Mutations in the Spliceosome Component *prp-6* and Overexpression of *cdh-5* Suppress Axon Guidance Defects of *cdh-4* Mutants in *Caenorhabditis elegans*

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ABSTRACT: During nervous system development, axons must navigate to specific target areas. In *Caenorhabditis elegans*, the cadherin CDH-4 is required for ventral nerve cord axonal navigation, and dorsal nerve cord fasciculation. How CDH-4 mediates axon navigation and fasciculation is currently unknown. To identify genes acting together with *cdh-4*, we isolated mutants suppressing the axon guidance defects of *cdh-4* mutants. These suppressors showed partial suppression of axonal defects in the dorsal and ventral nerve cords seen in *cdh-4* mutants. We identified one suppressor gene, *prp-6*, which encodes a component of the spliceosome. Complete loss-of-function alleles of *prp-6* are lethal, suggesting that the mutation isolated in our suppressor screen is a partial loss-of-function allele. A previous study found that RNAi-induced suppression of *prp-6* leads to changes in the expression of several 100 genes including the cadherin *cdh-5*. We found that overexpression of *cdh-5* mimics the suppression seen in *prp-6* mutants, suggesting that CDH-5 can partially compensate for the loss of CDH-4.

KEYWORDS: Cadherin, central nervous system, nervous system development, ventral nerve cord, axon guidance

Introduction

The *C. elegans* nervous system is relatively simple, with a small number of neurons[1] and invariant axon trajectories.[2] This simplifies the identification of developmental defects in the nervous system. The major longitudinal axon tracts in the nervous system are the dorsal (DNC) and ventral (VNC) nerve cords. The DNC consists of a single tightly fasciculated bundle of motoneuron axons, which innervate the dorsal body wall muscles. The VNC consists of 2 axon bundles flanking the ventral midline. About 50 axons are found in the right axon bundle, whereas the left bundle contains only 4 axons.[3] The VNC contains interneuron axons in the motor circuit, which form synapses with motoneurons within the VNC. Since synapses are formed "*en passant*" between adjacent neuronal processes, the precise location of neuronal processes within the VNC is essential for proper neuronal circuit formation. This means that even minor navigation defects along the VNC, such as crossing from the right into the left axon tract, can potentially lead to defects in neuronal circuit formation.

Several genes controlling axon navigation in the VNC have been identified. Among these is the Fat-like cadherin, *cdh-4*.[4] *cdh-4* mutants are characterized by fasciculation defects in the DNC, midline crossing defects in the VNC and migration defects of the Q neuroblasts. Outside the nervous system, *cdh-4* mutants have epidermal and pharyngeal cell attachment defects leading to incompletely penetrant embryonic and larval lethality. While most of the defects seen in *cdh-4* mutants can be interpreted as adhesion defects, the neuroblast migration defects and the midline crossing defects in the VNC cannot be fully explained by simple adhesion defects. These more complex defects point to a potential role for CDH-4 in cell migration and axon guidance.

Fat-like cadherins are characterized by an unusually large number of cadherin repeats in their extracellular domain[5] (Figure 1). Fat cadherins in *Drosophila* control cell proliferation in imaginal discs and planar cell polarity (PCP) in multiple tissues.[6-8] Different signaling pathways are used by Fat cadherins in PCP and cell proliferation.[9,10] The signaling pathway used by CDH-4 in *C. elegans* in the context of axon guidance and fasciculation is currently unknown and is likely very different from the signaling pathways used in controlling PCP and proliferation in *Drosophila*.

We performed a suppressor screen to identify genes that act together with *cdh-4* to guide axons. We identified 13 suppressors that partially suppress the axonal defects of *cdh-4* mutants. These suppressors only marginally suppress other defects seen in *cdh-4* mutants, suggesting that they specifically interact with *cdh-4* in the context of axon guidance. We identified one of these suppressor genes, *prp-6*, a core spliceosome component. Partial loss-of-function of *prp-6* causes misregulation of several 100 genes, including the cadherin *cdh-5*.[11] We found that overexpression of *cdh-5* can partially suppress axonal defects in *cdh-4* mutants, suggesting that *cdh-5* can partially compensate for the loss of *cdh-4*.

