. 5. Model for cues that dorsoventrally position SDQR. The UNC-6 guidance cue (filled squares) is distributed in a ventral to dorsal gradient along the body wall. A second cue(s) (open circles) is localized to the dorsal sublateral region. During the initial SDQR cell and axon migration (arrow), receptor complexes on SDQR mediate a repulsive response to UNC-6 that guides SDQR dorsally. SDQR interaction with the dorsal sublateral cue causes the response to UNC-6 to be modified so that SDQR is no longer repelled (horizontal bar). Within this zone, the SDQR axon migrates anteriorly. A third cue can attract UNC-6 ventrally only in the absence of UNC-6. Epidermal cells and muscle cells could be sources of localized cues. The borders of the underlying ventral, lateral, and dorsal epidermal cells are depicted by horizontal lines and the positions of the overlying muscle cells are shaded.

Genetic screens for mutations that disrupt nerve patterning have uncovered a gene that is required for positioning the dorsal sublateral nerve but does not affect circumferential migrations per se (S. K. and W. G. W., unpublished data). In the null mutant, the SDQR axon migrates to the dorsal midline in response to UNC-6, a phenotype that is never observed in *unc-6* mutants or in animals that ectopically express UNC-6. This phenotype is consistent with a loss of the ability to modify the response to UNC-6 at the dorsal sublateral position.

SDQR migrations are controlled by multiple signaling pathways

Two families of netrin receptors have been identified, DCC/UNC-40 and UNC-5. In culture, an antibody to DCC selectively blocks the outgrowth of rat commissural axons (Keino-Masu et al., 1996). Furthermore, turning of *Xenopus* growth cones in response to netrin-1 can be blocked by antibodies to DCC (de la Torre et al., 1997; Ming et al., 1997). This suggests that cell and axon responses to netrin UNC-6 in *C. elegans* are mediated by UNC-40. Since UNC-40 is involved in both dorsal and ventral migration, whereas the UNC-5 receptor is required only for dorsal migrations, and since guidance mediated by ectopic UNC-5 requires *unc-40*, repulsion from the ventral UNC-6 source may be controlled by UNC-5 working in association with UNC-40, perhaps as heteromeric receptor complex (Hedgecock et al., 1990; Leonardo et al., 1997; Colavita and Culotti, 1998). Our result would be consistent with the idea that UNC-40 regulates the strength of the UNC-5-mediated signal. Furthermore, our results suggest that UNC-5 may control some of the activities of UNC-40. While expression of the *mec-7::unc-6* transgene in other single and double mutant backgrounds is tolerated, expression in *unc-5(–); unc-6(–)* larvae causes embryonic and early larval lethality. It is possible that, without UNC-5, ectopically expressed UNC-6 causes inappropriate UNC-40-mediated signals that are deleterious to the animals. These

inappropriate signals are eliminated in *unc-5(+)* and *unc-40(–)* larvae. Our results further indicate that UNC-5 is not involved in mediating the response to cues that guides SDQR ventrally in the absence of UNC-6, while UNC-40 does play a role. Together our results provide evidence that through multiple receptors different guidance cues are simultaneously interpreted by SDQR in vivo and they suggest that the signaling pathways that direct a response are interwoven so that the response to UNC-6 is regulated by the combination of guidance cues that is encountered by SDQR.

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REFERENCES

Birchall, P. S., Fishpool, R. M. and Albertson, D. G. (1995). Expression patterns of predicted genes from the *C. elegans* genome sequence visualized by FISH in whole organisms. *Nat. Genet.* **11**, 314-320.

Brenner, S. (1974). The genetics of Caenorhabditis elegans. *Genetics* **77**, 71-94.

Chalfie, M. and Au, M. (1989). Genetic control of differentiation of the Caenorhabditis elegans touch receptor neurons. *Science* **243**, 1027-1033.

Chalfie, M. and Thomson, J. N. (1979). Organization of neuronal microtubules in the nematode Caenorhabditis elegans. *J. Cell Biol.* **82**, 278-289.

