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Suppressors of Ectopic UNC-5 Growth Cone Steering Identify Eight Genes Involved in Axon Guidance in *Caenorhabditis elegans*

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The UNC-5 guidance receptor, in response to the UNC-6/netrin path cue, orients growing axons in a dorsal direction along the epidermis of *Caenorhabditis elegans.* When ectopically expressed in the touch neurons, which normally extend ventrally or longitudinally, UNC-5 is able to reorient their axons toward the dorsal side in an UNC-6-dependent manner. This forms the basis of a genetic screen to identify other mutations that, like *unc-6* mutations, suppress *unc-5*-induced growth cone guidance. These mutations may identify new components required for pioneer axon guidance by *unc-5.* In this paper, we describe eight genes that are required for ectopic *unc-5*-induced growth cone steering. Mutations in four of these identify the previously known axon guidance genes *unc-6, unc-40, unc-34,* and *unc-44* and mutations in four others identify the novel genes *unc-129, seu-1, seu-2,* and *seu-3.* Several of these mutations cause axon guidance defects similar to those found in *unc-5* mutants. We propose that some or all of these genes may function in a developmentally important *unc-5* signaling pathway.

INTRODUCTION

Axons are able to navigate long distances through a complex extracellular environment to reach their proper synaptic targets. Elucidating the molecular mechanisms underlying this process has become a major focus of developmental neurobiology. Recently, several mechanisms have been described that involve both attractive and repulsive cues to guide axonal growth cones to target tissues (reviewed in Tessier-Lavigne and Goodman, 1996). We have taken a genetic approach to further our understanding of these processes by identifying new components involved in cell and growth cone migrations in the nematode *Caenorhabditis elegans.*

Three genes, *unc-5, unc-6,* and *unc-40,* are required for migrations of axon growth cones and motile cells along the dorsoventral axis of *C. elegans* (Hedgecock *et al.,* 1990). UNC-6 is a secreted laminin-related molecule expressed in neuroglia and neurons along the ventral midline (Ishii *et al.,* 1992; Wadsworth *et al.,* 1996). UNC-5 and UNC-40 are cell surface receptors that act cell autonomously to orient migrating cells in response to polarity information encoded by the UNC-6 cue (Leung-Hagesteijn *et al.,* 1992; Chan *et al.,* 1996). Genetic studies indicate that dorsal migrations require UNC-5, while ventral migrations and, to a lesser extent, dorsal migrations require UNC-40 (Hedgecock *et al.,* 1990). However, the signaling mechanisms that cells use to integrate guidance cue information and regulate cytoskeletal organization and motor activities required for directed outgrowth are poorly understood.

Vertebrate homologues of UNC-5, UNC-6, and UNC-40 have been identified, and their functions appear to be conserved. UNC-6 homologues, designated netrins, are diffusible molecules that can act as either attractive or repulsive guidance cues for different populations of neurons (Serafini *et al.,* 1994; Kennedy *et al.,* 1994; Colamarino *et al.,* 1995). UNC5H1, UNC5H2, and the product of the murine rostral cerebellar malformation (rcm) gene are homologues of UNC-5 (Leonardo *et al.,* 1997; Ackerman *et al.,* 1997) and the products of the deleted in colorectal cancer (DCC) gene and neogenin are homologues of UNC-40 (Keino-Masu *et al.,* 1996; Fazeli *et al.,* 1997). These proteins have been implicated as candidate netrin receptors (Keino-Masu *et al.,* 1996; Leonardo *et al.,* 1997).

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C. elegans unc-5 is able to induce dorsally directed axon outgrowth when ectopically expressed in the touch-sensitive mechanosensory neurons which normally extend along longitudinal or ventral trajectories (Hamelin *et al.*, 1993). This dorsal reorientation requires UNC-6 and is therefore consistent with a mechanism in which UNC-5 expression in the touch receptors makes their growth cones responsive to UNC-6 in the same way that motorneurons that normally express UNC-5 are responsive to UNC-6. We have used the ability of UNC-5 to steer growth cones as the basis for a suppressor screen to identify additional genes involved in *unc-5*-mediated guidance. In this paper, we describe mutations in eight genes, including *unc-6*, that are suppressors of ectopic UNC-5 function in the touch neurons. We propose that several of these genes encode previously unknown components of a developmentally important mechanism of UNC-5 receptor function and signaling.

MATERIALS AND METHODS

General Techniques and Strains

General techniques for the culture and handling of worms have been described (Brenner, 1974). The *C. elegans* Bristol (N2) stock was used as the wild-type strain. The phenotypes of mutations used in this study are Bli (*bli*ster), Dpy (*dumpy*), Fem (*fem*inization), Lin (*lin*eage abnormal), Mes (*m*aternal *e*ffect *s*terile), Pag (*pa*ttern of reporter *g*ene expression), Seu (suppressor of *e*ctopic *unc-5*), and Unc (*unc*oordinated). The mutations and rearrangements used were as follows: Linkage group I (LG I), *dpy-5(e61), unc-40(e1430)*; LG II, *dpy-10(e128);* LG III, *dpy-17(e164), pag-1(ls2);* LG IV, *dpy-13(e184), unc-5(e53), fem-1(e1991), unc-44(e362), bli-6(sc16), unc-24(e138), mes-6(bn66), fem-3(e2006), dpy-20(e1282), unc-22(s12), stDf8, eDf18;* LG V, *unc-34(e566), unc-60(m35), lin-40(e2173), unc-62(e644), unc-46(e177), dpy-11(e224), sDf34, nDf32;* and LG X, *unc-6(ev400).*

Mutant strains that were not derived in our laboratory were provided by A. Spence (University of Toronto), E. Aamodt (Louisiana State University), or the *Caenorhabditis* Genetics Center. Unless stated otherwise, worms were handled and maintained at 20°C.

Construction of Transgenic Lines

Standard germ-line transformation techniques were used as described by Mello and Fire (1995). *mec-7::unc-5* transgenic worms were generated by injecting a mixture of 100 μg/ml *mec-7::unc-5* plasmid DNA (Hamelin *et al.*, 1993), 50 μg/ml *mec-7::lacZ* plasmid DNA (Hamelin *et al.*, 1992), and 20 μg/ml of plasmid pMH86 containing the wild-type *dpy-20* gene (Han and Sternberg, 1991) into the distal gonad arms of *dpy-20* hermaphrodites. *evIs41* and *evIs68* were independently derived by integrating an extrachromosomal array carrying these constructs at random genomic sites by irradiation with approximately 4000 rads from a ^{137}Cs source. *mec-7::lacZ* (*evIs55*) worms were created in the same way except *mec-7::unc-5* plasmid DNA was not included in the injection mixture. *evIs41, evIs68,* and *evIs55* were passed into a *pag-1(ls2)* background by mating to establish lines with increased levels of *lacZ* expression (Xie *et al.*, 1995). *unc-6(ev400)* was also passed into the *evIs41;pag-1(ls2)* background by mating.

Identification of Suppressors of Ectopic UNC-5

evIs41;pag-1(ls2) worms were mutagenized with 50 mM ethyl methanesulfonate (EMS) as described by Brenner (1974). Upon overnight recovery, subpopulations of 25–50 worms were transferred to 100-mm-diameter seeded NGM plates and allowed to lay eggs overnight. A semisynchronized population of F_1 eggs was established by washing off the mutagenized P_o adults and newly hatched worms with M9 buffer. After 2 to 3 days, approximately 200 F_1 adults were transferred to each of approximately 20 large (100-mm-diameter) seeded NGM plates per screen and allowed to lay eggs. After 3 days, approximately 1000–2000 gravid F_2 worms were collected from each plate and stained for *lacZ* activity. Animals were fixed, permeabilized, and stained with X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) using a technique described by Xie *et al.* (1995) that allows for the histochemical staining of gravid worms without killing the majority of their eggs.

