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Developmental Biology 285 (2005) 447 – 461

DEVELOPMENTAL BIOLOGY

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The *C. elegans* Frizzled CFZ-2 is required for cell migration and interacts with multiple Wnt signaling pathways

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Received for publication 15 March 2005, revised 12 July 2005, accepted 15 July 2005
Available online 18 August 2005

Abstract

Members of the Frizzled family of integral membrane proteins are implicated in many developmental events, including specifying cell fate, orienting cell and planar polarity, and directing cell migration. Frizzleds function as cell surface receptors for secreted Wnt proteins. We report here the isolation of a mutation in *cfz-2*, a *Caenorhabditis elegans* Frizzled gene. Mutation of *cfz-2* causes defective cell migration, disorganization of head neurons, and can cause ectopic axon outgrowth. Analysis of mosaic animals shows that CFZ-2 functions cell nonautonomously, but does not rule out an autonomous role. CFZ-2 is expressed primarily in the anterior of embryos and in several cells in the head of adults. Our analysis of interactions between CFZ-2 and other Wnt pathways reveals that three Wnts, CWN-1, CWN-2 and EGL-20, and a Frizzled, MOM-5, function redundantly with one another and with CFZ-2 for specific cell migrations. In contrast, CWN-1, CWN-2, EGL-20, CFZ-2, and MOM-5 antagonize one another for other migrations. Therefore, CFZ-2 functions by collaborating with and/or antagonizing other Wnt signaling pathways to regulate specific cell migrations.

Keywords: *cfz-2*; *Caenorhabditis elegans*; Cell migration; Frizzled; Wnt; *cwn-1*; *cwn-2*; *egl-20*; *mom-5*; *lin-44*

Introduction

Directed cell migration is essential for metazoan development. In vertebrates, primordial germ cells, cardiac precursors, melanocytes, and neuronal growth cones often traverse long distances during development. Cell migration is also an important component of invertebrate development. For example, many cell types migrate long distances during *Caenorhabditis elegans* development (Fig. 1; Hedgecock et al., 1987; Sulston et al., 1983). The canal-associated neurons (CANs) and anterior lateral microtubule neurons (ALMs) migrate posteriorly to positions near the middle of the animal during embryogenesis. The hermaphrodite-specific neurons (HSNs) and BDU neurons migrate anteriorly, also in embryos. The left and right Q neuroblasts (referred to as QL and QR, respectively, hereafter) and their descendants migrate during the first larval stage.

Cell signaling molecules, including Wnts, have been implicated in cell migration. Wnt signaling has been divided into canonical and several noncanonical pathways. In the canonical pathway, Wnts bind to Frizzled proteins, seven-transmembrane domain (TMD)-containing receptors that transduce a signal that ultimately stabilizes cytoplasmic β-catenin, which moves to the nucleus in association with transcription factors of the TCF/LEF family (see Logan and Nusse, 2004 for recent review). In *C. elegans*, five genes, *cwn-1*, *cwn-2*, *egl-20*, *lin-44*, and *mom-2*, encode Wnt proteins (Herman et al., 1995; Maloof et al., 1999; Rocheleau et al., 1997; Shackleford et al., 1993; Thorpe et al., 1997). Mutations in three, *egl-20*, *lin-44*, and *mom-2*, have been reported. EGL-20 has been implicated in cell migration and in orienting cell polarity (Desai and Horvitz, 1989; Forrester et al., 2004; Harris et al., 1996; Maloof et al., 1999). MOM-2 participates in a pathway that includes MOM-5/Frizzled to specify endodermal cell fates (Rocheleau et al., 1997; Thorpe et al., 1997). LIN-44 orients cell polarity (Herman and Horvitz, 1994; Herman et al., 1995). Although mutations in *cwn-2* have not been reported, disrupting *cwn-2* by RNA-

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doi:10.1016/j.ydbio.2005.07.014

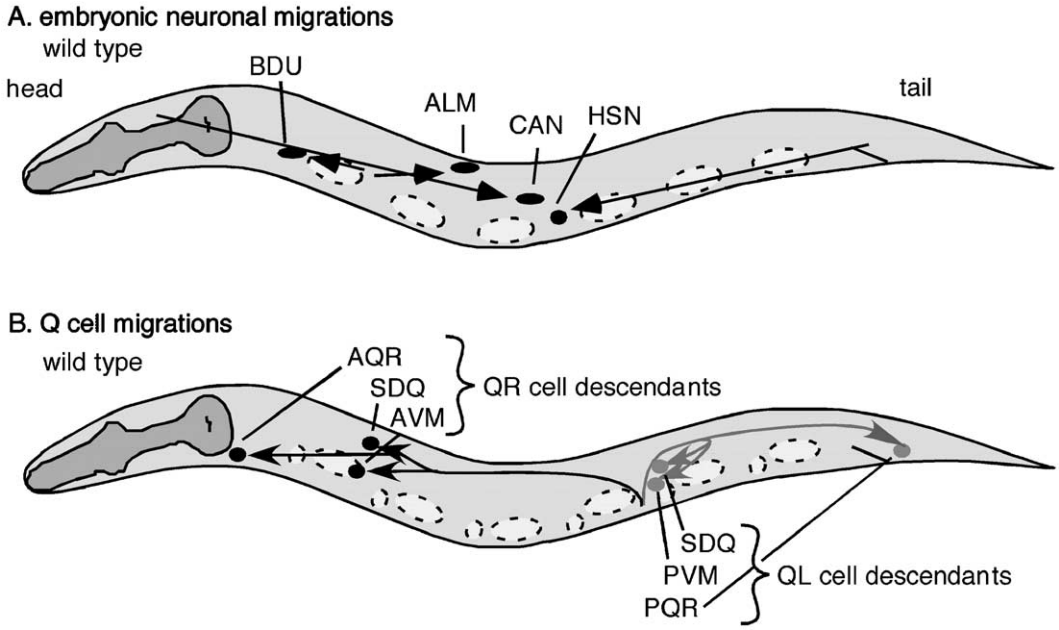


Fig. 1. *C. elegans* cell migrations. Anterior is to the left and dorsal is up in all figures. (A) Embryonic cell migrations. Schematic lateral view of a newly hatched first larval stage hermaphrodite. Both the final positions of the ALM, BDU, CAN, and HSN cell bodies (ovals and circles) and their migration routes (arrows) are indicated. Dashed ovals show the positions of some of the landmark hypodermal nuclei (V cells) used in assessing cell position. (B) Q neuroblast migrations. Schematic lateral view of an older first larval stage animal after the Q descendants have completed their migrations. Indicated are the final positions of the QR descendants, SDQ, AVM, and AQR (dark circles) and their migration routes (dark arrows), and of the QL descendants, SDQ, PVM, and PQR (gray circles) and their migration routes (gray arrows). Cell divisions and cell deaths in the Q lineages are not shown. Dashed ovals and circles show location of landmark hypodermal nuclei, Vn.a and Vn.p, used in assessing cell position.

mediated interference (RNAi) reveals that it controls specific cell fates during vulval development along with *lin-44* and *mom-2* (Inoue et al., 2004).

A canonical Wnt signal transduction pathway regulates the migration of *C. elegans* QL descendants. MAB-5, an Antennapedia homolog, functions as a key regulator of Q neuroblast migrations (Costa et al., 1998). In wild-type animals, the QL descendants migrate posteriorly, whereas the QR descendants migrate anteriorly (Sulston and Horvitz, 1977). The direction of migration of QL and QR neuroblasts and their descendants is controlled by *mab-5* expression. Expression of *mab-5* in QL results in posteriorly directed migrations, whereas the lack of *mab-5* expression in QR results in anteriorly directed migrations (Salser and Kenyon, 1992). EGL-20 and components of a canonical Wnt signaling pathway regulate *mab-5* expression in QL descendants (Harris et al., 1996; Korswagen et al., 2000; Maloof et al., 1999). Mutations that disrupt EGL-20 signaling eliminate *mab-5* expression in QL descendants, thereby causing them to migrate anteriorly (Harris et al., 1996; Maloof et al., 1999).

To further investigate cell migration, we screened for new genes required for CAN and/or HSN cell migration. Among the genes that we identified was *cfz-2*, a *C. elegans* Frizzled gene. We found that ALM and QR descendant cell migrations were defective in *cfz-2* mutants. Axon development also was defective; several neurons occasionally produced ectopic axons or axon branches. In *cfz-2* mutants, the organization of neurons in the head was disrupted. We found that *cfz-2* was primarily expressed in the anterior of embryos and in several cells in the head of adults and that its function was not required within the migrating ALM neuron, suggesting that *cfz-2* may function cell nonautonomously in directing cell migrations. We further showed that three Wnt genes, *cwn-1*, *cwn-2*, and *egl-20*, were required for cell migration (*egl-20* was previously implicated in cell migration, Forrester et al., 2004; Harris et al., 1996; Maloof et al., 1999), where they acted redundantly for the migrations of several cell types. Our results suggest that CWN-2 acts through CFZ-2 for specific cell migrations whereas CWN-1 and CWN-2 act through MOM-5/Frizzled to antagonize CFZ-2 for others.

