Multiple Signaling Mechanisms of the UNC-6/netrin Receptors UNC-5 and UNC-40/DCC *in Vivo*

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ABSTRACT

Cell and growth cone migrations along the dorsoventral axis of *Caenorhabditis elegans* are mediated by the UNC-5 and UNC-40 receptor subtypes for the secreted UNC-6 guidance cue. To characterize UNC-6 receptor function *in vivo*, we have examined genetic interactions between *unc-5* and *unc-40* in the migrations of the hermaphrodite distal tip cells. We report that cell migration defects as severe as those associated with a null mutation in *unc-6* are produced only by null mutations in both *unc-5* and *unc-40*, indicating that either receptor retains some partial function in the absence of the other. We show that hypomorphic *unc-5* alleles exhibit two distinct types of interallelic genetic interactions. In an *unc-40* wild-type genetic background, some pairs of hypomorphic *unc-5* alleles exhibit a partial allelic complementation. In an *unc-40* null background, however, we observed that *unc-5* hypomorphs exhibit dominant negative effects. We propose that the UNC-5 and UNC-40 netrin receptors can function to mediate chemorepulsion in DTC migrations either independently or together, and the observed genetic interactions suggest that this flexibility in modes of signaling results from the formation of a variety of oligomeric receptor complexes.

SEVERAL genes, including *unc-6*, *unc-5*, and *unc-40*, are known to interact in guiding circumferential cell and growth cone migrations in *Caenorhabditis elegans* (Hedgecock *et al.* 1990; McIntire *et al.* 1992). *unc-6* encodes a member of the secreted, laminin-related protein family called netrins (Ishii *et al.* 1992; Kennedy *et al.* 1994; Serafini *et al.* 1994). *unc-6* is expressed ventrally in *C. elegans*, and mutations cause defects in both dorsally and ventrally directed cell and growth cone migrations (Hedgecock *et al.* 1990; Wadsworth *et al.* 1996). Similar functions have been observed for insect and vertebrate netrins (Colamarino and Tessier-Lavigne 1995; Harris *et al.* 1996; Mitchell *et al.* 1996; Serafini *et al.* 1996). Although the mechanisms of function of the UNC-6/netrins are poorly understood, migrating cells and growth cones are thought to transduce relative differences in extracellular UNC-6/netrin concentration into local, intracellular changes in the actin cytoskeleton.

unc-5 and *unc-40* encode transmembrane receptors of the immunoglobulin (Ig) superfamily (Leung-Hagesteijn *et al.* 1992; Chan *et al.* 1996). Expression of the *C. elegans* UNC-5 protein is, in most cases, sufficient to cause repulsion of migrating cells or growth cones away from ventral concentrations of UNC-6 (Hamelin *et al.* 1993, Wadsworth *et al.* 1996; Su *et al.* 2000). Vertebrate homologues of UNC-5 include the murine *rostrocerebellar malformation* (*rcm*) gene product (Ackerman *et al.* 1997), now renamed UNC5H3 (Przyborski *et al.* 1998), and two rat homologues, UNC5H1 and UNC5H2 (Leonardo *et al.* 1997). *unc5h3* mutants have defects in cell migrations in the developing cerebellum (Ackerman *et al.* 1997; Przyborski *et al.* 1998), and all three homologues have been shown to bind directly to netrins (Leonardo *et al.* 1997).

C. elegans unc-40 is required primarily for ventrally oriented migrations, but also contributes to dorsally oriented and longitudinal migrations (Hedgecock *et al.* 1987, 1990). UNC-40 is related to the product of the mammalian *deleted-in-colorectal-cancer* (*Dcc*) gene (Chan *et al.* 1996), which is involved in axon guidance in the mouse spinal cord (Fazeli *et al.* 1997), and to the product of the *frazzled* gene of Drosophila, also involved in axon guidance (Kolodziej *et al.* 1996). DCC binds directly to netrins (Keino-Masu *et al.* 1996) and mediates responses to Netrin-1 of Xenopus retinal ganglion cells in culture (de la Torre *et al.* 1997). Thus, the UNC-6/netrin ligands together with the UNC-5 and UNC-40/DCC receptors compose a highly conserved system for the guidance of migrating cells and neuronal growth cones.

The functional relationships between the UNC-5 and UNC-40/DCC receptors are not entirely clear. Presumed null *unc-40* mutations in *C. elegans* disrupt the same dorsally oriented cell and growth cone migrations as do *unc-5* mutations, but with a lower penetrance (Hedgecock *et al.* 1990). These weak effects of *unc-40* mutations suggest that UNC-40/DCC is not strictly required for UNC-5 function. However, ectopic expres-

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sion studies in both *C. elegans* (Colavita and Culotti 1998) and Xenopus (Hong *et al.* 1999) indicate that, in these unusual situations, all UNC-5-mediated repulsion requires UNC-40/DCC. We are using the hermaphrodite distal tip cells (DTCs) of *C. elegans* as a model *in vivo* system for the study of the mechanism, guidance, and regulation of cell migrations. We have sought to clarify the roles of and interactions between *unc-40* and *unc-5* in the migrations of the DTCs, which are repelled by UNC-6 in the ventral-to-dorsal phase of their migration. We propose a model in which the UNC-5 and UNC-40 receptor subtypes are capable, to a limited extent, of mediating repulsion from UNC-6 independently of one another in the DTCs. However, the two netrin receptors function best in combination and the types of genetic interactions that we observe between hypomorphic *unc-5* alleles suggest that multimerization, possibly between UNC-5 receptors, is a key component of netrin receptor signal transduction.

MATERIALS AND METHODS

Culture conditions: Culturing, handling, and genetic manipulations were as previously described (Brenner 1974). The following genes and alleles were used:

Linkage group (LG) I: *unc-40(e1430)*, *unc-40(ev457)*, *unc-40 (ev643)* (D. C. Merz, unpublished results).
LG IV: *unc-5(e53)*, *unc-5(e152)*, *unc-5(ev432)*, *unc-5(ev435)*, *unc-5 (ev512)*, *unc-5(ev585)*, *unc-5(ev634)*, *unc-5(ev642)*, *unc-5 (ev644)* (this study), *gon-1(ev635)* (D. C. Merz, unpublished results), *dpy-20(e1282)*, *unc-22(e66)*.
LGX: *unc-6(ev400)*, *unc-6(rh202)*.

Some strains not derived in our lab were obtained from the *Caenorhabditis* Genetics Center, which is funded by the National Institutes of Health (NIH) National Center for Research Resources (NCRR). The isolation and phenotypic characterization of the *unc-5* alleles *e53*, *e152*, *e553*, *ev432*, and *ev435* have been previously described (Brenner 1974; Hedgecock *et al.* 1990; Leung-Hagesteijn *et al.* 1992). *ev512*, *ev585*, *ev634*, *ev642*, and *ev644* were isolated by EMS mutagenesis in several genetic screens (D. C. Merz, A. Colavita and J. G. Culotti, unpublished data). The allele *e53* is phenotypically identical to a group of severe *unc-5* alleles (Hedgecock *et al.* 1990). Genetic and molecular analyses suggest that these alleles represent the complete elimination of UNC-5 function. For example, *unc-5(e53)* results from a nonsense mutation predicted to terminate the protein (W283STOP) in the region encoding the extracellular domain (see results below). Using large-scale noncomplementation screens (>50,000 haploid genomes), we have been unable to generate *unc-5* alleles more severe than existing strong alleles such as *unc-5(e53)*.

unc-40(e1430) (Hedgecock *et al.* 1990) has been sequenced and is predicted to be a molecular null (Chan *et al.* 1996). *e1430* has the most 5′ nonsense mutation of characterized *unc-40* alleles. Several alleles with more 3′ truncations, including *unc-40(ev457)*, have slightly more severe phenotypes (Hedgecock *et al.* 1990), possibly due to the production of truncated, *trans*-interfering proteins (Chan *et al.* 1996). The genetic interactions described here for *unc-40(e1430)* are identical with *unc-40(ev457)*. On the basis of sequencing and phenotypic analysis, the *unc-6(ev400)* allele is considered a genetic and molecular null allele (Hedgecock *et al.* 1990; Wadsworth *et al.* 1996).