Suppressors of *unc-5*-induced growth cone guidance were identified by visual inspection of touch neurons using a Leitz Wild M3B microscope under 40× magnification and diffuse illumination. Worms in which at least two of the three anterior touch neurons projected longitudinally were identified as putative suppressors. To facilitate visualization of the *lacZ*-stained touch neurons and recovery of suppressors, stained worms were first transferred to 60-mm-diameter tissue culture plates prepared with a thin pad of 1% agarose on their bottom surface and partially filled with water. Once identified, an animal carrying a putative suppressor was picked using fine forceps and transferred to a 35-mm-diameter seeded NGM plate to establish a line from the living embryos trapped in its uterus. Each line was subsequently restained for *lacZ* to confirm the presence of a suppressor. To ensure that mutations were of independent origin, only one suppressor was retained from each subpopulation of mutagenized animals.

A suppressor was identified if at least 70% of the anterior touch neurons did not project in a dorsal direction. The strongest suppressor from each complementation group (see below) was scored for the penetrance of suppression of ectopic *unc-5*-growth cone steering. Populations of 400–500 worms were fixed and stained for *lacZ* as described, mounted on 1% agarose pads on glass slides, and observed using bright-field optics with a Leitz DMRXE microscope. The axonal processes of the anterior touch neurons, ALML/R and AVM, were scored as dorsalized if they displayed a dorsally oriented trajectory and entered the dorsal cord.

Genetic Mapping and Complementation Tests

Determination of LG, complementation tests, and mapping by three-factor crosses were performed using standard methods (Brenner, 1974).

Those suppressors that caused an obvious Unc phenotype were assigned to linkage groups by following the segregation of the uncoordinated defect in crosses with the reference mutants *dpy-5* (LG I), *dpy-10* (II), *dpy-17* (III), *dpy-13* (IV), and *dpy-11* (V). Suppressors were assigned to LG X if the phenotype was observed in hemizygous F_1 males. With the exception of *unc-129*, complementation groups for these mutants were readily assigned by complementation testing with canonical alleles of known genes on the same LG that cause similar phenotypes.

Other suppressors were assigned to LG by scoring segregation of the suppressor of *e*ctopic *unc-5* (Seu) phenotype by staining recombinants from heterozygotes of genotype *+seu/dpy+ Is41;pag-1*, where *dpy* is one of the tester mutations listed above. If the *seu*

mutation is linked to *dpy*, Dpy Seu recombinants should be difficult to recover. The segregation of the Seu phenotypes among the progeny of the above heterozygotes is consistent with recessive or loss-of-function mutations (data not shown).

The suppressors *ev520*, *ev529*, and *ev572* were designated alleles of *seu-1* based on failure to complement each other for a mild uncoordinated movement defect and were subsequently found linked to LG IV. Mapping was carried out by identifying and staining recombinants among progeny of genotype *+seu+/a + b;evIs41;pag-1*, where *a* and *b* are tester mutations listed above. *seu-2* was mapped in a similar manner.

Mapping of *seu-3* on LG V was complicated by the presence of the *evIs41* array, which also mapped to the left arm of LG V. To map *seu-3* in the *unc-60* to *lin-40* interval, *seu-3 +++ evIs41/+ unc-60 lin-40 dpy-11 +;pag-1/+* animals were produced by crossing homozygous *seu-3 evIs41;pag-1* males to *unc-60 lin-40 dpy-11/eT1*, and Unc non-Lin non-Dpy recombinants were identified among the F_2. Those recombinants that did not carry *evIs41*, as determined by *lacZ* staining, were mated to *evIs68;pag-1* homozygous males and the progeny of the resulting heterozygous worms tested for the presence of the Seu phenotype by staining for *lacZ*. *seu-3* was mapped in the *unc-34 dpy-11* interval in the same manner, with the exception that mating to *evIs68;pag-1* was not necessary as all recombinants carried the *evIs41* array.

Complementation Tests Using Deficiencies

unc-129 and *seu* mutants were placed *in trans* to deficiencies once their map positions had been determined by three-factor mapping. Heterozygous *unc-129(ev554) dpy-20/++* males were mated with *eDf18/unc-24 dpy-20* hermaphrodites and the resulting *unc-129 dpy-20/eDf18* animals identified by noncomplementation for the *unc-129* defect and subsequent segregation of the expected marker mutations among their self-progeny. Homozygous *seu-1(ev520)* males were crossed to *fem-1 unc-24 unc-22/stDf8* hermaphrodites and *seu-1/stDf8* animals identified by their failure to segregate Fem Unc animals. *seu-1/stDf8* animals were also compared to control *+/stDf8* animals generated by genetic crosses involving N2 instead of *seu-1* males. *seu-2* and *seu-3* were placed over deficiencies *nDf32* and *sDf34*, respectively, in the same way.

Construction of Worms Transgenic for Neuronal lacZ and GFP Reporters

Extrachromosomal arrays containing neuronal *lacZ* and green fluorescent protein (GFP) reporters were generated by standard germ-line transformation techniques described in Mello and Fire (1995). The *unc-5::lacZ* transgene was used to visualize the axonal trajectories of the DD and VD classes of motorneurons. *unc-129 dpy20* and *dpy-20* hermaphrodites were coinjected with 100 μg/ml of the *unc-5::lacZ* reporter plasmid pYZ129 (M. W. Su, Y. Zhou, and J. G. Culotti, unpublished results) and 31 μg/ml of pMH86 [*dpy-20(+)*]. *seu-1*, *seu-2*, and *seu-3* hermaphrodites were each transformed with a mixture containing 100 μg/ml of pYZ129 and 33 μg/ml of plasmid pRF4 containing the dominant cotransformation marker *rol-6(su1006)* (Kramer *et al.*, 1990). Transgenic animals were fixed and stained for *lacZ* as described in Xie *et al.* (1995).

Worms transgenic for the soluble GFP transcriptional reporter pAC12 (A. Colavita and J. G. Culotti, submitted for publication) were generated to visualize the axonal processes of the DA and DB classes of motorneurons. An extrachromosomal array containing the pAC12 reporter was generated by coinjecting 60 μg/ml of pAC12 and 20 μg/ml of pMH86 into the distal gonads of *dpy-20* hermaphrodites. This array was randomly integrated into the genome by gamma irradiation to generate two independent lines designated *evIs82A* and *evIs82B* (A. Colavita and J. G. Culotti, unpublished results). N2, *seu-1(ev520)*, *seu-2*, and *seu-3* worms were coinjected with 60 μg/ml of pAC12 and 44 μg/ml of pRF4 to generate stable transformed lines that express the pAC12 GFP reporter from extrachromosomal arrays. In all cases, the *seu* mutants were backcrossed to *evIs41;pag-1(ls2)* worms at least twice before establishing homozygous lines.

Construction of Double Mutants

Standard methods were used to construct *unc6(ev400);-unc-129(ev554)* and *unc-5(e53);-unc-129(ev554)* double mutants. Complementation tests were used to verify their genotypes.

Scoring Axon Guidance Defects

Integrated arrays containing pAC12 were crossed into lines carrying suppressors of ectopic *unc-5* that cause an Unc phenotype. These mutants had been back-crossed to wild-type males at least twice. The progeny of seven to eight transgenic worms were immobilized using 10 mM levamisole and transferred to a 1% agarose pad on a glass slide. GFP was visualized using epifluorescence. DA and DB axon morphologies were examined in 30 randomly chosen late larval and adult hermaphrodites. A representative subset of DA and DB motorneurons consisting of DA3, DA4, DA5, and DA6 of the DA class and DB4, DB5, DB6, and DB7 of the DB class were scored. The data from these neurons were subsequently pooled. Motorneurons were scored as having an outgrowth defect if a cell body was clearly visualized but the axon could not be seen. Motorneurons were scored as having an axon guidance defect if an axon failed to reach the dorsal cord and instead extended longitudinally along the lateral epidermis.

RESULTS

Identification of Eight Genes Required for unc-5-Induced Dorsal Steering of the Touch Neurons

Ectopic expression of *unc-5* in the touch neurons is sufficient to steer their ventrally or longitudinally directed axons in a dorsal direction (Fig. 1) (Hamelin *et al.*, 1993). The integrated array, *evIs41*, contains multiple copies of a *mec-7::unc-5* construct and a *mec-7::lacZ* reporter. In animals carrying *evIs41* and *pag-1(ls2)* (a mutation that increases expression from the *mec-7* promoter (Xie *et al.*, 1995)), 86% (n = 296) of the anterior touch neurons, ALML/R and AVM, have dorsally directed axons. In contrast, when *unc-6(ev400)*, a null mutation, was introduced into this genetic background, 0% (n = 100) of the anterior touch neuron axons extended in a dorsal direction.