Materials and methods

Strains and C. elegans culture

Strains were grown at 20°C and maintained as described (Brenner, 1974). In addition to the wild-type strain N2 and the wild isolate CB4856 (Hodgkin and Doniach, 1997), strains with the following mutations and transgenes were used in these studies, listed by chromosome:

LGI: *dpy-5(e61)* (Brenner, 1974), *mom-5(or57)* (Thorpe et al., 1997), *lin-44(n1792)* (Herman and Horvitz, 1994)
LGII: *cwn-1(ok546)*

LGIV: *cwn-2(ok895)*, *egl-20(mu27)* (Harris et al., 1996), *otIs33[kal-1::gfp]* (Bulow et al., 2002)
LGV: *cfz-2(cw49)*, *cfz-2(ok1201)*, *dpy-11(e224)* (Brenner, 1974), *mom-2(or309)*, *unc-60(m35)* (Brenner, 1974)
LGX: *gmIs18[ceh-23::gfp]* (provided by J. Withee and G. Garriga)
nuIS9[unc-5::gfp, myo-3::lacZ] (provided by E. Ryder and J. Culotti)

Screen for cell migration mutants

To identify cell migration defective mutants, *kal-1::gfp* (Bulow et al., 2002) animals were treated with ethyl methanesulfonate (EMS) as described (Brenner, 1974). Three to five progeny of EMS-treated parents were cultured together, and their offspring were screened for misplaced CAN and/or HSN cells using a Nikon SMZ1500 stereomicroscope equipped with epifluorescence. From a screen of approximately 13,000 haploid genome equivalents, we found twenty mutations that caused at least 30% of CAN and/or HSN cells to be misplaced.

Genetic mapping and cloning

cw49 was mapped genetically to the middle of the left arm of LGV between *dpy-11* and *unc-60*. *cw49* was found to be located between single nucleotide polymorphisms (SNPs) within cosmids F35F10 (pkP5106) and C02A12 (SNP at position 19717), by SNP mapping (Wicks et al., 2001). Examination of the predicted genes within this interval revealed the presence of *cfz-2*, a predicted Frizzled gene. To determine whether *cw49* might be a mutation within *cfz-2*, we asked whether wild-type *cfz-2* could rescue the defects of *cw49* mutants. A polymerase chain reaction (PCR) fragment that contained all of the predicted *cfz-2* coding region plus 4.7 kb of upstream sequence from wild type was used to generate transgenic animals by microinjection into *cw49* homozygous mutants (Mello et al., 1991). Of fourteen independent transgenic lines, ten were rescued and two were partially rescued. A smaller PCR fragment containing the wild-type *cfz-2* coding region with 1 kb of upstream sequence rescued three of eight lines and partially rescued three more.

The *cw49* mutation fails to complement *cfz-2(ok1201)*, a deletion within *cfz-2*. We crossed *cfz-2(cw49); kal-1::gfp* homozygous mutant males with *cfz-2(ok1201)* homozygous mutant hermaphrodites. ALMs were mispositioned anterior to the V2 nucleus in 18.6% (n = 43) of cross progeny.

DNA sequencing

To confirm that *cw49* represented a mutation in *cfz-2*, and to identify the molecular lesions present in *cfz-2*, *cwn-1*, and *cwn-2* genes, we sequenced the relevant genes from *cfz-2*, *cwn-1*, and *cwn-2* mutants, respectively, and from wild type. The complete DNA sequence of *cfz-2* was determined from at least two independent PCRs from each mutant strain and one from wild type. The same strategy was used to determine the complete DNA sequence of the mutant gene in *cwn-1(ok546)* and *cwn-2(ok895)*.

Phenotypic characterization

The extent of cell migration in wild type, mutant, and transgenic animals was determined by comparing the positions of nuclei relative to non-migratory hypodermal nuclei using Nomarski optics with a Nikon E600 microscope. For ALM, BDU, CAN, and HSN cells that migrate embryonically, we scored the positions of the nuclei of these cells relative to non-migratory hypodermal V and P nuclei in newly hatched first larval stage (L1) hermaphrodites. For the Q neuroblasts and their descendants, which migrate during the L1 stage, we scored the final positions of the Q descendant nuclei relative to the daughter hypodermal nuclei Vn.a and Vn.p derived from V1–6 in mid-L1 stage hermaphrodites. In *cwn-1; cwn-2* double mutants, we sometimes detected neurons that resembled CANs located posterior to the V4 nucleus. The neurons were located medially, about midway between the dorsal and ventral surfaces of the animal and their nuclei were oval shaped, both characteristics of CAN cells. The *ceh-23::gfp* transgene is expressed in CAN neurons, as well as several neurons in the head and tail (Zallen et al., 1999). The neurons found posterior to V4 in *cwn-1; cwn-2; ceh-23::gfp* animals expressed GFP, confirming that they were CANs (not shown).

Axon and nerve cord morphology was scored either in the presence of *kal-1::gfp* or *ceh-23::gfp* transgenes or by indirect immunofluorescence using antibodies (obtained from J. Steinbush, Free University, Amsterdam) that specifically recognized the neurotransmitter serotonin as described (Desai et al., 1988; McIntire et al., 1992).

cfz-2 mosaic analysis

We performed an analysis of animals mosaic for *cfz-2*, using *sur-5::gfp* as a cell autonomous marker as described (Yochem et al., 1998). To generate transgenic animals, a PCR fragment containing all of the predicted *cfz-2* coding region plus 4.7 kb of upstream sequence from wild type was coinjected with pTG96 (plasmid carrying a *sur-5::gfp* transgene) into *cw49* homozygous mutants (Mello et al., 1991). A GFP-positive transgenic line that rescued the ALM cell migration defect was used for further analysis. Of animals that retain the transgene in ALMs, an additional 3% of ALMs relative to wild type are misplaced. The cells used to assess losses were: for ABal lineage—CAN, m3L, m3VL; for ABar lineage—ALM, m3R, m3VR; for ABpl lineage—the excretory cell, P1/2 through P11/12 L; for ABpr—hyp10, P1/2 through P11/12 R; for E—gut; for MS—MS derived body wall muscle cells and m3DL or m3DR; for C lineage—C derived body wall muscle cells (hyp11 was also used in scoring some animals); for D

lineage—D derived body wall muscle cells. P4 losses were not assessed.

cfz-2 expression

To determine where *cfz-2* is expressed, we amplified 1.4 kb upstream of *cfz-2* including 117 nt of the coding region from wild type by PCR using 5′-CGGGGTACCATATTG-TAGCCAATACCTTTGC-3′ and 5′-CCTGCCAATTTCG-CATACACC-3′ primers. A *Kpn*I and *Hin*dIII fragment from the resulting PCR product was ligated into *Kpn*I/*Hin*dIII-cleaved pPD95.75 (gift from A. Fire lab), thereby inserting *gfp* downstream of the *cfz-2* promoter region. Following microinjection, we selected animals that stably transmitted the transgene to offspring (Mello et al., 1991). Although GFP fluorescence was not detected, we were able to detect its expression after staining with antibodies that recognized GFP (Molecular Probes). Embryos were fixed in methanol/acetone as described (Strome and Wood, 1983) and blocked in 1.5% BSA prior to antibody incubations. Antibody staining of adult animals was performed as previously reported (Desai et al., 1988), except that animals were fixed in a 1% glutaraldehyde and 1% paraformaldehyde mixture. Stacks of 0.5-μm optical sections were collected on a PerkinElmer spinning disc confocal microscope using Ultraview software (PerkinElmer Life Sciences) and displayed as projections.

Analysis of variance

Final positions of migrating cells were compared using statistical methods to assess the significance of the difference in cell positions among strains. To quantitate cell positions, the distance between the start of a migratory route and the farthest observed final wild-type cell position was divided into 100 increments (to reflect 100% migration if the cell reached the final position). Cells that migrated beyond the normal range of positions were assigned a value greater than 100% using the same scale. For ALM migration, these values were used for a one-way analysis of variance (ANOVA) using *JMP5.1* statistical software. Tukey HSD Post Hoc test was used to obtain pair-wise comparisons. Analysis of QR cell positions was the same except that the percent was averaged between the two QR cells scored within each animal.