Chalfie, M., Tu, Y., Euskirchen, G., Ward, W. W. and Prasher, D. C. (1994). Green fluorescent protein as a marker for gene expression. *Science* **263**, 802-805.

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.

Colamarino, S. A. and Tessier-Lavigne, M. (1995a). The axonal chemoattractant Netrin-1 is also a chemorepellent for trochlear motor axons. *Cell* **81**, 621-629.

Colamarino, S. A. and Tessier-Lavigne, M. (1995b). The role of the floor plate in axon guidance. *Annu. Rev. Neurosci.* **18**, 497-529.

Colavita, A. and Culotti, J. G. (1998). Suppressors of ectopic UNC-5 growth cone steering identify eight genes involved in axon guidance in Caenorhabditis elegans. *Dev. Biol.* **194**, 72-85.

de la Torre, J. R., Hopker, V. H., Ming, G. L., Poo, M. M., Tessier-Lavigne, M., Hemmati-Brivanlou, A. and Holt, C. E. (1997). Turning of retinal growth cones in a netrin-1 gradient mediated by the netrin receptor DCC. *Neuron* **19**, 1211-1224.

Deiner, M. S., Kennedy, T. E., Fazeli, A., Serafini, T., Tessier-Lavigne, M. and Sretavan, D. W. (1997). Netrin-1 and DCC mediate axon guidance locally at the optic disc: loss of function leads to optic nerve hypoplasia. *Neuron* **19**, 575-589.

Driscoll, M. and Chalfie, M. (1991). The mec-4 gene is a member of a family of Caenorhabditis elegans genes that can mutate to induce neuronal degeneration [see comments]. *Nature* **349**, 588-593.

Duggan, A., Ma, C. and Chalfie, M. (1998). Regulation of touch receptor differentiation by the *Caenorhabditis elegans mec-3* and *unc-86* genes. *Development* **125**, 4107-4119.

Durbin, R. (1987). Studies on the development and organisation of the nervous system of Caenorhabditis elegans. *PhD Thesis, Univ. of Cambridge, England*.

Fazeli, A., Dickinson, S. L., Hermiston, M. L., Tighe, R. V., Steen, R. G., Small, C. G., Stoeckli, E. T., Keino-Masu, K., Masu, M., Rayburn, H. et al. (1997). Phenotype of mice lacking functional Deleted in colorectal cancer (Dcc) gene. *Nature* **386**, 796-804.

Fire, A. (1986). Integrative transformation of *Caenorhabditis elegans*. *EMBO J.* **5**, 2673-2680.

Goodman, C. S. (1996). Mechanisms and molecules that control growth cone guidance. *Annu. Rev. Neurosci.* **19**, 341-377.

Hall, D. H., Gu, G., Garcia-Anoveros, J., Gong, L., Chalfie, M. and Driscoll, M. (1997). Neuropathology of degenerative cell death in Caenorhabditis elegans. *J. Neurosci.* **17**, 1033-1045.

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

Hamelin, M., Zhou, Y., Su, M.-W., Scott, I. M. and Culotti, J. G. (1993). Expression of the UNC-5 guidance receptor in the touch neurons of *C. elegans* steers their axons dorsally. *Nature* **364**, 327-330.

Harris, R., Sabatelli, L. M. and Seeger, M. A. (1996). Guidance cues at the Drosophila CNS midline: identification and characterization of two Drosophila Netrin/UNC-6 homologs. *Neuron* **17**, 217-228.

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.

Ishii, N., Wadsworth, W. G., Stern, B. D., Culotti, J. G. and Hedgecock, E. M. (1992). UNC-6, a laminin-related protein, guides cell and pioneer axon migrations in C. elegans. *Neuron* **9**, 873-881.