The ability of *unc-5* to steer touch receptor axons dorsally provides the basis of a genetic screen to identify additional components of the *unc-5*-mediated dorsal guidance pathway by isolating mutations that, like *unc-6(ev400)*, disrupt ec-

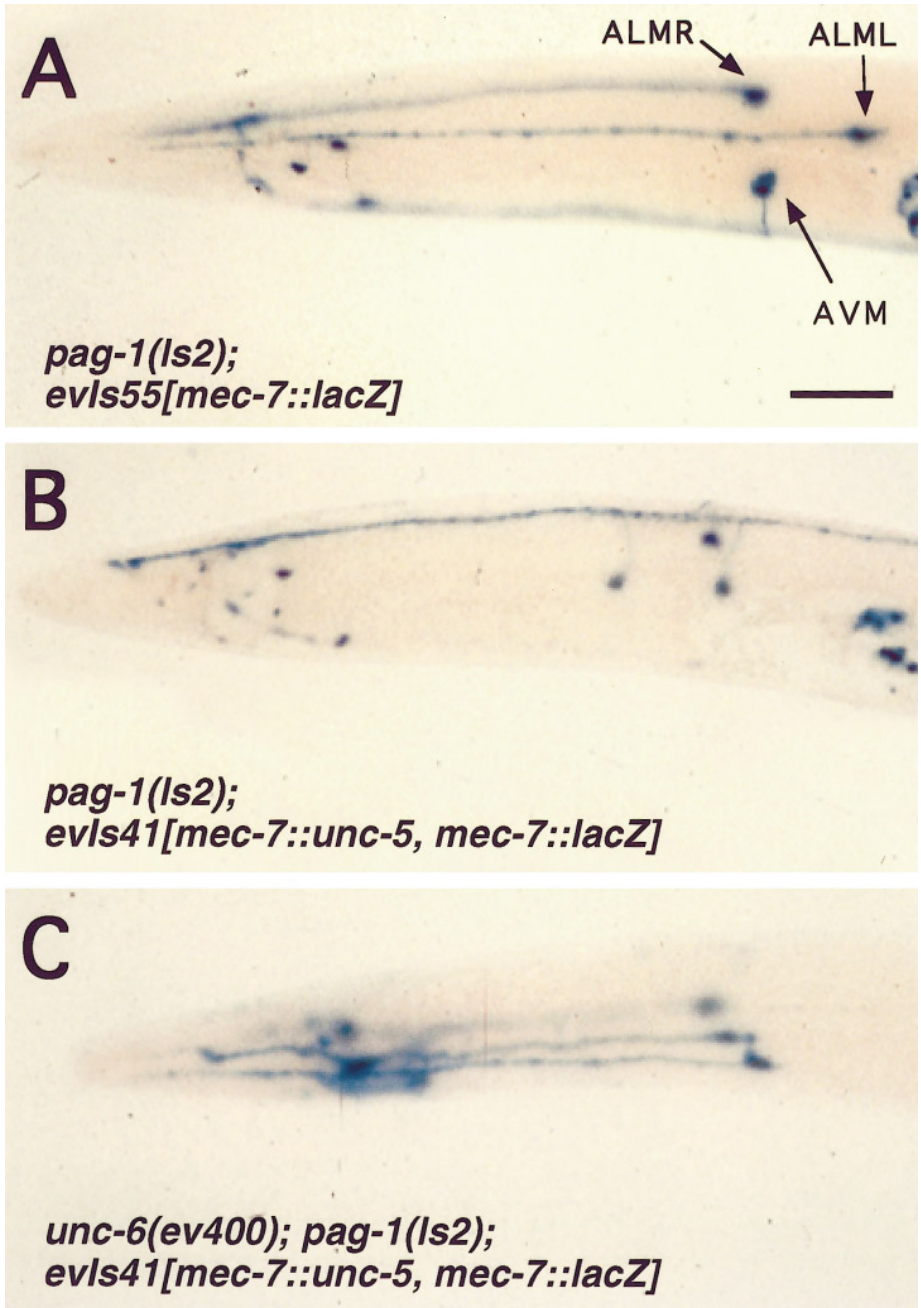

FIG. 1. Ectopic expression of *unc-5* in the touch receptor neurons steers their growth cones dorsally. Touch neurons are visualized in adult hermaphrodites by expression of *lacZ* from a *mec-7::lacZ* reporter gene. A *pag-1(ls2)* mutant background is used as defects in *pag-1* cause increased expression from the *mec-7* promoter. Anterior is to the left and dorsal is toward the top of each panel. (A) *pag-1(ls2)* mutant transgenic for a *mec-7::lacZ*-containing array *(evIs55)* showing ALML/R and AVM touch neurons. (B) *pag-1(ls2)* mutant transgenic for a *mec-7::lacZ* and *mec-7::unc-5*-containing array *(evIs41)* showing dorsally directed ALML/R and AVM touch neurons. (C) *unc-6(ev400)* suppresses the guidance defects caused by ectopic *unc-5*. Scale bar, 50 μm.

topic *unc-5* function in the touch neurons (Fig. 2). The F_2 progeny of EMS-mutagenized *evIs41;pag-1* worms were stained for *lacZ* to visualize the touch neurons and a visual screen was performed to identify worms with normal touch neuron trajectories.

In a screen of approximately 30,000–40,000 mutagenized

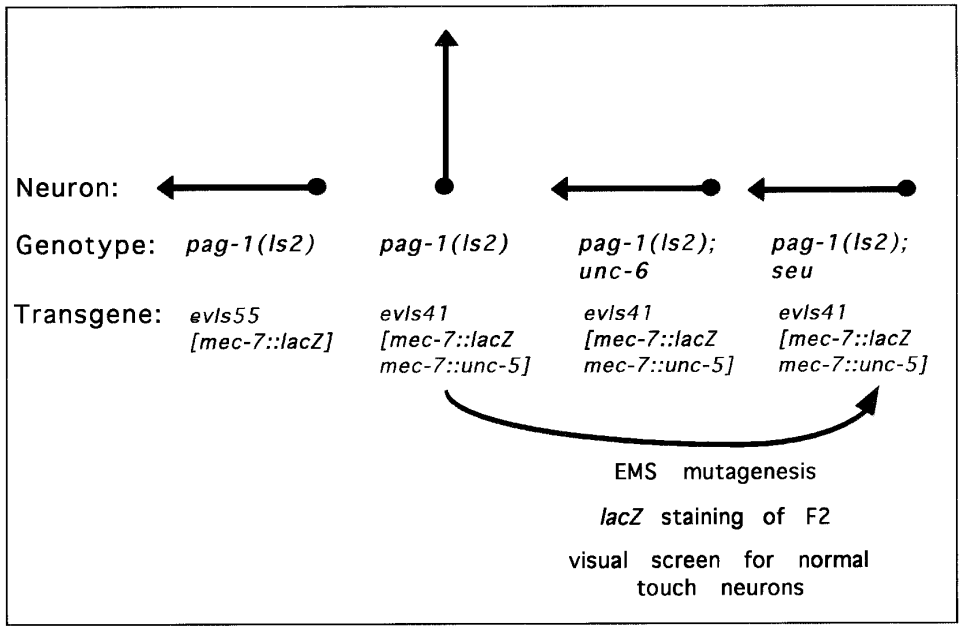

FIG. 2. Schematic outline of the genetic screen used to identify suppressors of ectopic *unc-5*. Worms in which the touch neurons express *unc-5* and extend axons in a dorsal direction are mutagenized with EMS. The F_2 progeny are then stained for *lacZ* using a technique that does not kill eggs, and a visual screen is performed to identify worms with normal touch neuron trajectories. The candidate suppressor is isolated and the viable eggs trapped *in utero* are used to establish a mutant line.