Scoring of DTC and Unc defects: Mutations in *unc-5*, *unc-6*, and *unc-40* disrupt specifically the ventral-to-dorsal second phase of DTC migrations (Figure 1). The misshapen gonad arms thus produced are readily visible and quantifiable with a dissecting microscope. Most strains could be scored in this manner at the L4 or young adult stages of development. Strains that are egg-laying defective (Egl), especially those including *unc-6* or *unc-40* mutations, were scored entirely at higher magnification under differential interference contrast (DIC) optics. Gonad arms were scored as defective if the turn from the centrifugal first to the centripetal third migration phase occurred on the ventral side, indicating that the ventral-to-dorsal second phase did not occur. Some differences with previously published frequencies of DTC defects were found (Hedgecock *et al* 1990). Scoring of DTC migrations under DIC facilitates distinctions between qualitatively different classes of migration errors. For example, there is a distinct type of DTC migration defect often observed in *unc-40* mutants in which the DTCs descend ventrally again immediately upon reaching the dorsal muscle band. Under low power microscopy, this defect is difficult to distinguish from a failure to migrate to the dorsal muscle band. The Unc-ness of worms was assayed using the standards of Hedgecock *et al.* (1990).

Standard errors for the proportions of defective DTC migrations in a population were calculated using the observed frequency and the actual sample size, assuming a binomial distribution, as previously described (Hedgecock *et al.* 1990).

Comparisons were done using a standard test (one-tailed) for comparing two proportions (Miller and Freund 1965). All P values represent the probability that the penetrance of DTC migration defects is lower in one strain than in another. A P value of 0.05 is considered significant. Where measures of statistical significance are reported in the text, the P value of the anterior or posterior DTC that is closer to 0.05 is given.

Transgenic strains: Transgenic lines were generated using standard germline transformation techniques (Mello and Fire 1995). pZH85 contains 2.0 kb of genomic sequence from immediately upstream of *unc-5* exon 2 (Leung-Hagesteijn *et al.* 1992) fused to a green fluorescent protein (GFP)-tagged *unc-40* cDNA (pZH22; Chan *et al.* 1996), to express functional UNC-40 (Chan *et al.* 1996) in cells that normally express *unc-5*. This 2.0-kb *unc-5* promoter fragment (*unc-5B2*), like the 4.6-kb promoter previously described (Su *et al.* 2000), can drive expression of reporter constructs in the DTCs and several classes of sensory neurons in the head. However, the 2.0-kb fragment does not drive expression in ventral cord motorneurons. pZH85 (20 or 80 ng/μl) was injected along with pMH86 (20 ng/μl), which contains the wild-type *dpy-20* gene. Four independent non-Dpy transgenic lines (two at 20 ng/μl pZH85 and two at 80 ng/μl pZH85; they are called *evEx117a–d*, respectively) were generated in a *dpy-20; him-5* background and passed into other mutant backgrounds, which were also usually in the *dpy-20* background in order to follow the transgenic array. GFP expression in the DTCs was confirmed for each line. For each mutant background (*e.g.*, *unc-5*) into which the extrachromosomal array containing pZH85 was passed, at least three of the four transgenic lines were used. No significant differences were observed between transgenic lines, and results are presented for *evEx117a* and *evEx117b*.

SSCP and sequencing of *unc-5* alleles: cDNA was prepared from RNA isolated from *unc-5* alleles. For *unc-5(e53)* and *unc-5 (e152)*, SSCP analysis was carried out essentially as previously described (Chan *et al.* 1996), and mutations were confirmed by sequencing. The complete coding sequence of *unc-5(e152)* was sequenced, while *unc-5(e53)* was sequenced 5′ to the mutation site. For *unc-5(ev585)*, single-stranded PCR products gen-

erated from cDNA were directly (and completely) sequenced for comparison to N2. The mutation was confirmed by sequencing of the opposing strand. A complete report of sequences of *unc-5* alleles will be published elsewhere (M. T. Killeen, A. Krizus, I. Scott, R. Wilk and J. G. Culotti, unpublished results).

Construction of heteroallelic strains: For scoring of DTC migration defects, heterozygous strains of *unc-5* were generated by crossing wild-type males with *unc-5 dpy-20* double mutant strains. Non-Dpy progeny of these crosses were scored for DTC migration defects.

unc-5 alleles (*e152* and *ev585*, for example) were placed *in trans* with one another as follows. *dpy-20(e1282); him-5(e1490)* males were crossed to *unc-5(e152)* hermaphrodites and male + *dpy-20(e1282)/unc-5(e152) +; him-5(e1490)/+* cross-progeny were picked. These were crossed to doubly homozygous *unc-5 (ev585) dpy-20(e1282)* hermaphrodites. The non-Dpy hermaphrodite progeny from this cross, which were *unc-5(e152) +/unc-5 (ev585) dpy-20(e1282)*, were scored for defects in the dorsal migrations of the anterior and posterior DTCs, as previously described (Hedgecock *et al.* 1990). The heterozygous *dpy-20* mutation (*dpy-20(e1282)/+*) did not affect the frequencies of DTC defects caused by *unc-5* mutations. For example, there were no significant differences in the defects of *unc-5(e152)* homozygotes compared with *unc-5(e152) +/unc-5(e152) dpy-20 (e1282)*. Due to the temperature sensitivity of *dpy-20(e1282)* and of *unc-5(ev585)*, all crosses were done at 25°. From each cross, the DTC migration defects of all of the non-Dpy hermaphrodite progeny were scored.

To examine the defects caused by heterozygous *unc-5* mutants in an *unc-40(e1430)* background, the balanced strains *unc-40(e1430); unc-5 + dpy-20(e1282) unc-22(e66)/+ gon-1 (ev635) ++* were constructed. Homozygous *unc-22(e66)* worms exhibit a Twitching (Twi) phenotype, while homozygous *gon-1(ev635)* worms exhibit a visible gonad (Gon) defect caused by failure of gonad arm extension. The *gon-1* phenotype is easily distinguishable from the Mig phenotype caused by *unc-5*. Non-Twi non-Gon worms were scored for defects in the ventral-to-dorsal phase of DTC migration. Heterozygous *gon-1* or *unc-22* mutants did not affect the penetrance of DTC defects in the *unc-40(e1430)* background.