Keino-Masu, K., Masu, M., Hinck, L., Leonardo, E. D., Chan, S. S.-Y., Culotti, J. G. and Tessier-lavigne, M. (1996). *Deleted in Colorectal Cancer* (DCC) encodes a netrin receptor. *Cell* **87**, 175-185.

Kennedy, T. E., Serafini, T., de la Torre, J. and Tessier-Lavigne, M. (1994). Netrins are diffusible chemotropic factors for commissural axons in the embryonic spinal cord. *Cell* **78**, 425-435.

Keynes, R. and Cook, G. M. W. (1995). Axon guidance molecules. *Cell* **83**, 161-169.

Kolodziej, P. A. (1996). *frazzled* encodes a Drosophila member of the DCC immunoglobulin subfamily and is required for CNS and motor axon guidance. *Cell* **87**, 197-204.

Lai, C. C., Hong, K., Kinnell, M., Chalfie, M. and Driscoll, M. (1996). Sequence and transmembrane topology of MEC-4, an ion channel subunit required for mechanotransduction in Caenorhabditis elegans. *J. Cell Biol.* **133**, 1071-1081.

Lauderdale, J. D., Davis, N. M. and Kuwada, J. Y. (1997). Axon tracts correlate with netrin-1a expression in the zebrafish embryo. *Mol. Cell. Neurosci.* **9**, 293-313.

Leonardo, E. D., Hinck, L., Masu, M., Keino-Masu, K., Ackerman, S. L. and Tessier-Lavigne, M. (1997). Vertebrate homolgues of *C. elegans* UNC-5 are candidate netrin receptors. *Nature* **386**, 833-838.

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 type1 domains, guides cell and pioneer axon migrations in *C. elegans*. *Cell* **71**, 289-299.

Livesey, F. J. and Hunt, S. P. (1997). Netrin and netrin receptor expression in the embryonic mammalian nervous system suggests roles in retinal, striatal, nigral, and cerebellar development. *Mol. Cell. Neurosci.* **8**, 417-429.

MacLennan, A. J., McLaurin, D. L., Marks, L., Vinson, E. N., Pfeifer, M., Szulc, S. V., Heaton, M. B. and Lee, N. (1997). Immunohistochemical localization of netrin-1 in the embryonic chick nervous system. *J. Neurosci.* **17**, 5466-5479.

Maduro, M. and Pilgrim, D. (1995). Identification and cloning of *unc-119*, a gene expressed in the *Caenorhabditis elegans* nervous system. *Genetics* **141**, 977-988.

Mello, C. and Fire, A. (1995). DNA Transformation. In *Caenorhabditis elegans: Modern Biological Analysis of an Organism*, vol. 48 (ed. Epstein, H. F. and Shakes, D. C.), pp. 451-482. San Diego: Academic Press.

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.

Metin, C., Deleglise, D., Serafini, T., Kennedy, T. E. and Tessier-Lavigne, M. (1997). A role for netrin-1 in the guidance of cortical efferents. *Development* **124**, 5063-5074.

Ming, G. L., Song, H. J., Berninger, B., Holt, C. E., Tessier-Lavigne, M. and Poo, M. M. (1997). cAMP-dependent growth cone guidance by netrin-1. *Neuron* **19**, 1225-1235.

Mitani, S., Du, H., Hall, D. H., Driscoll, M. and Chalfie, M. (1993). Combinatorial control of touch receptor neuron expression in Caenorhabditis elegans. *Development* **119**, 773-783.

Mitchell, K. J., Doyle, J. L., Serafini, T., Kennedy, T. E., Tessier-Lavigne, M., Goodman, C. S. and Dickson, B. J. (1996). Genetic analysis of *Netrin*

genes in Drosophila: netrins guide CNS commissural axons and peripheral motor axons. *Neuron* **17**, 203-215.

Ren, X. C., Kim, S., Fox, E., Hedgecock, E. M. and Wadsworth, W. G. (1999). Role of netrin UNC-6 in patterning the longitudinal nerves of Caenorhabditis elegans. *J. Neurobiol.* **39**, 107-118.