Material and Methods

Strains and transgenes

The following alleles were used to isolate the suppressors and to confirm their identities: *cdh-4(hd40) III*, *prp-6 (gk527875)*.

CDH-4 domain organization (4328 amino acids)

| SP | 4x CA | 4x CA | 4x CA | 4x CA | 4x CA | 4x CA | 4x CA | 4x CA | 2x EGF | LG | 2x EGF | TM | 233 |

CDH-5 domain organization (1449 amino acids)

| SP | CA | CA | CA | CA | CA | CA | CA | CA | TM | 157 |

Figure 1. Protein domain organization of CDH-4 and CDH-5.
Abbreviations: CA single cadherin domain, "4x CA", 4 cadherin domains; "2x EGF", 2 epidermal growth factor domains; LG, laminin G domain; SP, signal peptide; TM, transmembrane domain; white box with number, size of intracellular domain in amino acids (contains no recognizable domain).

The following GFP transgenes were used to evaluate nervous system defects. *evIs111[rgef-1::GFP]* V, *hdIs36 [rgef-1::DsRed2]*. The following suppressors were isolated: *sup(hd158); sup(hd160); sup(hd161); sup(hd162); sup(hd163); sup(hd167); sup(hd168); sup(hd169); sup(hd170); sup(hd171); sup(hd172); sup(hd173); sup(hd174)*. A complete list of all strains used is in Supplemental Table S1. Animals were grown at 15°C or 20°C under standard conditions.[12]

Screening, mapping, and sequencing of the suppressors

To identify suppressors of *cdh-4(hd40)*, animals were mutagenized with 50 mM EMS for 4 hours. After mutagenesis animals were grown for 2 generations. L4 stage F2 animals were placed on individual plates. A population of *cdh-4(hd40)* mutant animals would take 14 days (3 generations) to exhaust the food on a plate due to the small survival rate (24%) of mutant animals. Mutagenized animals that exhausted the food on the plate in 10 days or less were considered candidate suppressors. Those candidate strains were grown further and assayed for DNC fasciculation defects. Strains with less than 35% fasciculation defects were kept as suppressors of axonal defects.

Suppressors were outcrossed 4 times with the parental strain *cdh-4(hd40); evIs111* prior to whole genome sequencing. Rescue of growth rate was used to identify the suppressors after each round of outcrossing. We noticed during outcrossing that all suppressors exhibited a recessive Mendelian segregation pattern. The presence of the suppressor mutation was confirmed by evaluating DNC fasciculation defects. Whole Genome Sequencing (WGS) was performed as described.[13] Briefly, sequencing was performed on an Illumina HiSeq sequencer with approximately 20-fold sequence coverage. The reference genome (WS240) was compared to the sequences of the *cdh-4(hd40)* suppressor strains to identify SNPs. SNPs found in more than 1 suppressor were considered background SNPs and eliminated. Only unique mutations in each suppressor strain were considered further. Single nucleotide polymorphism (SNP) mapping was done using the Hawaiian strain CB4856.[14]

Transgenic strains

To generate a strain overexpressing *cdh-5*, we generated transgenic animals expressing *cdh-5* from a multi-copy extrachromosomal array using the fosmid WRM0613cD01, which contains the genomic region of *cdh-5*. The *cdh-5* transgene *hdEx628* was crossed into *cdh-4(hd40); evIs111* to evaluate suppression effects.

Phenotypic characterization of suppressors

Axonal defects and Q cell migration defects. DNC fasciculation defects, VNC midline crossing defects, and Q cell migration defects were analyzed in late-stage larvae and adult animals expressing the pan-neuronal fluorescent marker *evIs111*.

DNC fasciculation defects are defined as axons splitting off the tightly fasciculated DNC axon bundle creating a visible second bundle and extending separately for at least 3 cell diameters (~15 μm) before rejoining the main bundle. VNC midline crossing defects are defined as axons crossing from the right axon bundle into left bundle (or vice versa). Q cells are 2 neuroblasts initially located near the midbody region. The Q cell on the left side (QL) migrates to the posterior, divides during the migration, and produces the SDQL and PVM neurons. The Q cell on the right side (QR) migrates to the anterior, divides during the migration and produces the SDQR and AVM neurons. Migration defects are defined as QL migrating anteriorly, so that the SDQL and PVM neurons end up in the anterior half of the animal or QR migrating posteriorly so that the SDQR and AVM neurons end up in the posterior half of the animal.