RESULTS

Background on DTC migration defects: The extension of each of the two arms of the bilobed hermaphrodite gonad during larval development is led by the migration of a DTC (Hedgecock *et al.* 1987). Mutations in *unc-5*, *unc-6*, or *unc-40* specifically disrupt the ventral-to-dorsal second phase of the DTC migration pattern (Figure 1). The centrifugal first and centripetal third phases are normal in timing and extent. In these mutants, however, failures to execute the ventral-to-dorsal phase of migration result in both the first and third longitudinal phases of DTC migration occurring on the ventral side. *unc-6* null mutants are not fully penetrant for these defects (Hedgecock *et al.* 1990), indicating that UNC-6-independent mechanisms must also exist to guide this DTC migration phase. The UNC-5 and UNC-40 receptors are expressed by the DTCs during their migrations (Chan *et al.* 1996; Su *et al.* 2000). Consistent with the idea that these receptors transduce UNC-6-mediated directional information, additional mutations in *unc-5* and *unc-40*

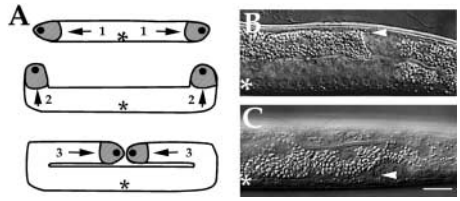

Figure 1.—DTC migrations and defects. (A) An illustration of the morphology of the developing hermaphrodite gonad. The arrows indicate the migration pathway of each DTC in each migration phase (numbered 1–3). DTC migrations begin at the ventral midbody (asterisks) and end near the dorsal midbody (B and C) Migrating posterior DTCs (position and direction of migration are indicated by arrowheads) are shown at the beginning of the third migration phase early in L4 in wild-type (B) and *unc-6* (C) genetic backgrounds. The second, ventral-to-dorsal, phase is disrupted in the *unc-6* mutant background, resulting in the third, centripetal, migration phase taking place ventrally (C). The proximal portion of the gonad arm is out of the plane of focus in C, although the first migration phase is normal in these mutants. Anterior DTCs follow a mirror image migration pattern. In all panels, ventral is down and anterior to the left. Asterisks indicate the position of the ventral midbody. Bar, 10 μm.

do not enhance the second migration phase defects of an *unc-6* null mutation (Hedgecock *et al.* 1990). Thus, the penetrance of DTC defects observed in an *unc-6* null background represents a complete loss of function in this pathway, and a comparison of the penetrance of this specific defect in other *unc-6*, *unc-5*, or *unc-40* mutants with that of the *unc-6* null provides a measure of the efficacy of the repulsive UNC-6 signaling pathway.

UNC-5 and UNC-40 function at the same time and place: The functional and biochemical relationships between the UNC-5 and UNC-40 receptors are unclear. We sought to examine in detail the role of each receptor in the second, ventral-to-dorsal, DTC migration phase. As described above, the identical DTC migration defect is caused by mutations in *unc-5*, *-6*, or *-40*. Previous mosaic and transgenic studies have demonstrated that UNC-5 acts cell autonomously within the DTCs to initiate the ventral-to-dorsal migration phase (Leung-Hagesteijn *et al.* 1992; Su *et al.* 2000). Reporter construct analyses suggest that *unc-40* has a broad pattern of expression, including the DTCs and surrounding tissues throughout the time of the DTC migrations (Chan *et al.* 1996). Although UNC-40 can act cell autonomously to guide neuronal axons (Chan *et al.* 1996), its site and time of action in DTC migrations have not been determined. A functional GFP-tagged *unc-40* transgene was placed downstream of a fragment of the *unc-5* promoter to express UNC-40 in the DTCs, but not in surrounding tissues, at the time of the initiation of the ventral-to-dorsal migration phase (Figure 2; Su *et al.* 2000). This GFP-tagged UNC-40 transgene was previously shown to be capable of rescuing an *unc-40* mutant when expressed under the control of its endogenous promoter (Chan *et al.* 1996). When expressed in the DTCs downstream of the

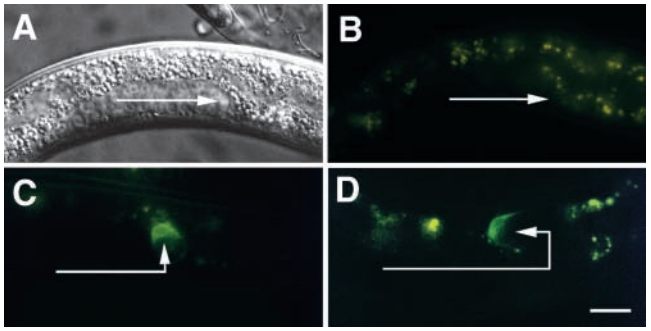

FIGURE 2.—A GFP-tagged *unc-40* cDNA placed downstream of a fragment of the *unc-5* promoter is expressed in the DTCs beginning at the time of the ventral-to-dorsal second migration phase. All panels are images of migrating posterior DTCs in an *unc-40(e1430); evEx117a* background. The arrows indicate the migration pathway of the DTCs. (A) A DIC image of a DTC in the first phase of migration. (B) An epifluorescence image of the same DTC, with no detectable *unc-40::GFP* expression. (C and D) During the second and third migration phases GFP-tagged UNC-40 is expressed in the DTCs but not in surrounding tissues. Background staining results from gut autofluorescence and is visible in nontransgenic strains. In all panels, anterior is to the left and dorsal is up. Bar, 10 μm.

unc-5B2 promoter, this *unc-40* transgene (in the *evEx117* lines) was able to rescue the DTC migration defects of an *unc-40* null mutant (Figure 3), as the frequency of defects in *evEx117; unc-40(e1430)* lines was significantly lower than in *unc-40(e1430)* alone ($P < 0.01$; Figure 3A). The dumpy (Dpy) and uncoordinated (Unc) phenotypes of *unc-40* were not affected. Expression of UNC-40 from this transgene was not able to rescue the DTC migration defects of the *unc-6(ev400)* null mutant ($P > 0.05$; Figure 3B), indicating that the function of the transgenic UNC-40GFP in the DTCs, like endogenous UNC-40, requires the UNC-6 guidance cue. From these results, we conclude that UNC-40, like UNC-5, acts cell autonomously within the DTCs at the time of the ventral-to-dorsal migration to mediate a response to UNC-6.

UNC-5 and UNC-40 can function independently of one another: As previously described, the relatively weak defects in ventral-to-dorsal migrations caused by *unc-40* mutations suggest that UNC-5 does not absolutely require UNC-40 to signal repulsion (HEDGECOCK *et al.* 1990). In ectopic expression assays *in vitro* or *in vivo*, however, UNC-5 does absolutely require UNC-40/DCC for growth cone repulsion (COLAVITA and CULOTTI 1998; HONG *et al.* 1999). The penetrance of DTC defects caused by a null mutation in *unc-6* is significantly greater than that caused by null mutations in either *unc-5* or *unc-40* ($P < 0.001$; Figure 4A). However, the null allele *unc-40(e1430)* in combination with the null allele *unc-5 (e53)* resulted in a frequency of DTC migration defects identical to that caused by a complete loss of *unc-6* function ($P > 0.09$; Figure 4A). This demonstrates that, in DTC migrations, UNC-5 can transduce in part the UNC-6 signal independently of UNC-40 (Figure 4B). In addition, UNC-40 can signal repulsion independently of UNC-5, indicating that UNC-5 is not absolutely required for UNC-40 to assume a repulsive role (Figure 4B). This guidance system is, however, fully functional only if both receptors are present.

We asked whether the residual function of UNC-40 in the absence of UNC-5 could be mimicked by expression of the *unc-40GFP* transgene in the DTCs. In addition we asked whether a high level of UNC-40 expression in the DTCs could partially rescue the DTC migration defects of an *unc-5* mutation. As shown in Figure 3C, the *unc-5B2::unc-40GFP* transgene of *evEx117* was capable of rescuing the DTC migration defects of an *unc-40(e1430); unc-5(e53)* double mutant strain to a level approximately equal to that of an *unc-5(e53)* mutant alone, but could not fully rescue the DTC migration defects, presumably due to its inability to substitute fully for UNC-5. Consistent with this interpretation, transgenic expression of UNC-40 together with wild-type endogenous UNC-40 was also unable to compensate for a null mutation in *unc-5* (Figure 3D). Therefore, in addition to acting together to mediate repulsion from UNC-6, UNC-5 and UNC-40 can act independently to carry out this same function and do so in non-interchangeable ways.