Richards, L. J., Koester, S. E., Tuttle, R. and O'Leary, D. D. M. (1997). Directed Growth of Early Cortical Axons Is Influenced by a Chemoattractant Released from an Intermediate Target. *J. Neurosci.* **17**, 2445-2458.

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

Savage, C., Hamelin, M., Culotti, J. G., Coulson, A., Albertson, D. G. and Chalfie, M. (1989). *mec-7* is a beta-tubulin gene required for the production of the 15-protofilament microtubules in *Caenorhabditis elegans*. *Genes Dev.* **3**, 870-881.

Savage, C., Xue, Y. Z., Mitani, S., Hall, D., Zakhary, R. and Chalfie, M. (1994). Mutations in the *Caenorhabditis elegans* β-tubulin gene *mec-7*: effects on microtubule assembly and stability and on tubulin autoregulation. *J. Cell Sci.* **107**, 2165-2175.

Serafini, T., Colamarino, S. A., Leonardo, E. D., Wang, H., Beddington, R., Sharnes, W. C. and Tessier-Lavigne, M. (1996). Netrin-1 is required for commissural axon guidance in the developing vertebrate nervous system. *Cell* **87**, 1001-1014.

Serafini, T., Kennedy, T. E., Galko, M. J., Mirzayan, C., Jessell, T. M. and Tessier-Lavigne, M. (1994). The netrins define a family of axon outgrowth-promoting proteins homologous to C. elegans UNC-6. *Cell* **78**, 409-424.

Seydoux, G. and Fire, A. (1994). Soma-germline asymmetry in the distribution of embryonic RNAs in *Caenorhabditis elegans*. *Development* **120**, 2823-2834.

Seydoux, G. and Fire, A. (1995). Whole-mount in situ hybridization for the detection of RNA in *C. elegans* embryos. In *C. elegans: Modern Biological analysis of an Organism*, (ed. Epstein, H. and Shakes, D.), pp. 323-337. San Diego: Academic Press.

Shirasaki, R., Katsumata, R. and Murakami, F. (1998). Change in chemoattractant responsiveness of developing axons at an intermediate target. *Science* **279**, 105-107.

Strahle, U., Fischer, N. and Blader, P. (1997). Expression and regulation of a netrin homologue in the zebrafish embryo. *Mech. Dev.* **62**, 147-160.

Sulston, J. and Hodgkin, J. (1988). Methods. In *The nematode Caenorhabditis elegans*, (ed. Wood, W. B.), pp. 587-606. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Sulston, J. and Horvitz, R. (1977). Postembryonic cell lineages of the nematode *Caenorhabditis elegans*. *Dev. Biol.* **56**, 110-156.

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

Wadsworth, W. G., Bhatt, H. and Hedgecock, E. M. (1996). Neuroglia and pioneer neurons express UNC-6 to provide global and local netrin cues for guiding migrations in C. elegans. *Neuron* **16**, 35-46.

Wadsworth, W. G. and Hedgecock, E. M. (1992). Guidance of neuroblast migrations and axonal projections in *Caenorhabditis elegans*. *Curr. Opin. Neurobiol.* **2**, 36-41.

Wadsworth, W. G. and Hedgecock, E. M. (1996). Hierarchical guidance cues in the developing nervous system of C. elegans. *BioEssays* **18**, 355-362.

Walthall, W. W. and Chalfie, M. (1988). Cell-cell interactions in the guidance of late-developing neurons in Caenorhabditis elegans. *Science* **239**, 643-645.

Way, J. C. and Chalfie, M. (1988). mec-3, a homeobox-containing gene that specifies differentiation of the touch receptor neurons in C. elegans. *Cell* **54**, 5-16.

Winberg, M. L., Mitchell, K. J. and Goodman, C. S. (1998). Genetic analysis of the mechanisms controlling target selection: complementary and combinatorial functions of netrins, semaphorins, and IgCAMs. *Cell* **93**, 581-591.