Results

A genetic screen for cell migration defects

To identify new genes required for cell migration, we conducted a genetic screen for mutants with misplaced CAN and/or HSN cells (Materials and methods). To facilitate the identification of CANs and HSNs, we used a *kal-1::gfp* reporter transgene that expressed green fluorescent protein (GFP) in those cells (Bulow et al., 2002). Among the mutations that we isolated was *cw49*, which caused defects in both CAN and HSN cell migration.

cw49 is a mutation in cfz-2

To begin to clone the gene that is mutated in *cw49* animals, we genetically mapped the mutation using single nucleotide polymorphism (SNP) mapping (Wicks et al., 2001). *cw49* was located between SNPs contained within cosmids F35F10 and C02A12, an approximately 160-kb interval. Within this interval resides *cfz-2*, a gene that encodes a *C. elegans* Frizzled homolog (Fig. 2A). Because Frizzleds have been implicated in cell migration (Maloof et al., 1999; Ulrich et al., 2003; Whangbo and Kenyon, 1999), we determined whether *cfz-2* was mutated in *cw49* mutants. The *cfz-2* gene from *cw49* mutants contains a mutation at position 1642 (where nucleotide 1 is the A of the ATG initiator Met codon, nucleotide −117 of Wormbase sequence F27E11.3a), which changes a G to an A, thereby changing the tryptophan at amino acid position 330 to a stop codon (Fig. 2B). We believe that the actual ATG lies upstream of that predicted in Wormbase because Wormbase-predicted sequence lacks conserved amino acids within the cysteine-rich domain (Fig. 2). To confirm that *cw49* phenotypes resulted from the mutation within *cfz-2*, we introduced by microinjection (Mello et al., 1991) into *cw49* mutants the wild-type *cfz-2* genomic DNA along with approximately 4.7 kb of upstream DNA (Fig. 2A). The *cfz-2* transgene fully rescued the defects of *cw49* mutants (Fig. 2A). Furthermore, wild-type *cfz-2* including 1 kb of upstream DNA also rescued (Fig. 2A). Because *cw49* maps to the interval that includes *cfz-2*, is mutated within *cfz-2* and is rescued by wild-type *cfz-2*, we conclude that *cw49* is a mutation in *cfz-2* and refer to it as *cfz-2(cw49)* hereafter.

Recently, an allele of *cfz-2, ok1201*, which deleted much of the gene, was isolated by the International *C. elegans* Gene Knockout Consortium. *cfz-2(ok1201)* caused phenotypes similar to those of *cfz-2(cw49)* (see below) and failed to complement *cfz-2(cw49)* (Materials and methods), further supporting our conclusion that *cw49* was an allele of *cfz-2*. *cfz-2(ok1201)* deleted from nt 1645 to nt 2819, thereby removing 194 predicted amino acids (Fig. 2B). The deletion resulted in a frameshift that added 26 amino acids not normally present in CFZ-2 followed by a stop codon. Both mutations are predicted to truncate the third transmembrane domain (TMD) of CFZ-2 and remove the last four TMDs. By analogy to other mutations in Frizzleds as well as other serpentine receptors, both *cfz-2* mutations are predicted to reduce or eliminate gene function (Chen et al., 2004; Heymann and Subramaniam, 1997; Ray et al., 1997; Sawa et al., 1996; Unson et al., 1995). Consistent with this, both mutations are recessive.

CFZ-2 is most similar to Drosophila Frizzled 2, human Frizzled 8 and Xenopus Frizzled 5. The *Genefinder* computer program predicts two CFZ-2 isoforms, which differ in the final exon. However, only one of the two

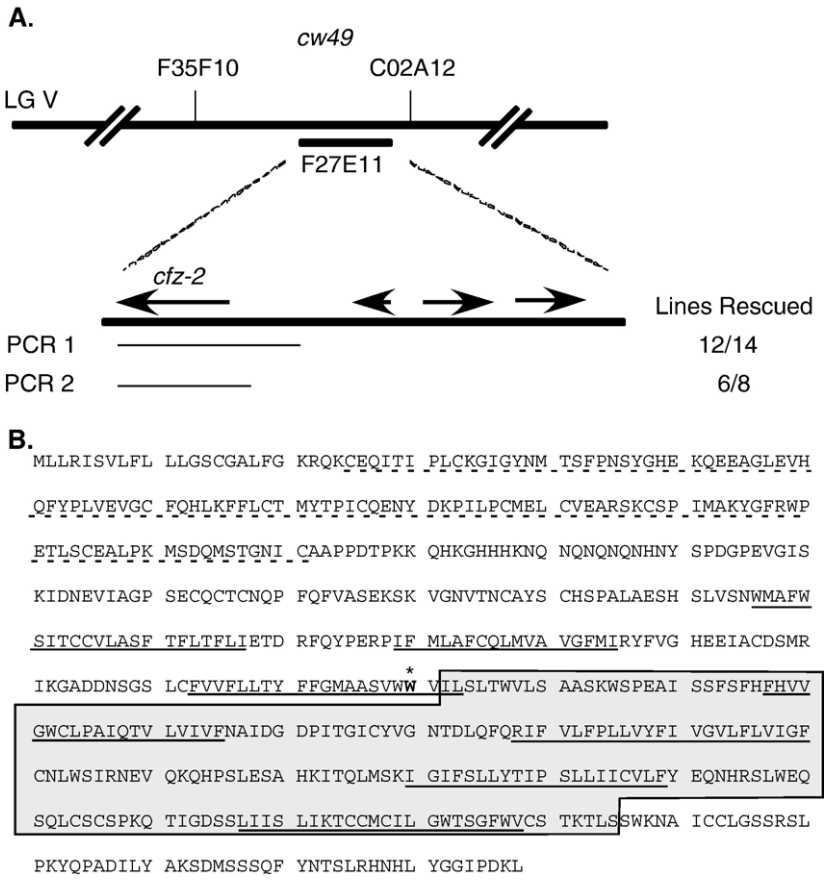


Fig. 2. (A) *cw49* mapped genetically to a region on chromosome V between SNPs within cosmids F35F10 and C02A12 that included *cfz-2*. PCR fragments that rescued *cw49* mutant phenotype are indicated. (B) The predicted amino acid sequence of CFZ-2. *cfz-2(cw49)* changes Trp330 (*) to a stop codon. *cfz-2(ok1201)* is predicted to delete the boxed amino acids and cause a frameshift that introduces 26 novel amino acids followed by a stop codon. Solid lines indicate the seven transmembrane domains as predicted by *PRED-TMR*. Dashed line indicates the predicted cysteine-rich domain.

predicted isoforms of the gene, the one shown in Fig. 2, is found among 16 cDNAs (Yuji Kohara, personal communication). Frizzled proteins contain an N-terminal cysteine-rich domain, seven predicted membrane-spanning domains, and a KTXXXW motif that is required to activate the β-catenin pathway (Umbhauer et al., 2000). CFZ-2 contains each of these motifs (Fig. 2).

cfz-2 is required for cell migration

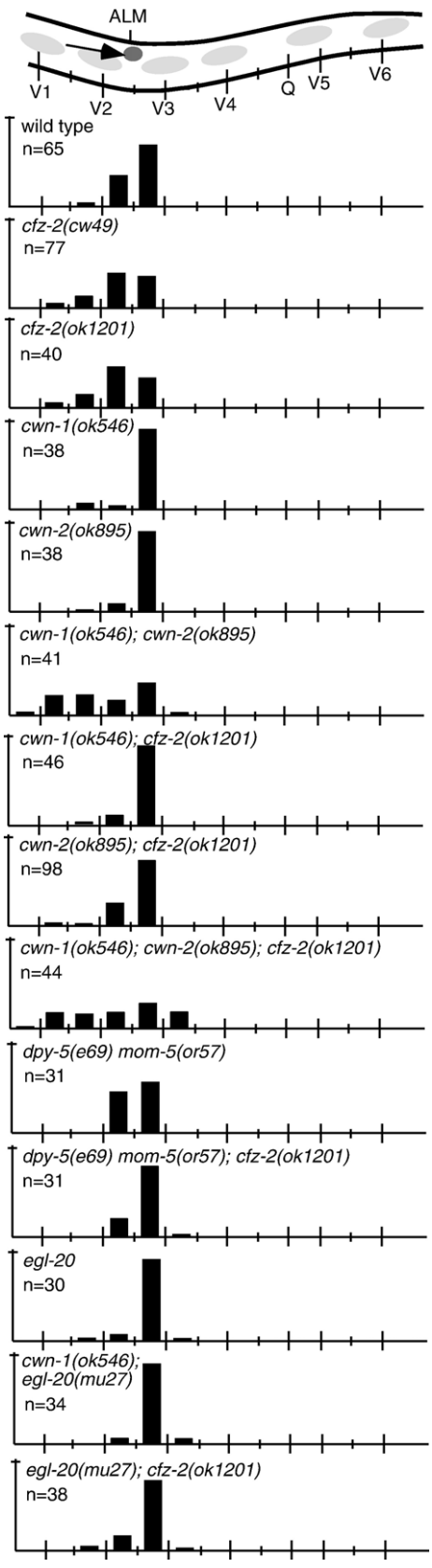
Our analysis of *cfz-2* mutants reveals that *cfz-2* is required for several cell types to migrate normally. The ALM cells migrate posteriorly during embryogenesis to occupy a range of final positions between the two hypodermal cells, V2 and V3 (Figs. 1 and 3) (Sulston et al., 1983). In *cfz-2* mutants, 18% of ALM cells are located anterior to V2 (Fig. 3, Table 1). In addition, even ALM cells that migrate to within the normal range of positions tend to be shifted anteriorly on average (Fig. 3).

QL and its descendants migrate posteriorly whereas QR and its descendants migrate anteriorly (Fig. 1; Sulston and Horvitz, 1977). In *cfz-2* mutants, the migrations of QR descendants terminated posterior to their normal position 11.8% of the time (Fig. 4, Table 1). QL descendant migration in *cfz-2* mutants is indistinguishable from wild type (Table 1).