Several hypomorphic *unc-6* alleles, including *unc-6 (rh202)*, selectively disrupt ventral-to-dorsal migrations (HEDGECOCK *et al.* 1990). These alleles result from deletions of the V-2 module of the UNC-6 EGF-like repeats and are predicted to disrupt UNC-6-UNC-5 interactions (WADSWORTH *et al.* 1996), although this has not been tested biochemically. *unc-6(rh202)* mutants are, like *unc-5 (e53)*, less severe than an *unc-6* null allele ($P < 0.001$; Figure 4A). However, double mutants of *unc-40(e1430); unc-6(rh202)* were not significantly different from the *unc-6* null allele *ev400* in the penetrance of DTC defects ($P > 0.25$; Figure 4A), nor were they significantly different from the *unc-40; unc-5* double null mutant ($P > 0.19$). This is consistent with the idea that *unc-6(rh202)* selectively eliminates UNC-5-dependent functions of UNC-6, but leaves intact a repulsion induced by UNC-6-UNC-40 interactions.

***unc-5* interactions in an *unc-40(+)* background:** Genetic interactions were examined between *unc-5* alleles predicted to partially reduce UNC-5 function. Most of the *unc-5* alleles examined were fully recessive and exhibited more frequent DTC defects when placed *in trans* to a null *unc-5* allele [*unc-5(e53)*; Figure 5]. Rare (<1%) posterior DTC defects were observed only in *unc-5(e53)/+* or *unc-5(e152)/+* strains. *unc-5(ev634)* has the same frequency of DTC migration defects as *unc-5(e53)* and this frequency is not increased when *ev634* is placed *in trans* to *unc-5(e53)* (Figure 5). This strain is, however, less uncoordinated than *unc-5(e53)*, suggesting some residual UNC-5 function, at least in the nervous system. Thus, we consider this and the other *unc-5* alleles examined [excepting *unc-5(e53)*] to be hypomorphic alleles.

When placed *in trans* to one another, some but not

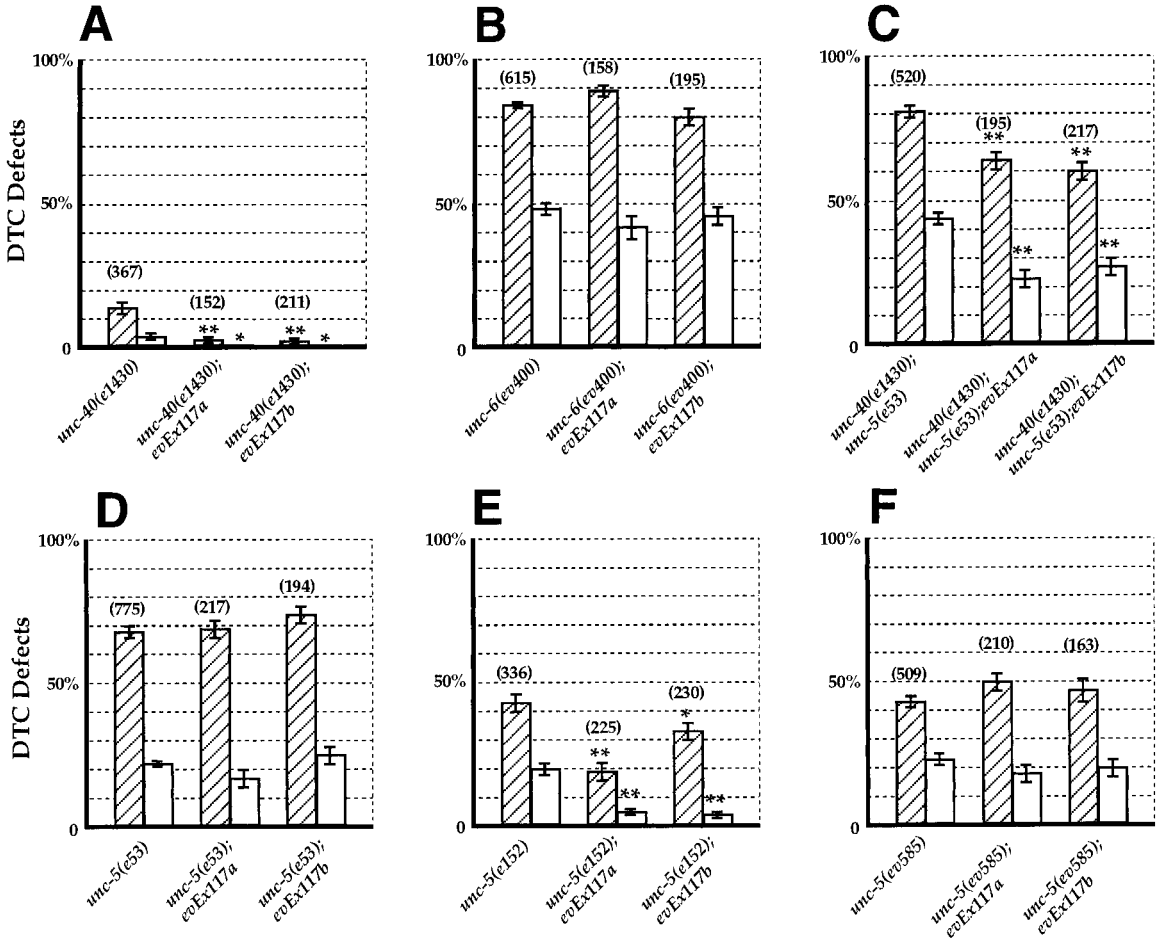

FIGURE 3.—Effects of expression of a transgene encoding a GFP-tagged UNC-40 in the DTCs. Extrachromosomal arrays containing *unc-40::GFP* downstream of the *unc-5B* promoter fragment (*evEx117a* and *evEx117b*) were passed into mutant backgrounds to assess the ability of these arrays to rescue DTC migration defects. Hatched columns represent the frequency of defects in the migrations of posterior DTCs, while open columns represent the frequency of defects of anterior DTCs. A significant reduction in DTC migration defects (relative to each mutant background without *evEx117a* or *-b*) was observed for the null allele *unc-40(e1430)* (A), the double null mutant strain *unc-40(e1430); unc-5(e53)* (C), and hypomorphic mutation *unc-5(e152)* (E). No effects were found in the null allele *unc-6(ev400)* (B), *unc-5(e53)* (D), or the hypomorphic *unc-5(ev585)* (F) backgrounds. Numbers above bars indicate the number of worms scored, each for both the anterior and posterior DTCs. Asterisks indicate significant differences at the $P < 0.05$ (single asterisk) or $P < 0.001$ (double asterisk) levels. Vertical bars indicate standard errors.

all pairs of two different hypomorphic alleles exhibited a partial complementation. For example, the *unc-5(ev585)* allele *in trans* to the *unc-5(e152)* allele resulted in a significantly ($P < 0.0001$) lower frequency of DTC defects than that observed in either homozygous *ev585* or homozygous *e152* mutants (Figure 5). Whereas homozygous *ev585* or *e152* hermaphrodites both exhibited defects in ~20% of anterior DTCs and 40% of posterior DTCs, the *trans*-heterozygotes (*ev585/e152*) had defects in only 5/226 (2%) of anterior and 54/226 (24%) of posterior DTCs.

A similar partial complementation was observed for other pairs of *unc-5* alleles (Figure 5). This interallelic complementation was never complete, but was in all cases a partial amelioration of DTC migration defects. Six of eight of these *unc-5* hypomorphs could be placed into one of two groups in which partial complementation was observed between but not within each group. Thus, the group *ev435*, *ev512*, *ev585*, and *ev642* partially complemented *e152* and *ev644*. In cases for which the site of the molecular lesion is known, the former group comprises alleles with UNC-5 ectodomain mutations, while the latter group comprises cytodomain domain mutations (M. T. KILLEEN, A. KRIZUS, I. SCOTT, R. WILK, M. NYGIEM and J. G. CULOTTI, unpublished results). For example, *unc-5(ev585)* results from a missense mutation in the second Ig domain (C181Y), while *unc-5(e152)* results from a nonsense mutation (Q507STOP) predicted to truncate the cytodomain. One of the exceptions to this grouping of alleles was *unc-5(ev634)*, which partially complemented *ev432*, *ev512*, and *ev644*. Another exception was *unc-5(ev432)*, which complemented only *ev634*.