genomes, we identified 33 mutations in eight genes that suppressed the guidance defects induced by ectopic *unc-5* expression in the touch neurons (Table 1). Two of these genes, *unc-6* and *unc-40*, like *unc-5*, are known to play a key role in axon guidance and cell migrations along the dorsoventral axis (Hedgecock *et al.*, 1987, 1990). Mutations in two other genes, *unc-34* and *unc-44*, are known to cause axon and cell migration defects along both the anterior–posterior and dorsoventral axes, but have not previously been shown to be required for *unc-5* function (Siddiqui and Culotti, 1991; McIntire *et al.*, 1992; Forrester and Garriga, 1997). Finally, we have identified alleles of four new genes,

TABLE 1

Suppressors of *unc-5* Induced Reorientation of the Touch Neurons

Strain background[a]	Suppressor	Alleles[b]	% Anterior touch neurons with normal trajectories	n[c]
evIs55; pag-1	None	—	100	100
evIs41; pag-1	None	—	14	296
evIs41; pag-1	*unc-6*	*ev517, ev521, ev540, ev551, ev552, ev556,* ***ev558,*** *ev563, ev565, ev567, ev568, ev569*	99	144
evIs41; pag-1	*unc-40*	*ev541,* ***ev542,*** *ev543, ev544, ev545, ev546, ev547*	91	338
evIs41; pag-1	*unc-34*	***ev553,*** *ev561, ev562, ev564*	72	348
evIs41; pag-1	*unc-44*	***ev570,*** *ev571*	85	335
evIs41; pag-1	*unc-129*	***ev554,*** *ev566, ev557*	97	335
evIs41; pag-1	*seu-1*	***ev520,*** *ev529, ev572*	94	340
evIs41; pag-1	*seu-2*	***ev523***	72	358
evIs41; pag-1	*seu-3*	***ev555***	82	366

[a] All suppressors are in a genetic background containing *evIs41[mec-7::unc-5 mec-7::lacZ dpy-20(+)]* and *pag-1(ls2).* The *evIs55* array contains *[mec-7::lacZ dpy-20(+)].*

[b] Alleles that were scored for suppression of ectopic *unc-5* function are indicated in bold.

[c] Total number of axons scored includes only the anterior touch neurons ALML/R and AVM.

TABLE 2

Three-Factor Map Data for *unc-129* and the *seu* Genes

Suppressor[a]	Parental genotype	Recombinant phenotype[b]	Genotype of chromosome	No. of recombinants
unc-129	*unc-24 dpy-20/unc-129*	Dpy non-Unc	+ *unc-129 dpy-20*	15/20
			+ + *dpy-20*	5/20
	unc-24 unc-129/mes-6	Unc-129 non-Unc-24	+ *mes-6 unc-129*	6/24
			+ + *unc-129*	18/24
	unc-24 fem-3/unc-129	Fem non-Unc	+ *unc-129 fem-3*	1/4
			+ + *fem-3*	3/4
seu-1	*unc-44 bli-6/seu-1 (ev520)*	Bli non-Unc	+ *seu-1 bli-6*	2/35
			+ + *bli-6*	33/35
	bli-6 unc-24/seu-1(ev520)	Bli non-Unc	+ *seu-1 bli-6*	0/30
			+ + *bli-6*	30/30
	unc-44 bli-6/seu-1(ev572)	Bli non-Unc	+ *seu-1 bli-6*	0/5
			+ + *bli-6*	5/5
	bli-6 unc-24/seu-1(ev572)	Bli non-Unc	+ *seu-1 bli-6*	0/9
			+ + *bli-6*	9/9
	dpy-13 unc-24/seu-1(ev520)	Unc non-Dpy	+ *seu-1 unc-24*	1/6
			+ + *unc-24*	5/6
	dpy-13 unc-24/seu-1(ev529)	Unc non-Dpy	+ *seu-1 unc-24*	1/6
			+ + *unc-24*	5/6
seu-2	*unc-46 dpy-11/seu-2*	Dpy non-Unc	+ *seu-2 dpy-11*	22/26
			+ + *dpy-11*	4/26
seu-3	*unc-62 dpy-11/seu-3*	Dpy non-Unc	+ + *dpy-11*	0/37
			seu-3 + *dpy-11*	37/37
	lin-40 dpy-11/seu-3	Dpy non-Lin	+ + *dpy-11*	0/19
			seu-3 + *dpy-11*	19/19
	unc-60 lin-40 dpy-11/seu-3	Unc non-Lin non-Dpy	*unc-60* + + +	0/10
			unc-60 seu-3 + +	0/10
	unc-34 dpy-11/seu-3	Dpy non-Unc	*seu-3* + *dpy-11*	16/17
			+ + *dpy-11*	1/17

[a] *unc-129* was mapped based on its Unc phenotype. The *seu* mutants were mapped based on direct visualization of touch neuron morphology by *lacZ* staining.

[b] *seu* genes were mapped in a genetic background containing the *evIs41[mec-7::unc-5 mec-7::lacZ dpy-20(+)]* transgene and *pag-1* (see Materials and Methods).

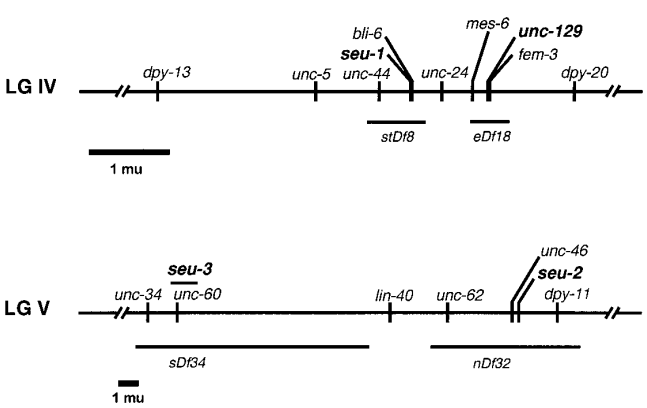

FIG. 3. Genetic map positions of the genes identified in this study and the markers and deficiencies used for mapping. New genes are highlighted in bold. The map position of *seu-3* is approximated by a bar since its position relative to *unc-60* is not known. Bar below each map represents 1 map unit (mu).

unc-129, *seu-1*, *seu-2*, and *seu-3* (suppressor of *e*ctopic *unc-5*), that potentially define new components of an *unc-5* signaling pathway. Genetic and phenotypic characterization during outcrossing and mapping indicated that all of the mutations are recessive, consistent with a loss of gene function.

Twelve new alleles of *unc-6* represent the single largest group of suppressors recovered in the screen. These mutations result in nearly complete suppression (99%) of ectopic *unc-5* function (Table 1). Since positional information encoded by *unc-6* is required for *unc-5*-induced dorsal guidance (Hamelin *et al.*, 1993), mutations in *unc-6* were expected if the screen were working as designed to identify components of the *unc-5* guidance pathway. An unexpected result was the observation that *unc-6* is haplo-insufficient in its ability to mediate dorsal guidance of the touch neurons by ectopic *unc-5*. Three *unc-6* loss-of-function alleles, *ev517*, *ev568*, and *ev569*, with phenotypes less severe than an *unc-6* null mutant (data not shown), were identified as dominant suppressors in this screen. In contrast, the visible