Ironically, although *cfz-2(cw49)* was isolated in a screen for CAN and HSN cell migration defects, after backcrossing to remove unlinked mutations and the *kal-1::gfp* reporter transgene, CAN and HSN migrations are nearly normal (Fig. 5, Table 1). The *kal-1::gfp* reporter transgene apparently is responsible for this enhancement; crossing it back into outcrossed *cfz-1(cw49)* mutants restores the CAN migration defects (not shown). Similarly, minor defects in CAN and HSN migration are seen in *cfz-2(ok1201)* mutants (Fig. 5, Table 1). However, CFZ-2 does function in CAN migrations. Whereas *mom-5* and *cfz-2* Frizzled mutants have weak CAN defects on their own, *mom-5; cfz-2* double mutants display much more severe CAN migration defects (Fig. 5, Table 1), demonstrating that CFZ-2 and MOM-5 function redundantly in CAN migration.

cfz-2 influences axon development

Mutations in *cfz-2* affect HSN and CAN axon development. The ventral nerve cord (VNC) is composed of two



bundles of axons, one on the left side and one on the right, that are separated by a ridge of hypodermal tissue (White et al., 1986). The HSNs each extend an axon that grows ventrally to the VNC, where it turns anteriorly to extend ipsilaterally to the nerve ring in the head (Garriga et al., 1993; White et al., 1986). Each HSN axon defasciculates from the VNC to skirt the vulva, where it forms one or two short branches that make synaptic contacts with the egg laying muscles (Garriga et al., 1993; White et al., 1986). In adult hermaphrodites, the HSNs express the neurotransmitter serotonin (Desai et al., 1988). We examined the morphology of the HSN axons by immunofluorescent histochemistry using anti-serotonin antibodies. In *cfz-2* mutants, approximately 8% of HSNs extended a second axon, generally from the posterior side (Table 2). The ectopic axons sometimes appeared to merge with the primary axons, after which they entered the VNC (Fig. 6, Table 2). More penetrant was a misrouting defect, where approximately 24% of HSN axons inappropriately crossed the ventral midline to extend anteriorly on the contralateral side (Fig. 6, Table 2).

In males, six Pn.aap neuroblasts divide asymmetrically to produce two daughter cells, CA and CP (Sulston et al., 1980). In wild type, CP neurons extend a single axon towards the posterior and express high levels of serotonin (Loer and Kenyon, 1993). We examined the morphology of the CA and CP neurons and their axons by immunofluorescent histochemistry using anti-serotonin antibodies. We found that in 12% of *cfz-2* homozygous mutant males CP neurons produced an anteriorly extending ectopic axon in addition to the normal posteriorly extending axon and 5% had branched axons (Fig. 6, Table 2).

Each CAN produces two axons, one that extends anteriorly to the nerve ring and one that extends posteriorly to the tail (Durbin, 1987; White et al., 1986). Both *kal-1::gfp* and *ceh-23::gfp* reporter transgenes express GFP in CAN cells and their axons, as well as in other neurons (Bulow et al., 2002; Zallen et al., 1999). We used these two reporter transgenes to independently assess CAN morphology in *cfz-2* mutant animals. Most CANs appeared morphologically normal, but occasional axonal defects were detected. On average, 11% of CAN axons produce ectopic branches (Fig. 6, Table 2).

Mutations in cfz-2 disrupt the organization of the anterior ganglion

Loss of CFZ-2 resulted in disorganization of the anterior ganglion. The *kal-1::gfp* reporter transgene is expressed in

Fig. 3. ALM cell migration. At the top is a schematic of the middle section of an animal with the ALM cell (dark circle) and its migration route (arrow). Light ovals and long hash marks below show V cells (named) and Q. Bars represent percentage of ALM cells located at that position along the anterior–posterior axis of L1 larvae as assessed by Nomarski optics. Long hash marks on the *x* axis denote location of V and Q cell nuclei and short hash marks denote the location of P cell nuclei. Hash mark on the *y* axis indicates 100%. *n* = number of ALM cells tallied.

Table 1
Cell migrations[a]

Strain	ALM[b]		BDU[c]	CAN[d]		HSN[e]	QL[f]	QR[g]
	Ant.	Post.		Ant.	Post.			
wild type[h]	3.1 (65)	0 (65)	0 (65)	1.5 (65)	0 (65)	1.5 (65)	0 (57)	3.1 (32)
cfz-2(cw49)	18.2 (77)	0 (77)	0 (77)	2.6 (77)	0 (77)	1.3 (77)	0 (34)	11.8 (34)
cfz-2(ok1201)	20.0 (40)	0 (40)	0 (40)	2.5 (40)	0 (40)	2.5 (40)	0 (58)	11.7 (60)
cwn-1(ok546)	7.9 (38)	0 (40)	18.4 (38)	0 (38)	0 (38)	0 (36)	0 (32)	72.7 (33)
cwn-2(ok895)	2.6 (38)	0 (38)	44.7 (38)	23.7 (38)	0 (38)	0 (38)	0 (30)	46.7 (30)
egl-20(mu27)	3.3 (30)	3.3 (30)	0 (30)	0 (30)	0 (30)	80.0 (30)	96.3 (54)	93.8 (32)
lin-44(n1792)	0 (31)	0 (31)	0 (31)	0 (31)	0 (31)	0 (31)	0 (29)	5.4 (37)
mom-2(or309)	0 (28)	0 (28)	0 (28)	0 (28)	0 (28)	0 (28)	0 (36)	0 (36)
cwn-1(ok546); cfz-2(ok1201)	2.2 (46)	0 (46)	15.2 (46)	2.2 (46)	0 (46)	4.3 (46)	0 (42)	94.3 (35)
cwn-2(ok895); cfz-2(ok1201)	5.1 (98)	0 (98)	45.5 (99)	39.4 (99)	0 (99)	2.1 (97)	0 (30)	51.6 (31)
cwn-1(ok546); cwn-2(ok895)	48.8 (41)	2.4 (41)	71.4 (42)	35.7 (42)	33.3 (42)	60.0 (40)	0 (32)	90.3 (31)
cwn-1(ok546); egl-20(mu27)	2.9 (34)	5.9 (34)	12.1 (33)	5.9 (34)	0 (34)	100 (34)	9.3 (43)	100 (30)
cwn-1(ok546); cwn-2(ok895); cfz-2(ok1201)	36.4 (44)	18.2 (44)	77.3 (44)	75.0 (44)	0 (44)	67.5 (43)	0 (30)	95.0 (40)
dpy-5(e61) mom-5(or57)[i]	0 (31)	0 (31)	6.7 (30)	6.3 (32)	0 (32)	13.3 (30)	0 (33)	86.7 (30)
dpy-5(e61) mom-5(or57); cfz-2(ok1201)	3.2 (31)	0 (31)	3.4 (29)	29.0 (31)	0 (31)	0 (31)	0 (43)	100 (30)
egl-20(mu27); cfz-2(ok1201)	5.3 (38)	0 (38)	0 (36)	5.3 (38)	0 (38)	72.2 (34)	90.6 (32)	56.0 (25)

[a] Cell positions were assessed by Nomarski optics. ALM, BDU, CAN, and HSN positions were determined in newly hatched hermaphrodite larvae (L1). QR and QL descendant positions were determined in older L1 stage hermaphrodites after the V cells had divided. Numbers are percentage of cells that failed to migrate to their normal position. Numbers in parentheses are number of cells scored.

[b] An ALM was scored as anteriorly misplaced (Ant.) if its nucleus was anterior to the V2 nucleus and posteriorly misplaced (Post.) if posterior to the V3 nucleus.

[c] A BDU was scored as defective if its nucleus was posterior to the V1 nucleus.

[d] A CAN was scored as anteriorly misplaced (Ant.) if its nucleus was anterior to the V3 nucleus and posteriorly misplaced (Post.) if posterior to the V4 nucleus.

[e] An HSN was scored as defective if its nucleus was posterior to the V4 nucleus.

[f] A QL cell descendant was scored as misplaced anteriorly if its nucleus was anterior to V4.p. Because they occupy positions near each other, the data for SDQL and PVM were combined. The position of PQR, a third QL descendant, was not included because it migrates to a location near other nuclei with similar morphology.

[g] A QR cell descendant was scored as defective if its nucleus was posterior to the V2.a nucleus. Because they occupy positions near each other, the data for SDQR and AVM were combined. The position of AQR, a third QR descendant, was not included because it migrates to a location near other nuclei with similar morphology.

[h] Some of these data have been reported elsewhere (Kim and Forrester, 2003). They are presented here for comparison.