***unc-5* interactions in an *unc-40(−)* background:** Al-

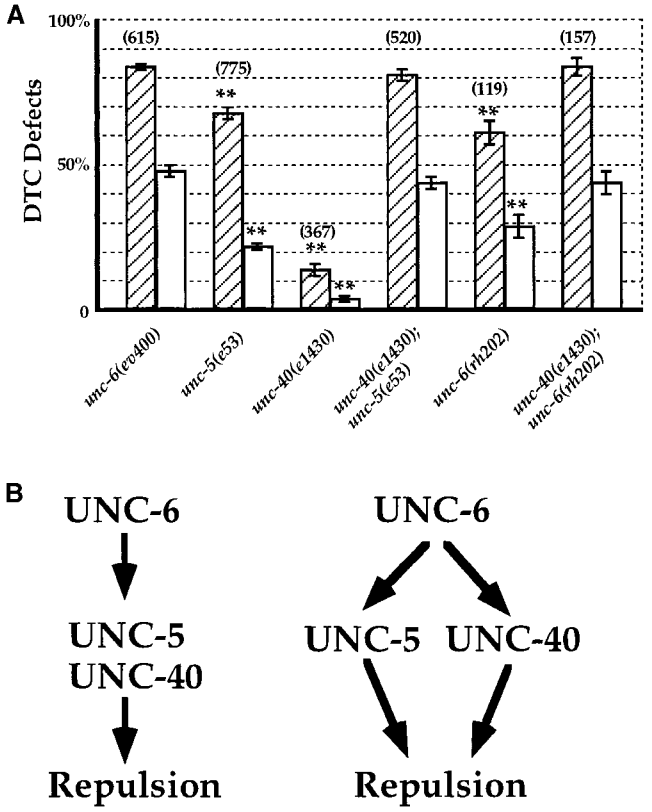

FIGURE 4.—Mutations in both *unc-5* and *unc-40* are required to eliminate UNC-6-dependent DTC migrations. Hatched columns represent the frequency of defects in the migrations of posterior DTCs, while open columns represent the frequency of defects of anterior DTCs. (A) *unc-5* null, *unc-40* null, and *unc-6(rh202)* mutants have significantly lower percentages of DTC migration defects than an *unc-6* null. However, double mutant strains of *unc-40* and *unc-5* nulls or of an *unc-40* null with *unc-6(rh202)* are indistinguishable from the *unc-6* null. Numbers above bars indicate the number of worms scored, each for both the anterior and posterior DTCs. Double asterisks indicate a statistically significant difference at a *P* value of 0.001. (B) As shown on the left, previous reports suggest that UNC-5 function is dependent upon UNC-40. However, in DTC migrations, examination of null mutants suggests that netrin receptor function is more complicated and indicates that UNC-5 and UNC-40 each retain some function in the absence of the other, as depicted on the right.

though all *unc-5* alleles examined were recessive and some *unc-5* hypomorphic alleles exhibited interallelic complementation, different genetic properties were observed when these *unc-5* alleles were examined in an *unc-40* null background. For example, an *unc-40(e1430); unc-5(e53)/+* strain was more severely defective in DTC migrations than *unc-40(e1430)* alone ($P < 0.0001$; Figure 6A). As *unc-5(e53)* is a null allele, this indicates the haploinsufficiency of *unc-5* in the absence of UNC-40. The *unc-5* hypomorphic alleles *e152* and *ev585* exhibited stronger dominant enhancement of the DTC defects of *unc-40(e1430)* than did the *unc-5* null allele *e53* (Figure 6A). Both *unc-40(e1430); unc-5(e152)/+* and *unc-40 (e1430); unc-5(ev585)/+* strains had significantly higher frequencies of DTC migration defects than *unc-40 (e1430)* alone ($P < 0.0001$). In addition, both had significantly more DTC defects than *unc-40(e1430); e53/+* [$P < 0.0001$ for *unc-40(e1430); unc-5(e152)/+* and $P < 0.012$ for *unc-40(e1430); unc-5(ev585)/+*]. The most severe effects were observed with *unc-40(e1430); unc-5 (e152)/+*, in which 43/136 (32%) of anterior and 110/136 (81%) of DTC migrations were defective (Figure 6A). These worms were also more uncoordinated than *unc-40(e1430)* alone. Thus, in an *unc-40(−)* background, *unc-5* null alleles can exhibit haploinsufficiency while hypomorphic alleles also exhibit dominant negative effects.

UNC-5 can act through an UNC-40-dependent pathway: As described above, null mutant phenotypes suggest that the UNC-5 and UNC-40 receptor subtypes can function independently of one another. However, complete function requires both receptors, and recent biochemical and *in vitro* assays on vertebrate homologues suggest direct interactions between the two receptor subtypes (HONG *et al.* 1999). We asked whether, for any *unc-5* hypomorphs, the residual function in DTC migrations was dependent upon UNC-40. Double mutant strains comprising a null allele of *unc-40* and an *unc-5* hypomorph were all more severe than the *unc-5* hypomorph alone (Figure 6B). Therefore, eliminating *unc-40* function enhances the defects of *unc-5* mutants. Some, but not all, alleles were enhanced to the severity of *unc-6* null mutants. In particular, a double mutant comprising *unc-40(e1430)* and *unc-5(e152)* was not significantly different from an *unc-6* null ($P > 0.39$) or from *unc-40(e1430); unc-5(e53)* ($P > 0.26$), either in the frequency of DTC migration errors (Figure 6B) or in the severity of uncoordination (data not shown). Similar results were obtained with other *unc-40* alleles together with *unc-5(e152)*. Two other strong and putative null *unc-40* alleles, *ev457* and *ev643*, isolated in separate genetic screens and backgrounds, also eliminated UNC-6-dependent DTC migrations as double mutants with *unc-5(e152)*, as the frequency of DTC migration defects was not significantly different from that of *unc-6 (ev400)*. An *unc-40(ev457); unc-5(e152)* strain ($N = 129$) exhibited 49% anterior ($P = 0.46$) and 88% posterior ($P = 0.13$) DTC defects. *unc-40(ev643); unc-5(e152)* ($N = 122$) had 47% anterior ($P = 0.42$) and 84% posterior ($P = 0.5$) DTC defects.

Most other *unc-5* hypomorphic alleles did not exhibit this interaction (Figure 6B). For example, although the *e152* and the temperature-sensitive *ev585 unc-5* alleles are quantitatively similar in their DTC and axon guidance defects at 25° (Figure 5 and D. C. MERZ, unpublished data), a double mutant of *unc-40(1430)* together with *unc-5(ev585)* exhibited a less severe phenotype than an *unc-6* null (Figure 6B). Thus, in an *unc-40* null background, the function retained by some hypomorphic *unc-5* alleles, such as *e152*, is dependent on UNC-40, while the function retained by other *unc-5* alleles is not