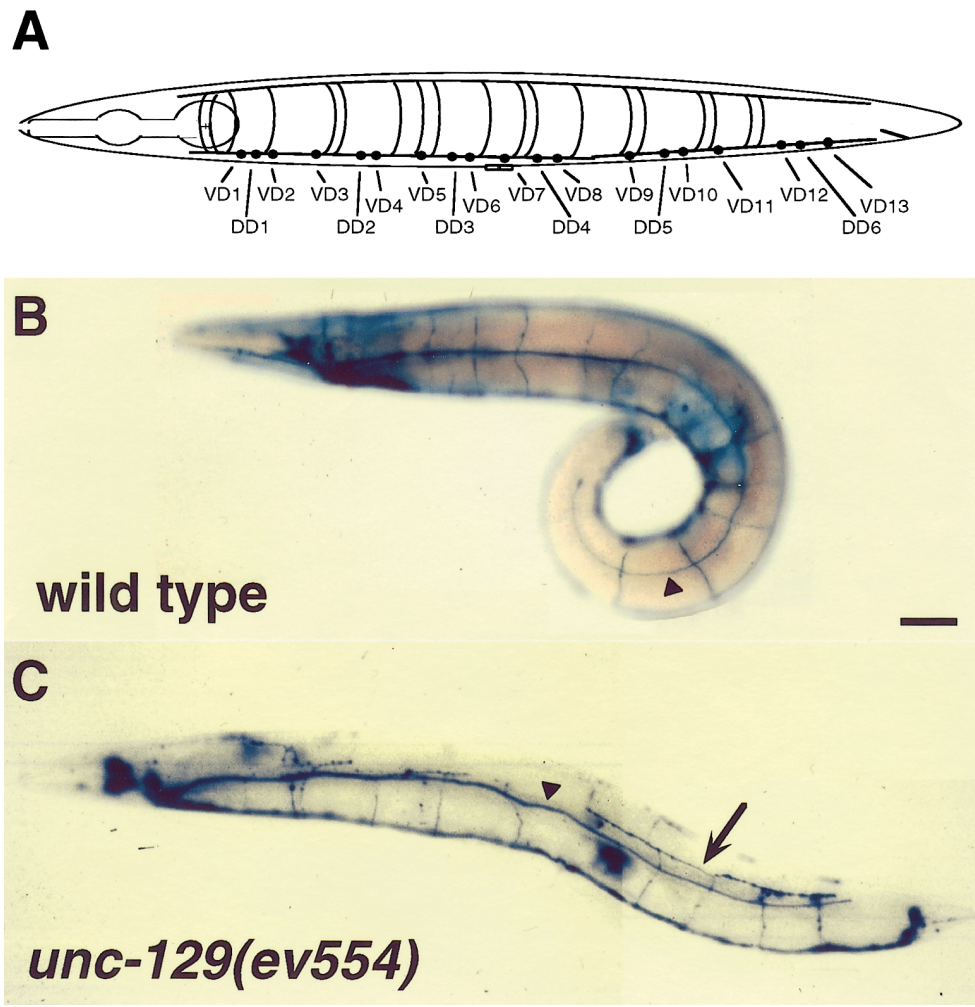

FIG. 4. DD and VD axon morphology in wild-type and *unc-129(ev554)* hermaphrodites. Axons are visualized using *lacZ* expression from an *unc-5::lacZ* transgene. Anterior is to the left and dorsal is toward the top of each panel. (A) Schematic drawing of the DD and VD classes of motorneurons in an adult hermaphrodite. (B) DD and VD axon morphology in a wild-type adult hermaphrodite. (C) DD and VD axons in an *unc-129(ev554)* adult hermaphrodite deviate from their normal trajectories to extend longitudinally at lateral axial positions (arrow). Arrowheads indicate the excretory cell process. Scale bar, 50 μm.

phenotypic defects present in *unc-6* mutants, uncoordination, egg-laying defects, and distal tip cell migration defects, are completely recessive (Hedgecock *et al.*, 1990). These results indicate that *evIs41;pag-1* represents a sensitized background from which to recover components of the *unc-5* signaling pathway. Dorsally directed touch neurons may be more sensitive to the dose of UNC-6 as their cell bodies are located along the lateral body wall where they are exposed to lower levels of UNC-6, which is most concentrated along the ventral midline (Wadsworth *et al.*, 1996).

We identified seven mutations in *unc-40*, several of which are among the weakest so far identified for axon and cell migration defects when compared to previously characterized alleles (Hedgecock *et al.*, 1990; D. Merz and J. G. Culotti, unpublished results). *unc-40(ev542)*, one of the most severe uncoordinated *unc-40* mutations isolated in this screen, is 91% (n = 338) effective at suppressing *unc-5*-induced dorsal reorientation of the touch neurons (Table 1).

Suppression of ectopic *unc-5*-induced dorsal reorientation varied from nearly complete suppression in *unc-6* (99%) and *unc-129* (97%) mutants to less severe effects in *unc-34* (72%) and *seu-2* (72%) mutants (Table 1). This variation in the penetrance of suppression may be due to identification of partial loss of function instead of null mutations at some loci, redundant mechanisms where another gene or signaling pathway may partially compensate, or a combination of both.

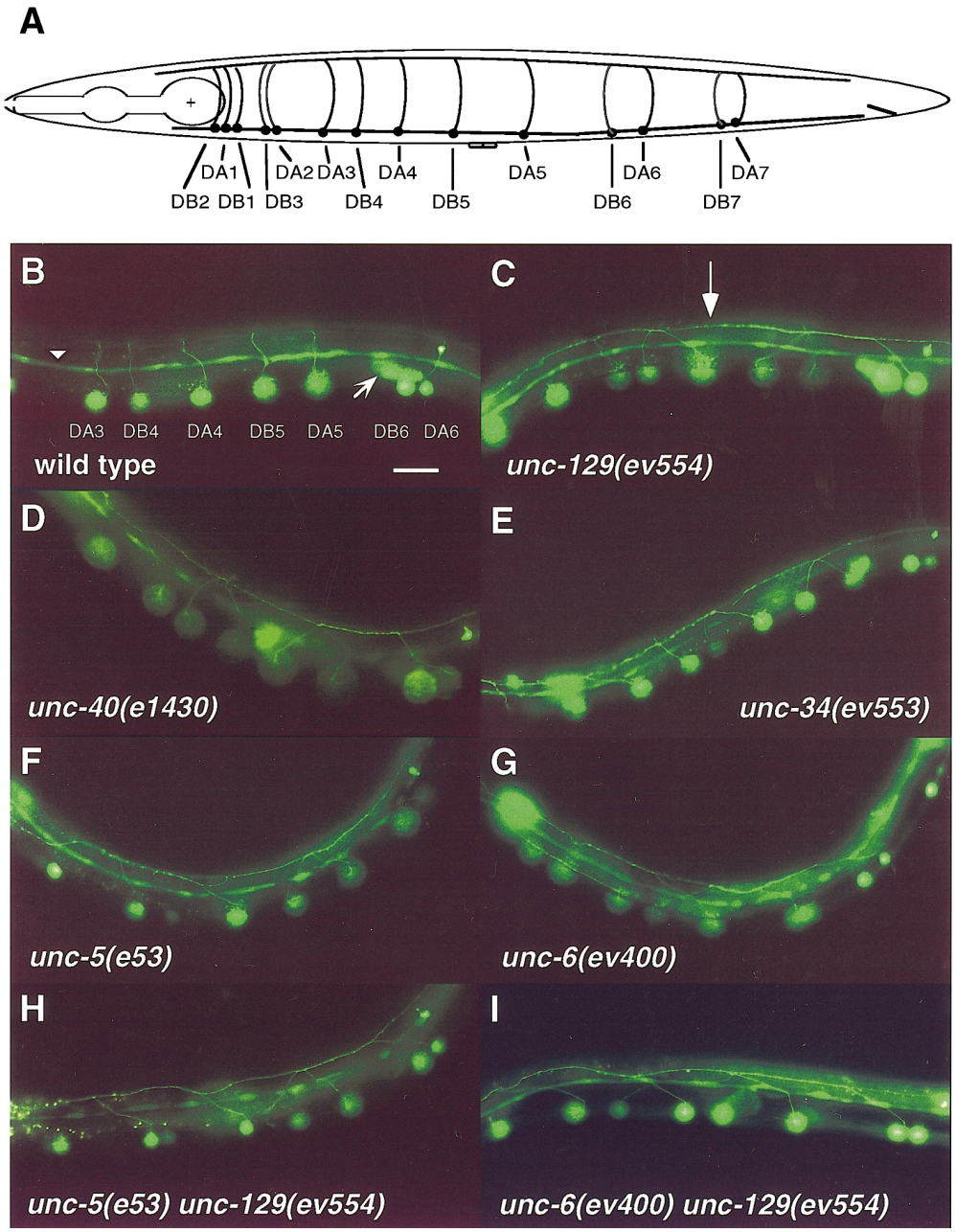

FIG. 5. DA and DB axon morphologies in wild-type and *unc* hermaphrodites. Axons are visualized using GFP expression from a transgenic array containing pAC12, a reporter gene expressed in a discrete set of cells including the DA and DB classes of motorneurons. Anterior is to the left and dorsal is toward the top of each panel. (A) Schematic drawing of the DA and DB class of motorneurons in an adult hermaphrodite. (B–I) Fluorescence micrographs of the middle portion of L4 stage larvae showing the axon morphology of the set of DA and DB motorneurons characterized in this study. In the *unc* mutants, axons deviate from their wild-type trajectories and extend along subdorsal longitudinal trajectories. Note that the dorsal and ventral nerve cords are not in the plane of focus. (B) Wild type: arrowhead indicates row of seam cells, and arrow indicates spermatheca. (C) *unc-129(ev554):* arrow indicates subdorsal longitudinal cord. (D) *unc-40(e1430),* (E) *unc-34(ev553),* (F) *unc-5(e53),* and (G) *unc-6(ev400).* (H, I) DA and DB axon morphologies in *unc5 unc-129* and *unc-6 unc-129* double mutants are qualitatively indistinguishable from that in the single mutants. Scale bar, 50 μm.