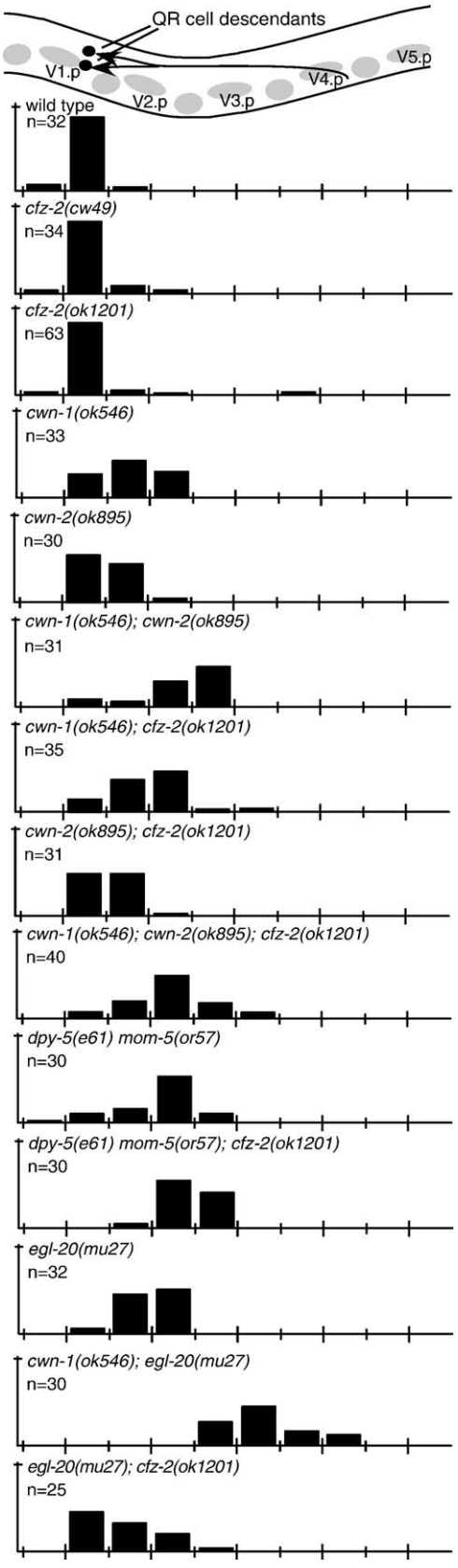
[i] These animals were homozygous *dpy-5 mom-5* mutant progeny of *dpy-5 mom-5/nt2* parents. Cell migration is normal in *dpy-5* homozygous mutant animals.

several cells of the anterior ganglia (Bulow et al., 2002). In wild-type animals, the neurons are organized relatively compactly to form the head ganglion (Fig. 7; White et al., 1986). Located halfway between the anterior and posterior bulb of the pharynx is the nerve ring, the major neuropil in the head (White et al., 1986). We noticed that in *cfz-2* mutant animals, several *kal-1::gfp*-expressing cell bodies were shifted anterior 75% of the time, a phenomenon seen in only 13% of the wild-type animals (Fig. 7). Furthermore, the nerve ring was shifted anteriorly in 85% of the animals and was spread more diffusely over a larger area of the head of the animal (Fig. 7).

cfz-2 may act cell nonautonomously for ALM cell migration

To determine whether *cfz-2* acted autonomously within ALM for cell migration or instead acted cell nonautonomously, we examined animals mosaic for *cfz-2*. *cfz-2* mutant animals containing a mitotically unstable transgene that included wild-type *cfz-2* along with the *sur-5::gfp* cell autonomous marker (Yochem et al., 1998) were generated. We identified both animals in which ALM retained GFP expression, indicating that wild-type *cfz-2* was present, and others, in which GFP expression had been lost from ALMs, indicating that ALMs had lost wild-type *cfz-2*. We then assessed ALM position.

In order to ascertain whether or not CFZ-2 functions cell autonomously, we used several strategies. First, we looked for animals with misplaced ALM neurons. These animals were then scored for the presence of the extrachromosomal array within the ALMs by assessing GFP expression. Of 27 animals with misplaced ALM neurons, 23 retained the array. The remaining four with misplaced ALMs that lacked the array had lost the transgene from other cell lineages as well. Second, we looked for mosaic animals in which the extrachromosomal array was lost from ALM neurons. All eleven ALMs found using this strategy were positioned normally. Lastly, we specifically looked for mosaic animals that had lost the array from any lineage without first assessing ALM position or GFP expression. Using this strategy, we found three animals with simultaneous losses from the AB lineage and the MS, C, or D cell lineages. In all



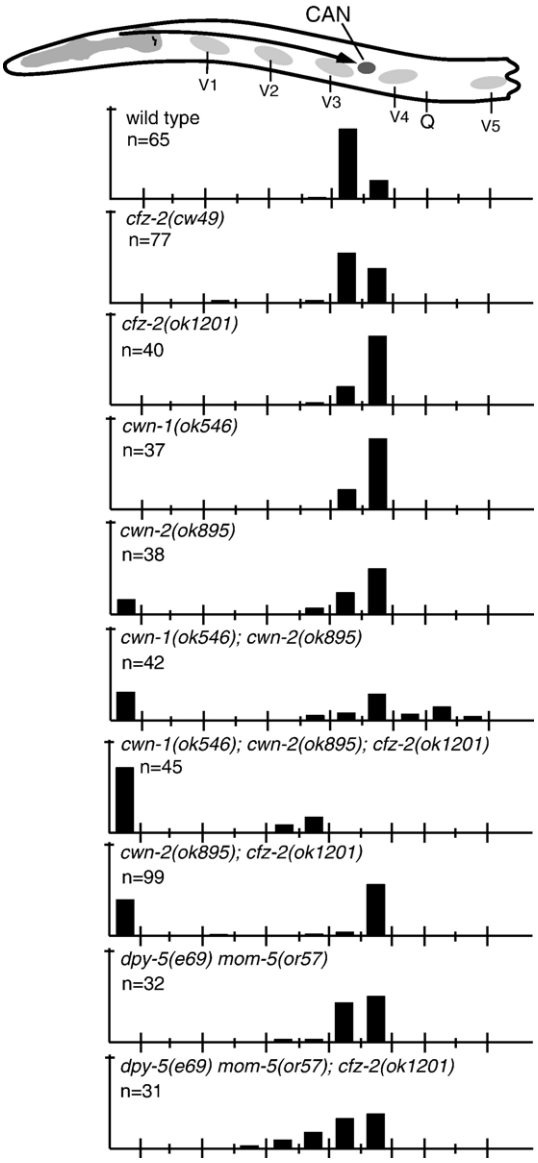


Fig. 5. CAN cell migration. Shown at the top is a schematic of the anterior section of an animal with the CAN cell (dark circle) and its migration route (arrow). Data are presented as described in the legend to Fig. 3.

three animals, ALM neurons were misplaced but retained the array. An additional 25 mosaic animals were found to have lost the array from ALM as well as other lineages. ALM was misplaced in only one of the 25 animals.

Fig. 4. QR descendant migration. At the top is a schematic lateral view of the middle section of a late first larval stage (L1) animal. Light gray circles and ovals show position of landmark Vn.a and Vn.p nuclei (each Vn.p is named). The final positions of the cell bodies of the QR descendants, SDQ and AVM (dark circles) and their migration routes (dark arrows) are indicated. Bars represent percentage of QR descendants located at that position along the anterior–posterior axis of L1 larvae. The long hash marks on the x axis indicate the location of Vn.p nuclei and the short hash marks indicate the location of Vn.a nuclei. The hash mark on the y axis denotes 100%. Data for SDQ and AVM were combined. AQR was not included because it migrates to a location near other neurons, making its position difficult to score.

Table 2
Axon development[a]

Strain	HSN			CP		CAN
	Ectopic axon[b]	Posterior axon[c]	Crossover[d]	Anterior axon[e]	Branching[f]	Branching[f]
wild type	0 (34)	0 (34)	0 (34)	0 (39)	0 (39)	n/a[g]
kal-1::gfp	n/a	n/a	n/a	n/a	n/a	0 (70)
ceh-23::gfp	n/a	n/a	n/a	n/a	n/a	3.7 (54)
cfz-2(cw49)	9.1 (77)	0 (52)	21.2 (52)	12.2 (41)	4.9 (41)	11.4 (50)[h]
cfz-2(ok1201)	7.9 (38)	2.6 (38)[i]	25.9 (27)	n/a	n/a	n/a
cwn-1(ok546)	0 (38)	7.9 (38)[j]	33.3 (15)	66.7 (6)	0 (6)	n/a
cwn-2(ok895)	0 (33)	0 (33)	0 (12)	31.3 (16)	12.5 (16)	9.7 (62)[k]
cwn-1(ok546); cwn-2(ok895)	0 (34)	8.8 (34)	58.3 (24)	n/a	n/a	n/a

[a] Axon morphology was examined by indirect immunofluorescence using anti-serotonin antibody (HSN and CP) or two independent GFP-expressing reporter transgenes, *ceh-23::gfp* and *kal-1::gfp* (CAN). Numbers are percentage of axons that produced the phenotype. Numbers in parentheses indicate number of neurons scored.
[b] Percentage of HSNs that produced an ectopic axon that extended anteriorly.
[c] Percentage of HSNs that produced an ectopic axon that extended posteriorly.
[d] Percentage of HSN axons that crossed to the contralateral side of the VNC.
[e] Percentage of CPs that produced an ectopic short anterior projection.
[f] Percentage of CP or CAN axons that produced an ectopic branch.
[g] Not assessed.
[h] Percentage is the average obtained with the two reporter transgene strains. Number in parentheses is the average number of CANs scored with each of the two reporter transgenes.
[i] This animal had a posteriorly directed HSN axon only.
[j] Apart from ectopic posterior axon formation, an additional 7.9% of the animals produced an HSN axon that was misguided posteriorly before rerouting in the anterior direction.
[k] Assessed only with *ceh-23::gfp* transgene.