For imaging, animals were incubated with 10 mM NaN_3 in M9 buffer for 1 hour and mounted on agar pads prior to analysis. Defects were scored using a 40x objective lens on a Zeiss Axioscope (Carl-Zeiss AG, Germany). Images were acquired on a Zeiss Axioplan II microscope connected to a Quorum WaveFX spinning disc system (Quorum Technologies, Canada). Stacks of confocal images with 0.2 to 0.5 μm distance between focal planes were recorded. Image acquisition and analysis was carried out using Volocity software (Perkin-Elmer, Waltham, MA). Images in the figures are maximum intensity

projections of all focal planes. Figures were assembled using Keynote (Apple).

Lethality and brood size. Five to 12 L4 stage animals were placed on a plate to allow them to lay eggs. Animals were transferred daily to a fresh plate for 2 days. The number of eggs was counted to determine the brood size. The number of animals reaching adulthood 2 days after larvae hatched in relation to the number of eggs laid was used as a measure of lethality.

Movement assay. L4 stage animals were placed in an eyeglass filled with buffer solution. The number of body bends within 30 seconds was recorded. In addition, the direction of bending (ventral vs dorsal) was observed. Wild type animals alternate between ventral and dorsal body contractions in a regular fashion. *cdh-4* mutants frequently fail to bend in either dorsal or ventral direction, or do not bend at all. Thirty to 100 animals were evaluated for each strain.

Animals with "normal movement" had only "normal" body bends for 30 seconds. Animals with 1 or more abnormal body bends were scored as "abnormal." "Percent normal movement" is the fraction of animals with "normal movement," that is, animals with "normal movement" divided by total number of animals.

Statistical analysis. We used the following tests (depending on the type of data) to determine the statistical significance of differences in phenotype penetrance: chi-square test (χ^2), unpaired *t*-test, and 2-sample Poisson test.

Data availability. Strains are available upon request. The authors affirm that all data necessary for confirming the conclusions of the article are present within the article, figures, and tables.

Results

Isolation of cdh-4 suppressors

cdh-4 mutants are characterized by a variety of defects, including substantial embryonic and larval lethality, with only 24% of animals reaching reproductive age. Embryonic lethality is due to epidermal defects during morphogenesis. Additional larval lethality is mainly caused by a detachment of the pharynx from the mouth preventing larvae from feeding.[4] *cdh-4* mutants also produce fewer progeny than wild type. Together, this results in a very slow population growth, where the progeny of an individual take more than 16 days to exhaust the food on a 60 mm culture plate. This slow growth phenotype was the basis for the suppressor screen. We mutagenized *cdh-4* mutant animals and subcloned individual F2 animals to determine how long it took them to exhaust the food. Populations that starved within 10 days or less were considered to carry a suppressor mutation that improved either survival or increased the number of progeny (or both). Identified suppressors were then evaluated for

suppression of DNC fasciculation defects. We identified 13 suppressors that significantly improved growth and suppressed DNC fasciculation defects.

Identification of prp-6 as one of the suppressors

The suppressors were outcrossed 4 times to remove background mutations. Whole genome sequencing was used to identify unique point mutations in each strain by elimination mutations found in several strains. SNP mapping was used to narrow down the interval containing the suppressor. For *sup(hd170)* the gene *prp-6* was left as the only candidate after mapping. We therefore focused on *sup(hd170)*. *prp-6* encodes a component of the spliceosome, specifically a component of the U5 subunit, which is part of the active spliceosome. *hd170* is a missense mutation that changes glutamic acid to lysine at amino acid position 110. An independently isolated allele from the Million Mutation Project,[13] *gk527875*, causes a similar glutamic acid to lysine change at amino acid position 93. *cdh-4(hd40); prp-6(gk527875)* double mutants have 25% DNC fasciculation defects and 25% VNC defects, showing suppression of axonal defects very similar to *prp-6(hd170)*, which has 27% DNC fasciculation defects and 24% VNC defects. *cdh-4(hd40)* mutants have 53% DNC fasciculation defects and 50% VNC defects. This confirms that the suppression of axonal defects is caused by a mutation in *prp-6*. Complete loss-of-function of *prp-6* is lethal,[11] since splicing is an essential cellular process. Therefore, *prp-6(hd170)* is likely to be a partial loss-of-function allele.