	+	*e53*	*e152*	*ev432*	*ev435*	*ev512*	*ev585*	*ev634*	*ev642*	*ev644*
ev644	A 0±0 P 0±0	A 0±0 P 25±4	A 2±2 P 11±3	A 1±1 P 19±4	A 0±0 P 10±3	A 0±0 P 8±3	A 0±0 P 8±3	A 2±1 P 6±2	A 1±1 P 4±2	A 1±1 P 17±3
ev642	A 0±0 P 0±0	A 17±4 P 65±5	A 1±1 P 23±4	A 9±3 P 42±5	A 1±1 P 25±3	ND	A 15±3 P 62±4	A 8±3 P 36±5	A 2±1 P 37±5	
ev634	A 0±0 P 0±0	A 23±4 P 57±5	A 17±4 P 64±5	A 0±0 P 19±3	A 2±1 P 18±3	A 0±0 P 1±1	A 11±3 P 38±5	A 25±2 P 64±2		
ev585	A 0±0 P 0±0	A 22±4 P 62±5	A 2±2 P 24±3	A 8±2 P 29±3	A 1±1 P 36±4	A 6±2 P 43±4	A 23±2 P 43±2			
ev512	A 0±0 P 0±0	A 12±3 P 36±5	A 1±1 P 17±4	A 6±2 P 35±4	A 1±1 P 19±5	A 5±1 P 33±1				
ev435	A 0±0 P 0±0	A 7±2 P 59±5	A 1±1 P 11±2	A 4±2 P 27±4	A 1±1 P 20±3					
ev432	A 0±0 P 0±0	A 13±3 P 45±5	A 21±5 P 50±6	A 10±3 P 46±4						
e152	A 0±0 P 1±1	A 29±3 P 49±3	A 20±2 P 43±3							
e53	A 0±0 P 1±1	A 22±3 P 68±4								
+	A 0±0 P 0±0									

FIGURE 5.—Some *unc-5* hypomorphic alleles exhibit a partial interallelic complementation. *Trans*-heterozygotes comprising two different alleles of *unc-5* were constructed and scored for the frequency with which DTCs failed to migrate dorsally. Both the anterior (A) and the posterior (P) DTCs were scored, with a minimum sample size of 100 each. The first row and column are the alleles tested. + indicates a wild-type *unc-5* allele. The shaded boxes highlight strains in which a partial complementation was observed, *i.e.*, in which the frequency of DTC defects (for the anterior or the posterior DTC) was significantly lower ($P < 0.05$) in the heteroallelic strain than in either homoallelic strain. Some data for homoallelic pairs (*e.g.*, *e53/e53*) are the same as in Figure 3.

fully dependent on UNC-40. These two groups of alleles, therefore, distinguish UNC-40-dependent and UNC-40-independent signaling functions of UNC-5. Expression of UNC-40 GFP in the DTCs from a multicopy array (*evEx117*) partially rescued the DTC defects of *unc-5 (e152)*, but not *unc-5(ev585)* or *unc-5(e53)* (Figure 3, D–F), providing additional evidence that the cytoplasmically truncated UNC-5 receptor is dependent upon UNC-40 for its residual function.

DISCUSSION

Extracellular guidance cues such as UNC-6/netrins and semaphorins are thought to act on migrating cells and growth cones by increasing or decreasing the extension or stabilization of membrane structures such as lamellipodia and filopodia. The mechanisms by which local concentration differences in a ligand are interpreted at the leading edge of a migrating cell or growth cone are not understood. To learn how the UNC-6 directional signal is transduced by the UNC-5 and UNC-40 receptors at the cell surface, we have examined genetic interactions between *unc-5* and *unc-40* in the ventral-to-dorsal migration phase of the DTCs. Transgenic and mosaic experiments indicate that each of the UNC-6 receptor subtypes can act cell autonomously within the DTCs at the time of this migration phase. This allows us to propose from the genetic interactions a model for how the UNC-5 and UNC-40 receptor proteins function.

The UNC-6/netrins are capable of acting as either attractant or repellant guidance cues. Of the two known UNC-6 receptors, UNC-5 is associated only with repulsive migrations, *i.e.*, away from sources of UNC-6/netrin. UNC-40, on the other hand, is involved in both attraction toward and repulsion away from UNC-6 in *C. elegans* (HEDGECOCK *et al.* 1990). This suggests that the two receptor subtypes may act together to mediate repulsion, and evidence for this interaction has been observed in *in vitro* studies of axon guidance functions of vertebrate homologues (HONG *et al.* 1999). Indeed, DTC migration phenotypes indicate that a complete response to UNC-6 requires both receptors. We observe, however, that UNC-5 can partially function in the absence of UNC-40, consistent with previous *in vivo* observations (HEDGECOCK *et al.* 1990). In addition, UNC-40 can partially function in the absence of UNC-5 to mediate repulsion of the DTCs away from UNC-6. This indicates that there are multiple mechanisms by which the UNC-6 signal can be transduced to produce DTC repulsion (Figure 4B).

Two distinct and opposing types of genetic interactions were observed between *unc-5* hypomorphic alleles. First, in the presence of wild-type UNC-40, some *unc-5* alleles exhibit a partial allelic complementation. In cases where the site of the molecular lesion is known (M. T. KILLEEN, A. KRIZUS, I. SCOTT, R. WILK, M. NYGIEM and J. G. CULOTTI, unpublished results), complementing pairs usually comprise one allele with an extracellular and one allele with an intracellular mutation. Noncomplementing pairs comprise two extracellular or two intracellular mutations. For example, *unc-5(e152)*, which encodes a protein with a predicted cytodomain truncation, complements *unc-5(ev585)*, which causes a missense mutation in the second Ig domain of the UNC-5

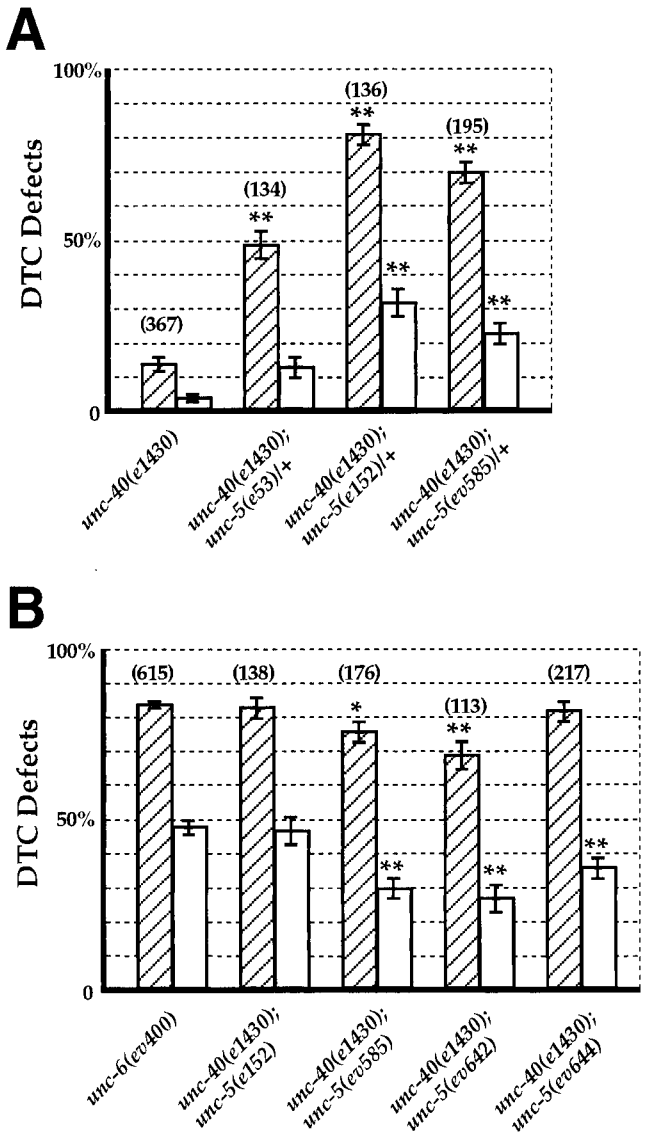

FIGURE 6.—*unc-5* mutant phenotypes in an *unc-40* null mutant background. Hatched columns represent the frequency of defects in the migrations of posterior DTCs, while open columns represent the frequency of defects of anterior DTCs. (A) Both null (*e53*) and hypomorphic (*e152* and *ev585*) *unc-5* alleles act as haploinsufficient or dominant enhancers of the DTC defects of an *unc-40* null allele [*unc-40(e1430)*]. Each of these alleles is fully recessive in a wild-type *unc-40* background. (B) The residual function in DTC migrations of the *unc-5 (e152)* cytoplasmic truncation allele is eliminated by a null mutation in *unc-40*, as *unc-40(e1430); unc-5(e152)* is identical in penetrance of DTC defects to an *unc-6* null. Double mutants of other hypomorphic *unc-5* alleles (*ev585*, *ev642*, and *ev644*) together with *unc-40* null have significantly lower penetrance of DTC defects relative to the *unc-6* null. Numbers above bars indicate the number of worms scored, each for both the anterior and posterior DTCs. Asterisks indicate significant differences at the $P < 0.05$ (single asterisk) or $P < 0.001$ (double asterisk) levels. Vertical bars indicate standard errors.