In summary, we found 26 misplaced ALMs that retained the array, five misplaced ALMs that had lost the array and 35 normally positioned ALMs that had lost the array. Of 26 misplaced ALMs that retained the array, we could identify sites of loss in ten. All had losses from multiple lineages, but nine had in common losses within the MS lineage, suggesting a possible focus of CFZ-2 function. Of 40 animals that had lost GFP expression from ALMs, five, or 12.5%, were mispositioned. In all five, the transgene had been lost from other lineages besides the ALM lineage. Therefore, our data suggest a cell nonautonomous role for CFZ-2 in ALM cell migration, but do not rule out a cell autonomous function.

cfz-2 expression

To identify the sites of *cfz-2* expression, we generated a reporter transgene that fused 1.4 kb of DNA upstream of *cfz-2* to *gfp*. This promoter fragment was sufficient to provide rescue when upstream of *cfz-2* (Fig. 2). GFP expression was first seen early in embryogenesis (~100 cell stage) in the anterior of the embryo (data not shown). By the time of embryonic elongation, staining was observed in specific cells in the anterior of the embryo and a few cellular projections (Fig. 8). Several cells in the posterior and the midbody of the embryo also expressed GFP at this stage (Fig. 8). By hatching, the GFP expression became restricted to a few cells in the head, and a pair of cells in the tail (data not shown). We did not detect GFP in QR descendants, but could not unambiguously determine whether GFP was expressed in the ALMs during embryonic elongation. GFP was not detected in ALMs at the threefold stage.

Adult animals that contained the transgene produced GFP within two pairs of head neurons and within at least two cells in the posterior pharynx bulb (Fig. 8). Diffuse staining was also observed throughout the pharynx (Fig. 8). Although we could not identify the specific neurons, the morphology and location of one pair suggested that they might be IL or URA neurons. IL1s, UNC-5::GFP-expressing neurons (E. Ryder, personal communication), were shifted anterior in *cfz-2* mutant animals (data not shown). Identical patterns of expression were detected in two independently produced transgenic lines.

Candidate CFZ-2 ligands

Frizzled proteins are receptors for Wnt molecules (Bhanot et al., 1996; Dann et al., 2001; Hsieh et al., 1999). The *C. elegans* genome contains five Wnt genes, *egl-20*, *lin-44*, *mom-2*, *cwn-1*, and *cwn-2* (Herman et al., 1995; Maloof et al., 1999; Rocheleau et al., 1997; Shackleford et al., 1993; Thorpe et al., 1997). Mutations in three, *egl-20*, *lin-44*, and *mom-2*, have been described previously (Herman et al., 1995; Maloof et al., 1999; Rocheleau et al., 1997; Thorpe et al., 1997). Recently, mutations in *cwn-1* and *cwn-2* have been isolated by the International *C. elegans* Gene Knockout Consortium. To molecularly characterize the mutations in *cwn-1* and *cwn-2*, we amplified the *cwn-1* and *cwn-2* genes from the respective mutant strains by PCR and determined the

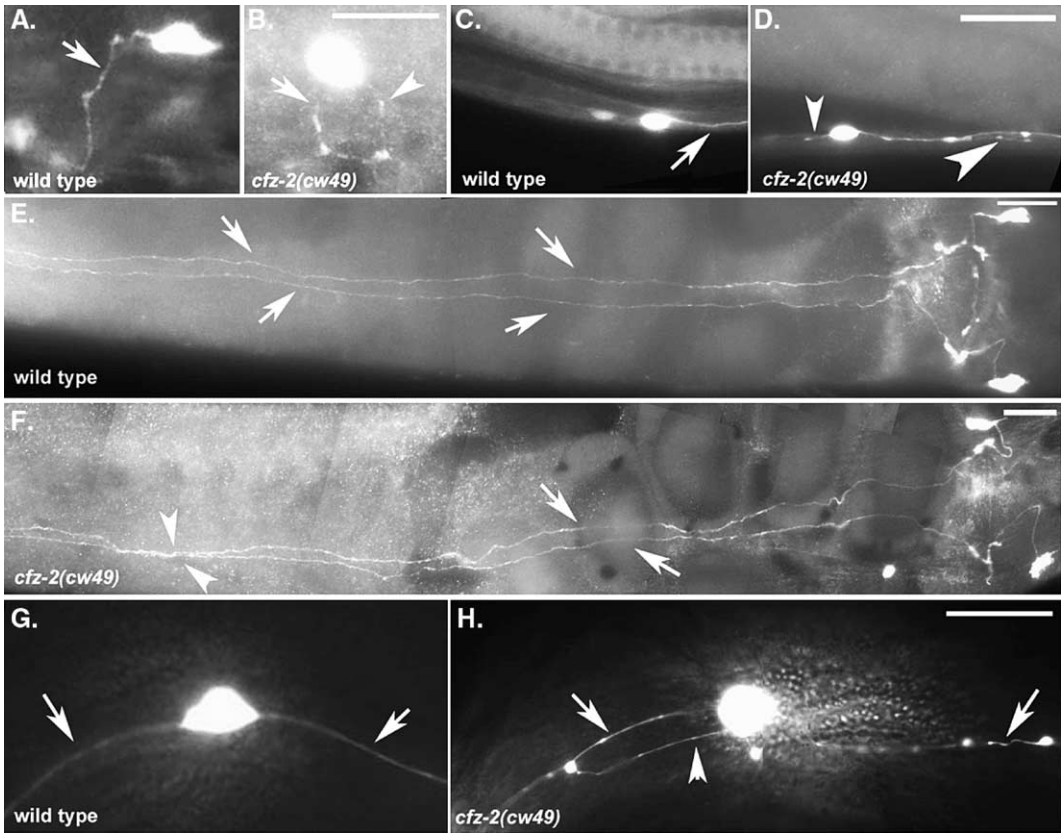


Fig. 6. Representative axon development defects. (A–F) Immunofluorescent photomicrographs of adult animals stained with anti-serotonin antiserum. (A) A single long HSN axon (arrow) is produced in wild type. (B) A second axon (arrowhead) is sometimes seen in mutants. (C) Male-specific CP neurons extend axons (arrow) posteriorly in wild type. (D) In mutants, CP neurons sometimes produce a second axon that extends anteriorly (small arrowhead) and ectopic branches (large arrowhead). (E) Each HSN axon (arrows) extends ipsilaterally to the head in wild type. (F) HSN axons sometimes cross to the opposite side in mutants (arrowheads). (G, H) Fluorescent photomicrographs of late fourth larval stage animals containing the *ceh-23::gfp* reporter transgene. (G) A single axon extends anteriorly and a second posteriorly (arrows) from each CAN in wild type. (H) CAN axons sometimes branch (arrowhead) in mutants. Scale bars indicate 20 μm.

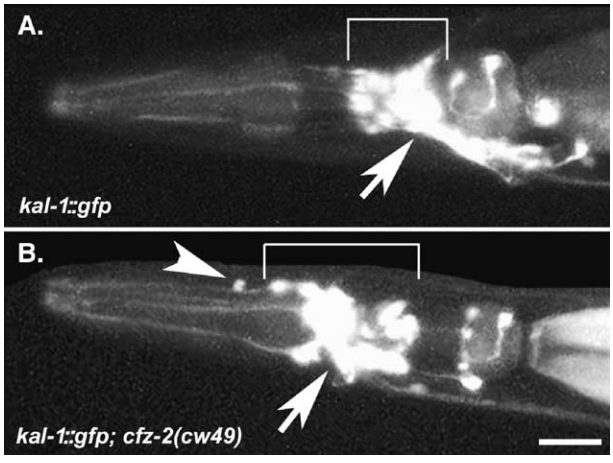


Fig. 7. The organization of neurons in the anterior ganglion is disrupted in *cfz-2* mutants. Fluorescent photomicrographs of adult animals containing the *kal-1::gfp* transgene. (A) In wild type, the neurons are in a relatively compact arrangement (bracket). (B) In *cfz-2(cw49)* mutants, anterior neurons (arrowhead) and the nerve ring (arrow) are often misplaced anteriorly. Scale bar indicates 20 μm.

sequence of the DNA. *cwn-1(ok546)* deletes from nucleotide 317 through 1101, thereby deleting exons 2, 3, and the majority of exon 4 (Wormbase sequence K10B4.6a and K10B4.6b). *cwn-2(ok895)* deletes from nucleotide 482 through 1386 thereby deleting part of exon 2 through exon 5 (Wormbase sequence W01B6.1). Because in each mutant one deletion endpoint is located within an intron, it is possible that a truncated protein is produced as a result of alternative splicing. However, because each deletion removes a large portion of the gene, each is likely to eliminate gene function.