Identification and Mapping of Four New Genes

Complementation testing and genetic mapping, summarized in Table 2, indicated that mutations in *unc-129, seu-1, seu-2,* and *seu-3* identify new genes. The positions of these genes on the *C. elegans* genetic map are shown in Fig. 3.

unc-129 mutants display a fully penetrant uncoordinated movement defect that manifests as a kink in the normal sinusoidal wavelike motion of wild type. This kink is especially evident during backward movement. While all *unc-129* worms were uncoordinated, the severity of the movement defect varies from worm to worm. Examination of axon morphology in *unc-129* mutants, described below, indicates that uncoordination is most likely caused by variable axon guidance defects that prevent motorneurons from reaching their final targets in the dorsal nerve cord. Observation of *unc-129* mutants using Nomarsky DIC optics did not reveal any other visible muscle or gross morphological defects that could account for the uncoordinated phenotype. Similar observations of *seu-1, seu-2,* and *seu-3* mutants did not reveal any visible morphological or movement defects except for a mildly uncoordinated phenotype in *seu-1* mutants.

To test whether these mutations cause a complete loss of gene function, they were made hemizygous using genomic rearrangements that result in chromosomal deficiencies. These tests may reveal residual gene function if the phenotype of the mutation *in trans* to a deficiency is more severe than the mutant phenotype on its own. The visible appearance and movement of *seu-1, seu-2,* and *seu-3* deficiency heterozygotes did not appear to differ from those of homozygous mutants. However, *unc-129(ev554) in trans* to a deficiency appeared to be slightly more uncoordinated than *unc-129(ev554)* homozygotes. This may suggest that *ev554* is not a null mutation or alternatively that loss of another gene uncovered by the deficiency may be additive with *unc-129* resulting in an apparently stronger phenotype.

Axon Guidance Defects: A Potential Role for unc-34 and unc-129 in unc-5-Mediated Guidance

The *unc-5* gene is specifically required to guide pioneer growth cones and migrating cells in a dorsal direction on the basal surface of the epidermis (Hedgecock *et al.,* 1990). Five classes of motorneurons with cell bodies in the ventral nerve cord (AS, DA, DB, DD, and VD) send commissural processes dorsally to synapse with muscle targets in the dorsal cord (White *et al.,* 1986). Position in the ventral cord, axon morphology, and connectivity can be used to identify these neurons (White *et al.,* 1986). A large proportion of these axons are misguided in *unc-5* mutants, often extending along aberrant longitudinal trajectories at lateral or dorsolateral axial positions (Hedgecock *et al.,* 1990; Siddiqui and Culotti, 1991). A similar phenotype is observed in *unc-6* mutants (Hedgecock *et al.,* 1987; 1990). If the ectopic *unc-5* suppressors identified in this study function in a physiologically relevant *unc-5* signaling pathway, then they might be expected to have defects similar to those found in *unc-5* mutants. To determine if this is the case, neurons were observed in late larval and adult hermaphrodites using an *unc-5::lacZ* reporter to characterize the DD and VD motorneurons and pAC12, a DA and DB neuron-specific GFP reporter, to characterize the DA and DB motorneurons.

Axon morphologies of the DD and VD motorneurons in *unc-40, unc-34,* and *unc-44* mutants have been described elsewhere (Hedgecock *et al.,* 1987; Siddiqui and Culotti, 1991; McIntire *et al.,* 1992; Forrester and Garriga, 1997). Qualitative observation of DD and VD axons in *unc-129(ev554)* mutants revealed a large number of axons that failed to reach the dorsal cord and instead extended along longitudinal paths at aberrant axial levels (Fig. 4). In some cases, these motorneurons displayed excessive branching and very disorganized trajectories. Similar, but more penetrant defects were seen in *unc-5* and *unc-6* mutants using the same reporter (data not shown). Observation of DD and VD axons in *seu-1, seu-2,* and *seu-3* mutants did not reveal any defects.

The pAC12 GFP reporter is expressed in the DA and DB class of motorneurons as well as a discrete set of other cells (A. Colavita and J. G. Culotti, submitted for publication). The strong fluorescence that is observed in the axons of living animals expressing this reporter makes it an ideal marker for studying the axon morphologies of the DA and DB neurons. Visualization of these neurons in Unc mutants that are suppressors of ectopic *unc-5* (Fig. 5) showed that *unc-6, unc-40, unc-34,* and *unc-129* are required for proper circumferential growth of the DA and DB motorneurons. In these mutants, a significant fraction of the DA and DB axons failed to grow to their targets in the dorsal cord and instead extended along longitudinal paths at dorsal lateral or lateral positions (Table 3 and Figs. 5A–5G). For example, in *unc-129(ev554)* mutants 39% (n = 120) of DA and 63% (n = 120) of DB processes failed to reach the dorsal cord. These defects were qualitatively identical to those seen in *unc-5* and *unc-6* mutants, but were not as penetrant. DA and DB axon morphologies appear normal in *seu-1, seu-2,* and *seu-3* mutants (Table 3). The touch neurons in outcrossed *unc-129* mutants appeared morphologically wild type (data not shown), indicating that ventral and longitudinal axon guidance, at least for these cells, does not require *unc-129.* This is also the case in *unc-5* mutants, although ventral guidance of AVM and PVM axons is perturbed in *unc-6* and *unc-40* mutants (Hedgecock *et al.,* 1990).

To examine possible genetic interactions between *unc-5* and either *unc-34* or *unc-129* or between *unc-6* and *unc-129,* double mutant combinations were constructed and the DA and DB motorneuron axons were examined. Dominant enhancement of axon guidance defects was not observed in *trans*-heterozygous combinations of the above mutants (data not shown). We also did not observe any additional synthetic defects in *unc-5; unc-129* or *unc-6; unc-129* doubles compared to *unc-5* and *unc-6* single mutants (Figs. 5H and 5I). Determination of additive genetic effects in these double mutants was complicated by the nearly complete

TABLE 3
Summary of DA and DB Axon Guidance Defects in *seu* Mutants

Strain	DA motor neurons Defects[b] (%)				DB motor neurons Defects[b] (%)			
	wt	Outgrowth	Guidance	*n*	wt	Outgrowth	Guidance	*n*
evIs82A	100	0	0	120	99.2	0.8	0	120
evIs82B	100	0	0	120	100	0	0	120
unc-129(ev554)	60.8	0	39.2	120	37.5	0	62.5	120
unc-129(ev557)	59.2	0	40.8	120	38.3	0	61.7	120
unc-129(ev566)	77.5	0	22.5	120	70.8	0	29.2	120
unc-40(e1430)	71.5	0.9	27.6	116	44.2	0.8	55	120
unc-34(ev553)	85.8	4.2	10	120	39.2	15.8	45	120
unc-34(e566)	85.8	4.2	10	120	51.7	3.3	45	120
unc-44[c]			nd				nd	
unc-5(e53)	1.7	1.7	96.6	120	2.5	16.7	80.8	120
unc-6(ev400)	0.8	5	94.2	120	0.8	44.2	55	120
unc-5(e53) unc-129(ev554)	0	2.5	97.5	120	0.8	30.8	68.4	120
unc-6(ev400); unc-129(ev554)	1.7	2.5	95.8	120	0	29.2	70.8	120
seu-1(ev520)	100	0	0	60	100	0	0	60
seu-2(ev523)	100	0	0	60	100	0	0	60
seu-3(ev555)	100	0	0	60	100	0	0	60

[a] All strains are in an *evIs82A* transgenic background, except for *unc-40(e1430),* which carries *evIs82B,* and the *seu* mutants, which are transgenic for an extrachromosomal array containing pAC12 and pRF4. All transgenic arrays contain multiple copies of pAC12, a GFP reporter expressed in DA and DB motorneurons.