ectodomain. Interallelic complementation usually signifies the functional importance of close or direct protein-protein interactions (RAZ *et al.* 1991; SIBLEY *et al.* 1994). We propose that complementation results from one UNC-5 receptor compensating for a defect in input through the extracellular domains and the other for a defect in output to cytoplasmic signaling pathways through the intracellular domains.

In contrast to these genetic interactions in a wild-type *unc-40* background, the dominant negative interactions observed in the absence of functional UNC-40 suggest that the products of hypomorphic *unc-5* alleles can, under these conditions, interfere with the functions of wild-type UNC-5. Together, these observations strongly argue for an intimate and possibly direct association between UNC-5 proteins, in addition to that between UNC-5 and UNC-40.

The formation by the UNC-6 receptors of a variety of functional oligomeric complexes may be required to allow the sensitivity of cells and growth cones to a wide range of UNC-6 concentrations. Alternatively, different concentrations of UNC-6 may activate, through the formation of different receptor complexes, distinct cellular responses.

The idea that UNC-5 and UNC-40 cannot substitute for one another despite having the same general role in the second DTC migration phase suggests that they may have distinct downstream targets. Although signaling pathways linking transmembrane guidance receptors like UNC-5 and UNC-40 to the cytoskeleton are not well understood, there are in principle several ways in which cell adhesion and cytoskeletal dynamics may be regulated in cell migration (LAUFFENBURGER and HORWITZ 1996). As motility requires the comprehensive and coordinated function of the cytoskeleton, guidance systems may have to send several parallel signals to various targets to effectively direct the cytoskeleton. It is also possible that the UNC-5 and UNC-40 receptor subtypes regulate distinct subsets of the motility apparatus. For example, one receptor subtype may be more important for filopodial extension and the other for filopodial retraction. Although we have provided evidence that both receptor subtypes act within the DTCs at the same time, we have not examined their subcellular localization during signaling. A combination of precise localization and functional studies in combination with biochemical assays will be necessary to address these issues.

In ectopic expression studies in different systems, UNC-5 appears to absolutely require UNC-40/DCC to mediate repulsion (COLAVITA and CULOTTI 1998; HONG *et al.* 1999). As we have shown, however, this is not the case in cells that normally express UNC-5 and that are repelled by UNC-6. One reason for such differences may be that ectopic expression studies reflect an artificial situation that results in enhanced reliance on some, but not all, components of the normal signaling pathway. For example, we have proposed that cells that normally express UNC-5, which include the DTCs, express some component, possibly a coreceptor or downstream signaling partner, that allows UNC-5 to function indepen-

dently of UNC-40/DCC. This component may be absent or inactive in cells that normally exhibit only attractive responses to UNC-6/netrin cues through the UNC-40/DCC receptor subtype. In such cells, therefore, UNC-40-independent repulsive functions of UNC-5 would be absent.

Further evidence for the sensitized nature of ectopic expression studies comes from a genetic screen for suppressors of the ventral-to-dorsal axon projections caused by ectopic expression of UNC-5. This screen identified mutations at eight loci (Colavita and Culotti 1998). Most of these loci, although required for UNC-5 function in this particular set of touch sensory neurons, are not absolutely required for UNC-5 function in commissural neurons or DTCs. We have proposed (Merz and Culotti 2000) that the touch sensory neurons do not possess the ability to generate a complete response downstream of UNC-5 and that this results in a dependence upon the products of *unc-40* and other genes identified as suppressors in this screen.

UNC-5/UNC-40 signaling models: Although null mutations in *unc-40* do not completely eliminate wild-type UNC-5 function in DTC migrations, they do completely eliminate the function of some *unc-5* hypomorphic alleles. The protein products of these particular *unc-5* alleles may be defective mainly in UNC-40-independent signaling, but retain UNC-40-dependent signaling. The *unc-5(e152)* cytoplasmic truncation allele, for example, appears to require UNC-40 for its residual function. In addition, excess UNC-40 in the DTCs expressed from a transgene partially rescues the DTC migration defects of this *unc-5* allele. This suggests that the UNC-5 cytoplasmic domain encoded by *unc-5(e152)* is involved in signaling functions that largely require UNC-40, whereas the deleted portion of the cytoplasmic domain is involved in signaling functions that do not require UNC-40. Conversely, other hypomorphic alleles that do not require UNC-40 presumably retain some signaling through an UNC-40-independent pathway.

A similar paradigm for interactions between receptor subtypes has been proposed for the $p75^{NTR}$ and trkC neurotrophin receptors. Truncated trkC receptors that lack cytodomain tyrosine kinase activity retain partial signaling functions, but this residual function is dependent upon the $p75^{NTR}$ coreceptor (Hapner *et al.* 1998). This indicates the function of distinct $p75^{NTR}$-dependent and -independent signaling mechanisms for the trkC cytodomain. A different signaling model has been proposed for the two receptor subtypes for the semaphorin guidance cues. The neuropilin receptor subclass appears to increase the affinity of the semaphorin ligands for the plexin receptor subclass (Takahashi *et al.* 1999). However, cytoplasmic signaling occurs only through the plexin receptor cytodomain and does not require the cytodomain of the neuropilin receptor (Takahashi *et al.* 1999; Tamagnone *et al.* 1999).

Hong *et al.* (1999) have reported that direct physical interactions between the cytoplasmic domains of UNC-5 and DCC are required for repulsion of growth cones from a netrin source *in vitro*. It was reported that a specific region of the cytodomain of UNC-5, the DB domain, is essential for both the functional and the biochemical UNC-5-DCC interactions. However, in *C. elegans* the *unc-5(e152)* mutation that retains full dependence upon UNC-40 contains a nonsense mutation (Q507STOP) predicted to truncate UNC-5 between the transmembrane region and the domain that has homology to ZO-1 (a.k.a. the ZU5 domain), thus eliminating the proposed DB domain.

It is unclear how an UNC-5 mutation that is predicted to eliminate the functional interaction between UNC-5 and UNC-40/DCC can retain full dependency on UNC-40 for repulsive guidance mechanisms. One possibility is that UNC-5 in *C. elegans* functions differently in this respect from vertebrate UNC-5s or that functional interactions between UNC-5 and UNC-40 in DTCs are different from that in neurons. It is also possible that *in vitro* and ectopic expression experiments like those carried out in Xenopus to define the DB domain of UNC-5 are artificially sensitized to perturbations in the UNC-5 protein that, in a normal situation, might not be as important to function.