We investigated the possibility that each of the five Wnts might act through CFZ-2 by examining cell migrations in individual Wnt mutants. Our expectation was that mutation of a Wnt that acted through CFZ-2 might cause phenotypes similar to those of *cfz-2* mutants. Mutations in *cwn-1*, *cwn-2*, and *egl-20* disrupted the migrations of QR descendant cells, as well as others, raising the possibility that they could function through CFZ-2 (Table 1; Desai et al., 1988; Forrester et al., 2004; Harris et al., 1996). In contrast, mutations in other Wnt genes either weakly affected QR descendant migration and no other migration

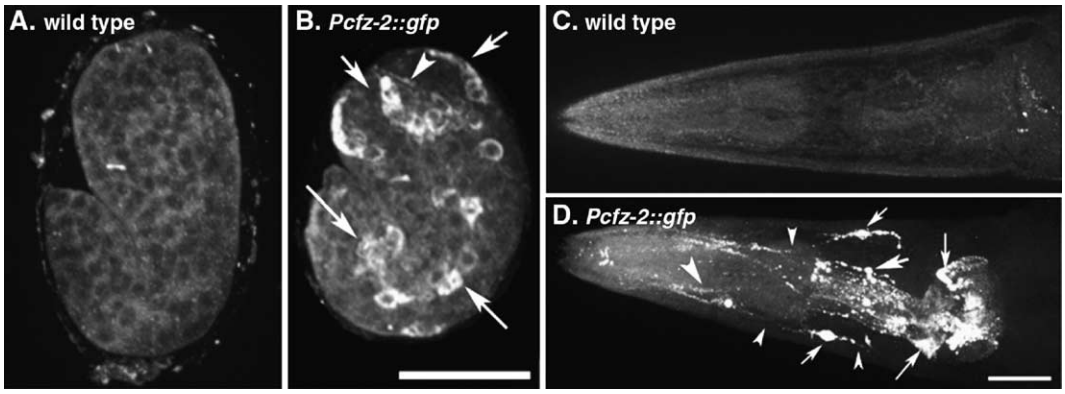


Fig. 8. *cfz-2* is expressed primarily in the anterior of the embryos and in two pairs of anterior neurons and within the pharynx in adults. Immunofluorescent photomicrographs of embryos and adult animals stained with anti-GFP antiserum. (A) Wild-type control. (B) *Pcfz-2::gfp*. GFP is expressed primarily in rows of cells in the anterior (arrows), as well as their processes (arrowhead). At this stage, GFP is also seen in a row of cells in the midbody region and the posterior of the embryo (long arrows). (C) Wild-type control. (D) *Pcfz-2::gfp*. A pair of neurons (arrows) extends an axon anteriorly and an axon posteriorly (arrowheads). A second pair of neurons expresses GFP, one cell is indicated (large arrow) and sends an axon anteriorly (large arrowhead). At least two other cells express GFP in the posterior bulb of the pharynx (long arrows). Diffuse staining is also detected throughout the pharynx. Scale bar indicates 20 μm.

(*lin-44*), or did not affect cell migrations (*mom-2*) (Table 1). We focused on *cwn-1*, *cwn-2*, and *egl-20* because they produced defects in QR descendant cell migration (Fig. 4, Table 1), a *cfz-2* phenotype. We found that both *cwn-1* and *cwn-2* mutations not only affected migrations of subsets of neurons (Table 1), but also resulted in defects in axon development and guidance (Table 2).

CWN-2 functions with CFZ-2 for some, but not all cell migrations

Because mutations in *cwn-1*, *cwn-2*, and *egl-20* affected migrations of some of the same neurons as did mutations in *cfz-2*, we next tested the possibility that they might signal through CFZ-2. If this model is correct, then we expect individual cell migrations in animals doubly mutant for Wnt and *cfz-2* to be similar in severity to either single mutant. This result was seen for some migrations. For example, QR descendant cell migration was similar in *cwn-2; cfz-2* double mutants to *cwn-2* single mutants ($P = 0.875$), suggesting that CWN-2 might act through CFZ-2 in QR descendant migration (Table 1). Interestingly, mutations in *cfz-2* significantly enhanced the CAN descendant migration defect of *cwn-2* ($P = 0.047$), suggesting that CWN-2 does not act through CFZ-2 for CAN migration (Table 1). In addition, we noted that CWN-1 and CWN-2 acted redundantly for many cell migrations, including QR descendant and CAN migrations (Table 1).

CWN-1, CWN-2, EGL-20, and MOM-5 act as CFZ-2 antagonists in directing ALM migration

We found that mutation in either *cwn-1*, *cwn-2*, or *egl-20* suppressed the ALM migration defect of *cfz-2* mutants (Fig. 3, Table 1). The ALM migration defect in animals mutant for *cwn-1*, *cwn-2*, or *egl-20* and *cfz-2* was significantly different from that of *cfz-2* alone ($P < 0.0001$), but not significantly different from wild type. These surprising results suggest that CWN-1, CWN-2, and EGL-20 might antagonize CFZ-2 function. In addition, mutation in *cfz-2* suppressed the QR descendant migration defect of *egl-20* mutants (Fig. 4, Table 1), indicating that while both CFZ-2 and EGL-20 function to direct QR cell migration, they may do so in opposing ways.

We wondered whether CWN-1 and CWN-2 might signal through a Frizzled other than CFZ-2 for ALM migration. A candidate was suggested by the cell migration defects of *mom-5* mutants. Mutation in *mom-5* is maternal-effect lethal. Therefore, we assessed cell position in homozygous mutant offspring of heterozygous mothers. Although ALM cell migration resembles wild type in *mom-5* mutants, we found that several other cell migrations were disrupted. As with Wnt mutations, we found that mutation in *mom-5* suppressed the ALM migration defect of *cfz-2* mutants (Fig. 3, Table 1). The migration defect of double mutant animals was significantly different from that of *cfz-2* alone ($P < 0.0001$; Table 1), but not from wild type, indicating that MOM-5 might antagonize CFZ-2 function, similar to CWN-1, CWN-2, and EGL-20. These data suggest that these Wnts might act through MOM-5 to antagonize CFZ-2 signaling.

CWN-1, CWN-2, and CFZ-2 function in specifying cell position

In addition to reading the cues that direct them, migrating cells must also recognize when they reach their proper destinations. Our data suggest that Wnt signaling functions in specifying final cell position. In *cwn-1; cwn-2* double mutants, ALM cells occasionally and CAN cells frequently migrate beyond their normal destinations, a defect not seen in wild type or in other mutants (Fig. 3, Table 1). This observation suggests that CWN-1 and CWN-2 act redundantly to prevent ALM and CAN from migrating too far. Furthermore, mutation in *cfz-2* enhanced the ALM over-

migration phenotype of *cwn-1; cwn-2* double mutants suggesting that a third pathway may be involved. Interestingly, mutation in *cfz-2* suppresses the CAN cell over-migration defects of *cwn-1; cwn-2* double mutants (Fig. 5, Table 1), suggesting that *cfz-2* antagonizes *cwn-1; cwn-2* function in CANs. Alternatively, it may simply reflect the enhanced CAN migration defect in the triple mutants; CANs are much more often shifted anteriorly in their final position in *cwn-1; cwn-2; cfz-2* triple mutants than in the *cwn-1; cwn-2* double mutant.

Mutations are likely to eliminate gene function

Most mutations used in these studies are likely to severely reduce or eliminate function. Each *cfz-2* mutation is predicted to remove approximately half of the protein. Our expectation is that such protein would not retain function. Consistent with this, mutations that truncate Drosophila Frizzled 2 after the fifth and last transmembrane domains or a mutation that truncates *C. elegans* LIN-17 after the third transmembrane domain produce phenotypes that are indistinguishable from null mutations (Chen et al., 2004; Sawa et al., 1996). Similarly, mutations that truncate other serpentine receptors eliminate their ability to transduce extracellular signals apparently by blocking protein localization to the cell surface (Heymann and Subramaniam, 1997; Ray et al., 1997; Unson et al., 1995).

Both *cwn-1(ok546)* and *cwn-2(ok895)* deletions are predicted to delete a large portion of each protein. Only the first 54 amino acids of CWN-1 or 76 amino acids of CWN-2 are predicted to be retained in the respective mutants. Therefore, *cwn-1(ok546)* and *cwn-2(ok895)* are likely to be null. *egl-20(mu27)* changes a highly conserved cysteine and produces phenotypes similar to those produced by mutations that are predicted to eliminate gene function, suggesting that it may be null (Maloof et al., 1999).

Discussion

In a screen for genes required for cell migration, we isolated the first mutation in *cfz-2*, a *C. elegans* Frizzled gene. CFZ-2 functions in cell migration, prevention of ectopic axon formation, and organization of head ganglia. CWN-1, CWN-2, and EGL-20 Wnts are required for some of the same developmental events as CFZ-2. CFZ-2 acts partially redundantly with MOM-5, a second Frizzled gene, for some cell migrations and antagonistically for others.