[b] Axon morphologies of DA3, DA4, DA5, and DA6 of the DA class of motor neurons and DB4, DB5, DB6, and DB7 of the DB class of motor neurons were examined by fluorescence microscopy. Motor neurons were scored as having an outgrowth defect if a cell body was clearly visualized but not the axon. Axon guidance defects were scored if an axon failed to reach the dorsal cord and instead extended longitudinally along the lateral epidermis. wt, wild-type.

[c] Axon morphologies in *unc-44* mutants were not determined (nd) as it was difficult to unambiguously identify neurons because of missing and mispositioned cell bodies.

penetrance of DA and DB guidance defects seen in the *unc-5* and *unc-6* single mutants. Worms homozygous for *unc-5(e53)* plus *unc-34(ev553)* were very abnormal and difficult to interpret.

Mutations in *unc-5, unc-6,* and *unc-34* also appeared to cause less frequent defects in initial axon outgrowth as brightly fluorescing cell bodies were sometimes observed to lack a process exiting the ventral cord (Table 3). Alternatively, these axonal growth cones may have failed to migrate dorsally, or they may have done so and then retracted and instead migrated longitudinally along other axons in the ventral nerve cord.

The morphological defects of the DA and DB neurons in *unc-44* mutants were not quantified as the ventral cord was often disorganized due to missing or mispositioned neuronal cell bodies. When commissures were observed, they appeared to reach the dorsal nerve cord but were often branched and took less direct paths.

Neuronal cell body positions in the ventral cord were sometimes, but infrequently, mispositioned in *unc-5, unc-6,* and *unc-34* mutants. In *unc-40* mutants these defects were slightly more common (data not shown). These defects were not observed in *unc-129, seu-1, seu-2,* or *seu-3* mutants.

DA and DB neurons appeared to differ in their requirement for functions of certain genes. Mutations in *unc-34, unc-40,* and *unc-129* caused the DB neurons to be misguided more frequently than the DA neurons (Table 3). For example, in *unc-34(ev553)* mutants 10% ($n = 120$) of DA neurons showed axon guidance defects compared to 45% ($n = 120$) of DB neurons. This observation also extended to the apparent axon outgrowth defects observed in *unc-5, unc-6,* and *unc-34* mutants (Table 3). For example, in *unc-34(ev553)* mutants 4% ($n = 120$) of DA neurons showed apparent axon outgrowth defects compared to 16% ($n = 120$) of DB neurons. DA and DB neurons have similar morphologies, appearing to differ only in the decision to turn and elongate toward the anterior (DA) or the posterior (DB) upon reaching the dorsal cord and in their pattern of synapses (White *et al.,* 1986). The differential requirement for these genes may therefore reflect underlying differences in guidance mechanisms or the presence of DA-specific genes that confer partial redundancy.

DISCUSSION

In a screen for suppressors of touch receptor axon guidance defects induced by ectopic UNC-5 expression, we have

identified eight genes involved in pioneer axon guidance in *C. elegans*. These include four genes previously known to be required for axon guidance, *unc-6, unc-40, unc-34,* and *unc-44,* as well as four new genes, *unc-129, seu-1, seu-2,* and *seu-3.* We propose that some or all of these genes are involved in axon guidance mediated by the UNC-5 receptor.

As *unc-5* is not normally expressed in the touch neurons (Hamelin *et al.,* 1993), these genes were identified under artificially induced conditions. However, the ability of *unc-5* to cause *unc-6*-mediated dorsal guidance of touch neuron axons indicates that some or all of the components necessary for dorsally directed axon outgrowth are present in the touch neurons. Presumably, the addition of the UNC-5 receptor to the touch neurons is sufficient to allow UNC-6 to trigger a mechanism that results in reorganization of the cytoskeleton and regulation of molecular motors involved in directed growth cone migration. The observation that ectopic *unc-5* guidance requires the path cue molecule UNC-6 is consistent with this mechanism being the same or similar to that used by endogenous UNC-5 (Hamelin *et al.,* 1993). Furthermore, the identification of suppressors of ectopic *unc-5* that also have axon guidance defects observed in *unc-5* and *unc-6* mutants (see below) suggests that a common mechanism is being revealed.

These genes may impinge directly on the *unc-5* pathway as is believed for the products of *unc-6* and *unc-40* or, alternatively, they may identify components of a partially redundant or parallel pathway. The incomplete penetrance of many of the axon and cell migration defects observed in null alleles of *unc-5, unc-6,* and *unc-40* mutants is evidence for a second, *unc-5*-independent pathway involved in dorsal migrations (Hedgecock *et al.,* 1990). Mutations in an alternative pathway may cause suppression of ectopic *unc-5* if signaling from both pathways contributes to a common mechanism for dorsally directed outgrowth. The observation that *unc-6* is haplo-insufficient for *unc-5*-mediated reorientation of the touch neurons suggests that suppressors were identified in a sensitized background and is consistent with the identification of genes with weak effects on dorsal outgrowth.

This screen was limited in the sense that only those mutations that did not adversely affect the fecundity of the founder parental worm or the viability of its progeny could be detected as suppressors. As a result, even though most of the suppressors were identified by multiple alleles, we do not believe that we have identified all of the genes that can be mutated to cause suppression of ectopic *unc-5* function in the touch neurons.

unc-40 Is Required for Guidance Mediated by Ectopic UNC-5

Vertebrate homologues of UNC-5 (UNC5H1, UNC5H2, and rcm=UNC5H3) and UNC-40 (DCC) have been shown to bind directly to netrins, providing a mechanism for their involvement in axon guidance (Keino-Masu *et al.,* 1996; Leonardo *et al.,* 1997). By inference, given the phylogenetic conservation of these genes, *unc-5* and *unc-40* in *C. elegans* are also likely to encode netrin receptors.

The UNC-40 receptor, unlike the UNC-5 receptor, is expressed in the touch neurons and has been shown to act cell autonomously in the AVM and PVM touch neurons to guide their axons toward ventral sources of UNC-6 (Chan *et al.,* 1996). However, the identification of *unc-40* as a suppressor of ectopic *unc-5* function is evidence that these receptors can also be required together to guide axons and motile cells away from ventral sources of UNC-6. This combined instructive role is consistent with the findings that *unc-5* and *unc-40* mutants share similar axon and cell migration defects and that these genes are coexpressed in all motile cells in which they are known to function in dorsally directed migrations (Hedgecock *et al.,* 1990; Chan *et al.,* 1996). Furthermore, double- and triple-mutant combinations of *unc-5, unc-6,* and *unc-40* that are not phenotypically more severe than the single mutants suggest that these genes may act in the same pathway (Hedgecock *et al.,* 1990).

We propose two simple, nonmutually exclusive models to account for the involvement of both receptors in mediating dorsal guidance. In the first model, UNC-5 and UNC-40 may associate laterally or multimerize on the cell membrane to form a single receptor complex that affects localized cytoskeletal reorganization and outgrowth in an UNC-6-dependent manner. In this model the UNC-40 receptor might be recruited by UNC-5 (or vice versa) in order to respond to UNC-6 as a repulsive guidance cue. Alternatively, UNC-5 and UNC-40 may exist as separate receptor complexes that signal independently to downstream components, but dorsally directed growth requires the combined input from both receptors. The important observation that dorsal guidance defects in *unc-40* mutants are much less severe than those in *unc-5* mutants suggests that UNC-5 is also able to signal in an UNC-40-independent manner. The UNC-40 receptor may therefore act to increase the efficacy of UNC-5-mediated signaling, possibly by presenting UNC-6 in a more favorable context on the cell surface or modulating some common downstream component. Similar models have been advanced by Leonardo *et al.* (1997).