Switching of UNC-40/DCC: Studies of Xenopus retinal ganglion cell growth cone responses to exogenously applied Netrin-1 *in vitro* have revealed that levels of cAMP within the growth cone can regulate the role of DCC (Ming *et al.* 1997). Prior incubation of the neurons with an inhibitor of protein kinase A or a nonhydrolyzable analogue of cAMP appeared to switch DCC from a netrin receptor that mediates attraction to one that mediates repulsion. Other experiments suggest that the expression of the UNC-5 receptor may also act as a switching mechanism for UNC-40. For example, touch sensory axons in *C. elegans* that normally project ventrally are redirected dorsally by ectopic expression of UNC-5 in these neurons (Hamelin *et al.* 1993). UNC-40 is required for both the abnormal dorsalward and the normal ventralward axonal projections of the touch neurons (Hedgecock *et al.* 1990; Colavita and Culotti 1998). It is not known whether the cAMP and UNC-5-mediated switching mechanisms are related.

In the DTCs, UNC-40 can mediate repulsion from UNC-6 to some extent in the absence of UNC-5. It is interesting to note that UNC-40 is present in the DTCs during the longitudinal first migration phase (Chan *et al.* 1996), yet the DTCs do not exhibit a repulsive response to UNC-6 at this time. UNC-40 is presumably inactive or ineffective in mediating a repulsive response to UNC-6 until the appropriate time of initiation of the second phase of migration. At the time of the initiation of the ventral-to-dorsal migration phase, UNC-40 generates a repulsive response to UNC-6 due to the expression at this time of UNC-5 (Su *et al.* 2000) and also due to

an unidentified UNC-5-independent switching mechanism.

The UNC-6 signaling model proposed here suggests genetic screening strategies capable of isolating particular signaling pathways in the DTCs downstream of UNC-5 and UNC-40. The isolation of mutations in genes encoding downstream components will permit further examination of UNC-6 signal transduction mechanisms through the UNC-5 and UNC-40 receptors.

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LITERATURE CITED

Ackerman, S. L., L. P. Kozak, S. A. Przyborski, L. A. Rund, B. B. Boyer *et al.*, 1997 The mouse rostral cerebellar malformation gene encodes an UNC-5-like protein. Nature **386:** 838–842.

Brenner, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77:** 71–94.

Chan, S. S.-Y., H. Zheng, M.-W. Su, R. Wilk, M. T. Killeen *et al.*, 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 M. Tessier-Lavigne, 1995 The axonal chemoattractant netrin-1 is also a chemorepellent for trochlear motor axons. Cell **81:** 621–629.

Colavita, A., and J. G. Culotti, 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., V. H. Hopker, G. Ming, M. Poo, M. Tessier-Lavigne *et al.*, 1997 Turning of retinal growth cones in a netrin-1 gradient mediated by the netrin receptor DCC. Neuron **19:** 1211–1224.

Fazeli, A., S. L. Dickinson, M. L. Hermiston, R. V. Tighe, R. G. Steen *et al.*, 1997 Phenotype of mice lacking functional *Deleted in colorectal cancer (Dcc)* gene. Nature **386:** 796–804.

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

Hapner, S. J., K. L. Boeshore, T. H. Large and F. Lefcort, 1998 Neural differentiation promoted by truncated trkC receptors in collaboration with p75(NTR). Dev. Biol. **201:** 90–100.

Harris, R., L. M. Sabatelli and M. A. Seeger, 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., J. G. Culotti, D. H. Hall and B. D. Stern, 1987 Genetics of cell and axon migrations in *C. elegans*. Development **100:** 365–382.

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

Hong, K., L. Hinck, M. Nishiyama, M-m. Poo, M. Tessier-Lavigne *et al.*, 1999 A ligand-gated association between cytoplasmic domains of Unc5 and DCC family receptors converts netrin-induced growth cone attraction to repulsion. Cell **97:** 927–941.

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

Keino-Masu, K., M. Masu, L. Hinck, E. D. Leonardo, S.-S. Y. Chan *et al.*, 1996 Deleted in Colorectal Cancer (DCC) encodes a netrin receptor. Cell **87:** 175–185.

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

Kolodziej, P. A., L. C. Timpe, K. J. Mitchell, S. R. Fried, C. S. Goodman *et al.*, 1996 *Frazzled* encodes a *Drosophila* member of the Deleted in Colorectal Cancer (DCC) immunoglobulin subfamily and is required for CNS and motor axon guidance. Cell **87:** 197–204.

Lauffenburger, D. A., and A. F. Horwitz, 1996 Cell migration: a physically integrated molecular process. Cell **84:** 359–369.

Leonardo, E. D., L. Hinck, M. Masu, K. Keino-Masu, S. L. Ackerman *et al.*, 1997 Vertebrate homologues of *C. elegans* UNC-5 are candidate netrin receptors. Nature **386:** 833–838.

Leung-Hagesteijn, C., A. M. Spence, B. D. Stern, Y. Zhou, M.-W. Su *et al.*, 1992 Unc-5, a transmembrane protein with immunoglobulin and thrombospondin type 1 domains, guides cell and pioneer axon migrations in *C. elegans*. Cell **71:** 289–299.

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

Mello, C. C., and A. Fire, 1995 DNA transformation, pp. 452–482 in *Caenorhabditis elegans: Modern Biological Analysis of an Organism*, edited by H. F. Epstein and D. C. Shakes. Academic Press, San Diego.

Merz, D. C., and J. G. Culotti, 2000 Genetic analysis of growth cone migration in *C. elegans*. J. Neurobiol. **44:** 281–288.

Miller, I., and J. E. Freund, 1965 *Probability and Statistics for Engineers*. Prentice-Hall, Englewood, NJ.

Ming, G., H. Song, B. Berninger, C. E. Holt, M. Tessier-Lavigne *et al.*, 1997 cAMP-dependent growth cone guidance by Netrin-1. Neuron **19:** 1225–1235.

Mitchell, K. J., J. L. Doyle, T. Serafini, T. E. Kennedy *et al.*, 1996 Genetic analysis of Netrin genes in *Drosophila*: netrins guide CNS commissural axons and peripheral motor axons. Neuron **17:** 203–215.

Przyborski, S. A., B. B. Knowles and S. L. Ackerman, 1998 Embryonic phenotype of *Unc5h3* mutant mice suggests chemorepulsion during the formation of the rostral cerebellar boundary. Development **125:** 41–50.

Raz, E., E. D. Schejter and B. Z. Shilo, 1991 Interallelic complementation among DER/flb alleles: implications for the mechanism of signal transduction by receptor-tyrosine kinases. Genetics **129:** 191–201.

Serafini, T., T. E. Kennedy, M. J. Galko, C. Mirzoyan, T. M. Jessell *et al.*, 1994 The netrins define a family of axon outgrowth-promoting proteins homologous to *C. elegans* UNC-6. Cell **78:** 409–424.

Serafini, T., S. A. Colamarino, E. D. Leonardo, H. Wang, R. Beddington *et al.*, 1996 Netrin-1 is required for commissural axon guidance in the developing vertebrate nervous system. Cell **87:** 1001–1014.

Sibley, M. H., P. L. Graham, N. van Mende and J. M. Kramer, 1994 Mutations in the alpha2(IV) basement membrane collagen gene of *Caenorhabditis elegans* produce phenotypes of differing severities. EMBO J. **13:** 3278–3285.

Su, M.-W., D. C. Merz, M. T. Killeen, Y. Zhou, H. Zheng *et al.*, 2000 Regulation of the UNC-5 netrin receptor initiates the first re-orientation of migrating distal tip cells in *C. elegans*. Development **127:** 585–594.

Takahashi, T., A. Fournier, F. Nakamura, L. Wang, Y. Murakami *et al.*, 1999 Plexin-neuropilin-1 complexes form functional semaphorin-3A receptors. Cell **99:** 59–69.

Tamagnone, L., S. Artigiani, H. Chen, Z. He, G. Ming *et al.*, 1999 Plexins are a large family of receptors for transmembrane, secreted, and GPI-anchored semaphorins in vertebrates. Cell **99:** 71–80.

Wadsworth, W. G., H. Bhatt and E. M. Hedgecock, 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.

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