CFZ-2 functions in cell migration and axon development

Mutations in *cfz-2* disrupt ALM and QR descendant cell migration, and only weakly affect CAN and HSN migration, despite having been found originally in a screen for mispositioned CANs and HSNs. The CAN cell migration defects in the original *cfz-2(cw49)* mutant strain can be largely attributed to an enhancement that results from the *kal-1::gfp* reporter. When the *kal-1::gfp* transgene is reintroduced into outcrossed *cfz-2(cw49)* mutants, CAN cells often are misplaced (not shown).

Besides cell migration defects, CANs, HSNs, and male CP neurons sometimes produced extra axon branches in *cfz-2* mutants and the neurons of the head ganglion were disorganized. These observations demonstrate that CFZ-2 is required for cell migration and neuronal development. Molecular analysis of *cfz-2(cw49)* and *cfz-2(ok1201)* suggests that both mutations are likely to eliminate gene function. Therefore, the phenotypes that we describe are likely to reflect the function of wild-type CFZ-2 during *C. elegans* development.

Whether the defects in *cfz-2* mutants reflect a single or multiple activities of CFZ-2 is unclear. Mosaic analysis revealed a nonautonomous requirement for CFZ-2 in ALM cell migration but did not rule out an autonomous function as well. One possibility is that nonautonomous CFZ-2 specifies the fate of some cell or cells that, in turn, provide guidance cues or substrate for migrating cells and autonomous CFZ-2 functions in migrating cells to specify fate or direct their migrations. In embryos, a *cfz-2::gfp* promoter fusion was expressed in multiple anterior cells and in a few cells each in the middle and posterior of the animal. The lack of detectable expression within Q cells or their descendants is consistent with a nonautonomous role in QR. The pattern of expression does not unambiguously support either autonomous or nonautonomous roles for other CFZ-2 functions.

Specific Wnts may function through CFZ-2 for individual cell migrations

Frizzled receptors bind secreted Wnt molecules via their CRDs (Bhanot et al., 1996; Dann et al., 2001; Hsieh et al., 1999; Lin et al., 1997, but for a different view, see Chen et al., 2004). Therefore, CFZ-2 is likely to function as a Wnt receptor. The *C. elegans* genome contains five predicted Wnt genes, *lin-44*, *egl-20*, *mom-2*, *cwn-1*, and *cwn-2* (Herman et al., 1995; Maloof et al., 1999; Rocheleau et al., 1997; Shackleford et al., 1993; Thorpe et al., 1997). Mutations in *egl-20* cause QR descendant and HSN cell migration defects (Forrester et al., 2004; Harris et al., 1996; Maloof et al., 1999). Mutations in *cwn-1* cause ALM and QR descendant cell migration defects. Mutations in *cwn-2* cause QR descendant cell migration defects and anterior neuron disorganization (Table 1 and data not shown). Therefore, mutations in *cwn-1*, *cwn-2*, or *egl-20* each produce a subset of *cfz-2* phenotypes, suggesting that they could act through CFZ-2 (Table 1).

So how might CWN-1, CWN-2, and EGL-20 act with CFZ-2? In the simplest model, these Wnts act as ligands for CFZ-2 for specific cell migrations. Our data support this simple model for CWN-2 and CFZ-2 in QR descendant migration. QR descendant cell migrations are no more severely defective in *cwn-2; cfz-2* double mutants than in

cwn-2 single mutants, suggesting that CWN-2 may function through CFZ-2. If so, CWN-2 presumably also acts via another receptor because the QR cell migration defects of *cwn-2* mutants are more severe than those of *cfz-2* alone.

However, our data do not support the simple model that CWN-1, CWN-2, and EGL-20 act through CFZ-2 for other migrations (Table 1). Mutations in *cfz-2* enhance the CAN migration defect of *cwn-2* mutants and the QR descendant migration defect of *cwn-1* mutants. This suggests a division of function, with CWN-2 regulating QR descendant cell migration via CFZ-2 and CAN cell migration via another receptor. Similarly, CWN-1 may specifically regulate QR descendant cell migration via another receptor. How specificity of Wnt function via different receptors is achieved is unclear.

Mutations in *cwn-1* or *cwn-2* produce BDU cell migration defects, a phenotype not seen in *cfz-2* mutants, suggesting that they act via a non-CFZ-2 signaling pathway in directing BDU migration. Similarly, mutation in *egl-20* causes QL descendants to reverse direction and migrate anteriorly (Table 1; Forrester et al., 2004; Harris et al., 1996; Maloof et al., 1999). For QL descendant migration, EGL-20 appears to signal through MIG-1 and LIN-17 Frizzled proteins (Harris et al., 1996; Maloof et al., 1999).

Wnt signaling specifies ALM and CAN cell position

Interestingly, *cwn-1* and *cwn-2* function redundantly to specify the final positions of specific cells, as do *cwn-1* and *egl-20*. ALMs and CANs sometimes migrate beyond their normal positions in *cwn-1; cwn-2* double mutants. Furthermore, the ALM overmigration phenotype is enhanced by mutation in *cfz-2*. Although *cfz-2* mutants alone have no ALM overmigration defect, the enhancement of the defect in the triple mutant demonstrates that CFZ-2 functions in specifying cell position. Mutation in any of the remaining three *C. elegans* Frizzleds does not produce ALM overmigration (Forrester et al., 2004; Harris et al., 1996; Maloof et al., 1999). However, the possibility that one or more might act redundantly in the process remains unexplored. Because *cfz-2* acts nonautonomously for ALM migration, the affect on ALM position is likely to be due, at least in part, to nonautonomous CFZ-2 function. Therefore, CWN-1 and CWN-2, acting either through multiple Frizzleds or other receptors, produce a redundantly functioning "stop migration" signal.

Interaction between Wnt signaling pathways

Mutations in *cwn-1*, *cwn-2*, or *egl-20* suppress the ALM migration defects of *cfz-2* (Table 1). A model that might explain the interactions is for CWN-1, CWN-2, and/or EGL-20 to act via CFZ-2 and a second receptor, perhaps another Frizzled, to direct cell migrations. In the absence of CWN-1, CWN-,2 and CFZ-2, another signaling pathway often drives ALMs to more posterior locations. In this model, the suppression of ALM defects in *cfz-2* mutants by *cwn-1*, *cwn-2*, or *egl-20* mutants might be due to compensatory regulation of another signaling pathway, perhaps involving another Wnt. Alternatively, the suppression might reflect antagonism between Wnt signaling pathways. In this view, a balance between the different pathways is essential for normal migration. Antagonism between other Wnt pathways has been demonstrated (Ishitani et al., 2003; Topol et al., 2003; Torres et al., 1996; Westfall et al., 2003).

Interestingly, mutation in *cwn-1* dramatically suppresses the QL migration defect of *egl-20* mutants. EGL-20 acts via a canonical Wnt pathway to activate the MAB-5 homeobox protein in QL and its descendants, thereby preventing them from expressing a QR-like fate (Harris et al., 1996; Maloof et al., 1999; Whangbo and Kenyon, 1999). One possible interpretation is that CWN-1 and EGL-20 act antagonistically in QL migration. An alternative possible interpretation is that *egl-20* prevents MAB-5 expression in QL, and mutation in both *cwn-1* and *egl-20* shifts the position of the cell posterior.

Redundancy in Wnt pathway components

Animals mutant for both *cwn-1* and *cwn-2* had dramatically enhanced ALM, BDU, CAN, HSN, and QR descendant migration cell migration defects. Similarly, in *egl-20* and *cwn-1* double mutants, HSN and QR descendant cell migration defects were enhanced relative to either single mutant. These observations demonstrate that CWN-1, CWN-2, and EGL-20 act redundantly for specific cell migrations. Why should there be redundancy between signaling molecules? One possibility is that multiple Wnts act in concert to direct migrating cells and to fine tune the precise position of migrating cells. In its simplest form, Wnts could each act as guidance cues and the precise balance of individual Wnts could regulate cell position. Consistent with this view, Wnts have recently been implicated in growth cone guidance. Drosophila Wnt5 appears to be a repellant for axons that express the Derailed RTK, ensuring that the axons cross the midline at the anterior and not the posterior commissure (Yoshikawa et al., 2003). This simplistic view is unlikely in light of our observation that CFZ-2 acts nonautonomously for ALM migration. Note, however, that although our mosaic analysis clearly demonstrates a nonautonomous role for CFZ-2, it does not rule out a possible autonomous role. Determining whether other Frizzleds act nonautonomously like CFZ-2, and therefore play a similar role, or act autonomously, will begin to distinguish between these possibilities.

Acknowledgments

We thank the Caenorhabditis Genetics Center, H. Buelow, O. Hobert, J. Withee, J. Culotti, and G. Garriga

for providing strains and Andrew Fire's lab for providing pPD95.75. We thank E. Ryder for strains and sharing data prior to publication. We thank Edmund Brodie, III for invaluable assistance with statistical analysis, C. Kim and J. Kean for technical assistance, N. Hawkins for critical reading of the manuscript, and G. Garriga for helpful discussions. We are indebted to the International *C. elegans* Gene Knockout Consortium for isolating mutations within *cfz-2*, *cwn-1*, and *cwn-2* genes and for making them freely available to the community. This work was supported by National Institutes of Health grant R01 HD37815.

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