UNC-44 (Ankyrin): A Putative Link between UNC-5 and the Cytoskeleton

unc-44, identified in this study as a suppressor of ectopic *unc-5*-mediated guidance, encodes a series of ankyrin-related proteins (Otsuka *et al.,* 1995). Ankyrins are spectrin binding proteins that link integral membrane proteins such as ion channels and some cell adhesion molecules to the underlying actin cytoskeleton (Bennett, 1992). Mutations in *unc-44* result in axon outgrowth and guidance defects in many classes of neurons (Hedgecock *et al.,* 1985; Siddiqui and Culotti, 1991; McIntire *et al.,* 1992), consistent with the ubiquitous expression of *unc-44* in the nervous system, including the touch neurons (A. Otsuka, personal communication). However, while *unc-44* mutants display aberrant DA and DB motor axon morphologies, they usually do not

exhibit axon guidance errors of the kind found in *unc-5* or *unc-6* mutants. Similar observations have been made concerning the DD and VD classes of neurons (McIntire *et al.*, 1992). These results may be explained if other genes, perhaps other ankyrin homologues, are able to partially compensate for the loss of UNC-44 function, or UNC-44 has other functions in addition to UNC-5 signaling, and these complicate the phenotype.

Cell adhesion molecules that have been shown to associate with ankyrin$_B$, the major isoform of ankyrin in brain, include neurofascin, L1, and NrCAM (Davis *et al.*, 1993; Davis and Bennett, 1994). These members of the Ig superfamily of cell adhesion molecules have been implicated in multiple aspects of neurite outgrowth and guidance (Doherty *et al.*, 1995; Stoeckli and Landmesser, 1995). Similarly, an ankyrin$_B$ isoform is present in developing brain during the time of axonogenesis, is localized to axons (Kunimoto, 1995a), and is able to promote neurite outgrowth in cell culture (Kunimoto, 1995b).

While *unc-5* and *unc-40* are also members of the Ig superfamily, they do not possess the conserved cytoplasmic ankyrin binding domain present in the cell adhesion molecules mentioned above. However, both *unc-5* and *unc-44* share a conserved death domain (DD) sequence located at their carboxy ends, a motif first identified in proteins involved in apoptosis (Hofmann and Tschopp, 1995). DD motifs have been implicated as sites for direct interaction involving other DD-containing proteins (reviewed in Hofmann and Tschopp, 1995). Therefore, it is possible that the genetic interaction observed between *unc-5* and *unc-44* is attributable to a direct physical interaction mediated by their DD motifs.

A possible model to explain the role of *unc-44* in *unc-5*-mediated guidance is that UNC-44 associates with either UNC-5 or UNC-40 (or both), linking them directly to the actin cytoskeleton. If activated UNC-5 binds UNC-44, this association may constitute part of a mechanism to generate localized actin polymerization or depolymerization and subsequent directed outgrowth of filopodia and lamellipodia. Evidence for a similar mechanism has recently been demonstrated in which activated neuroglian, a cell adhesion molecule, recruits ankyrin to the membrane, resulting in a localized distribution of ankyrin at sites of cell–cell adhesion (Dubreuil *et al.*, 1996). Alternatively, ankyrin may simply act as a structural element or scaffold stabilizing UNC-5 or UNC-40 at specific sites and preventing its lateral diffusion on the membrane.

unc-34, unc-129, seu-1, seu-2, and seu-3 Encode Potential New Components of unc-5-Mediated Guidance

The identification in this study of *unc-34* as a suppressor of ectopic *unc-5* suggests a potential new role in UNC-5-mediated signaling. This hypothesis is supported by the identification of DA and DB axon guidance defects in *unc-34* mutants that are qualitatively similar to those found in *unc-5* mutants. Similar defects in the circumferential axon growth of the DD and VD classes of motorneurons have been described previously (Siddiqui and Culotti, 1991; Forrester and Garriga, 1997).

A possible role for the involvement of *unc-34* in the interpretation of environmental cues has been suggested by McIntire *et al.* (1992). In that study, the HSN axon in *unc-34* mutants was shown to be defective in fasiculative outgrowth along longitudinal nerve fascicles but grew to normal length when forced to elongate longitudinally along the lateral epidermis. In addition, DD and VD motorneurons in *unc-34* mutants were shown to be defective in fasiculation and longitudinal outgrowth along the dorsal nerve cord. This result, combined with our own, indicates that *unc-34* is required for both fasciculative and pioneer guidance of the same axons over different substrates and along both body axes. If a common mechanism is employed to mediate these processes, it may involve a role in the regulation of membrane or intracellular components that respond to environmental cues rather than one that establishes or is part of an extracellular guidance system. If this assumption is valid and *unc-34* is involved in *unc-5*-mediated processes, these results are consistent with a model in which UNC-34 acts at the same genetic level as the UNC-5 receptor or downstream, as part of an intracellular signaling mechanism that regulates the cytoskeleton.

Three alleles identified in this study define a new axon guidance gene designated *unc-129*. Mutations in this gene disrupt dorsally directed axon growth cone migrations as a significant fraction of commissural axons fail to reach their targets in the dorsal cord and instead elongate along longitudinal trajectories at various axial positions. These defects, while less penetrant, are similar to those found in *unc-5* and *unc-6* mutants and are therefore consistent with *unc-129* functioning as part of an *unc-5* pathway. This idea is supported by examination of *unc-5, unc-129* and *unc-129; unc-6* double mutants in which these axon guidance defects do not appear qualitatively different from *unc-5* and *unc-6* single mutants. Furthermore, mutations in *unc-129* did not reveal any ventral guidance defects like those caused by mutations in *unc-6* and *unc-40;* therefore, like *unc-5,* appears to be specifically required for dorsal migrations. However, *unc-129* differs from *unc-5* in that it does not appear to function in the migration of the distal tip cells, mesodermal cells that display frequent dorsal guidance defects in *unc-5, unc-6,* and *unc-40* mutants (Hedgecock *et al.*, 1990). Molecular analysis of *unc-129* will be described in a separate manuscript (A. Colavita, S. Krishna, H. Zheng, R. Padgett, and J. G. Culotti, submitted for publication).

We have identified mutations in three other new genes, *seu-1, seu-2,* and *seu-3,* that are strong suppressors of *unc-5*-induced guidance of the touch neurons. However, apart from their effect on ectopic *unc-5* function, they seem to have no other readily identifiable phenotypes. These genes may therefore represent redundant components involved in *unc-5*-mediated signaling. The touch neurons presumably contain at least one functional pathway that allows the

UNC-5 receptor to transduce external cues into directed axon outgrowth. This pathway may be present in cells that normally require *unc-5* guidance functions, such as the commissural motorneurons, but the presence of other components in these cells may render it redundant. If this assumption is valid, then the *seu* genes may have been difficult to identify in a conventional *unc-5* enhancer or suppressor screen, as other genes may have been able to compensate for their loss in the motorneurons. Alternatively, the *seu* mutations may represent hypomorphs of essential genes or recessive gain-of-function mutations. These possibilities would appear to be most likely for *seu-2* and *seu-3,* which were each identified by a single mutant allele.

We have shown that mutations in eight genes, which include four new genes, disrupt the ability of *unc-5* to steer touch neurons in a dorsal direction on the epidermis of *C. elegans* and in some cases cause motor axon guidance defects that are similar to a subset of defects found in *unc-5* mutants. The latter observation provides good evidence that each gene functions in a physiologically important guidance mechanism that either involves the *unc-5* signaling pathway or acts in parallel with it. Characterization of the genes identified in this study will likely lead to greater insight into the mechanisms involved in pioneer axon guidance including those mediated by *unc-5.* These insights could have broad implications given the phylogenetic conservation of other genes in the *unc-5* pathway such as *unc-6* and *unc-40.*

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