Phenotypic characterization of suppressors

Most suppressors increased the number of progeny produced but not the fraction of surviving progeny (Table 1). Only *sup(hd161), sup(hd163), sup(hd167),* and *sup(hd174)* significantly reduced overall lethality. In other words, the majority of the mutants we isolated do not suppress defects causing lethality. This observation supports the hypothesis that lethality in *cdh-4* mutants is caused through a pathway distinct from the one controlling axon navigation and fasciculation.

cdh-4 mutants show migration defects of the QL neuroblast, which normally migrates posteriorly in wildtype animals, but frequently migrates anteriorly in *cdh-4* mutants. We sampled six suppressors for a suppression of QL migration defects and found that only two of them, *prp-6(hd170)* and *sup(hd161)*, marginally suppressed the QL migration defects to 78%-83% (Table 2, Figure 2). In four of the six suppressors we found that QR neuroblasts showed migration defects with 20% to 25% penetrance, substantially higher than what is observed in *cdh-4* mutants, where only 5% of QR neuroblasts have migration defects. *prp-6(hd170)* mutants in a wildtype background show no Q cell migration defects (Table 2). Since there are no Q cell migration defects in wild type, all the suppression effects are partial effects. Two of the descendants of the Q cells (AVM

Table 1. Lethality and brood size in the suppressors.

GENOTYPE	BROOD SIZE[a]	% LETHALITY
cdh-4(hd40)	62	76
cdh-4(hd40); prp-6(hd170)	166***	67
cdh-4(hd40); sup(hd158)	129***	80
cdh-4(hd40); sup(hd160)	126***	86
cdh-4(hd40); sup(hd161)	57	53#
cdh-4(hd40); sup(hd162)	99**	68
cdh-4(hd40); sup(hd163)	119***	54##
cdh-4(hd40); sup(hd167)	154***	44###
cdh-4(hd40); sup(hd168)	163***	90
cdh-4(hd40); sup(hd169)	135***	86
cdh-4(hd40); sup(hd171)	123***	91
cdh-4(hd40); sup(hd172)	81	65
cdh-4(hd40); sup(hd173)	99**	82
cdh-4(hd40); sup(hd174)	105**	58#

[a]The number of parental animals is 10 for *hd40* and 5 for the suppressors. The experiment was done once.
**$P < .01$ compared to *cdh-4(hd40)*.
***$P < .001$ compared to *cdh-4(hd40)*; 2-sample Poisson test.
#$P < .05$ compared to *cdh-4(hd40)*.
##$P < .01$ compared to *cdh-4(hd40)*.
###$P < .001$ compared to *cdh-4(hd40)*; (χ^2 test).

Table 2. Q cell migration defects in the suppressors.

GENOTYPE	% Q CELL MIGRATION DEFECTS (N)	
	QR	QL
cdh-4(hd40)	5** (100)	95** (105)
cdh-4(hd40); prp-6(hd170)	20** (100)	83*** (150)
prp-6(hd170)	0* (100)	0*** (100)
cdh-4(hd40); sup(hd158)	25*** (107)	89** (101)
cdh-4(hd40); sup(hd160)	11** (101)	89** (101)
cdh-4(hd40); sup(hd161)	22*** (102)	78*** (103)
cdh-4(hd40); sup(hd162)	9** (55)	95** (58)
cdh-4(hd40); sup(hd163)	24*** (100)	84*** (100)

*$P < .05$ compared to *cdh-4(hd40)*.
**$P < .01$ compared to *cdh-4(hd40)*.
***$P < .001$ compared to *cdh-4(hd40)*; (χ^2 test).

and PVM) are mechanosensory neurons. Migration defects of these cells could lead to mechanosensory defects.

In wild type DNC and VNC defects are in the range of 4% to 5%.[4] DNC fasciculation defects were partially suppressed in all suppressors (Table 3, Figure 3), with defects ranging from 18% to 37% penetrance compared to 53% in *cdh-4(hd40)*.
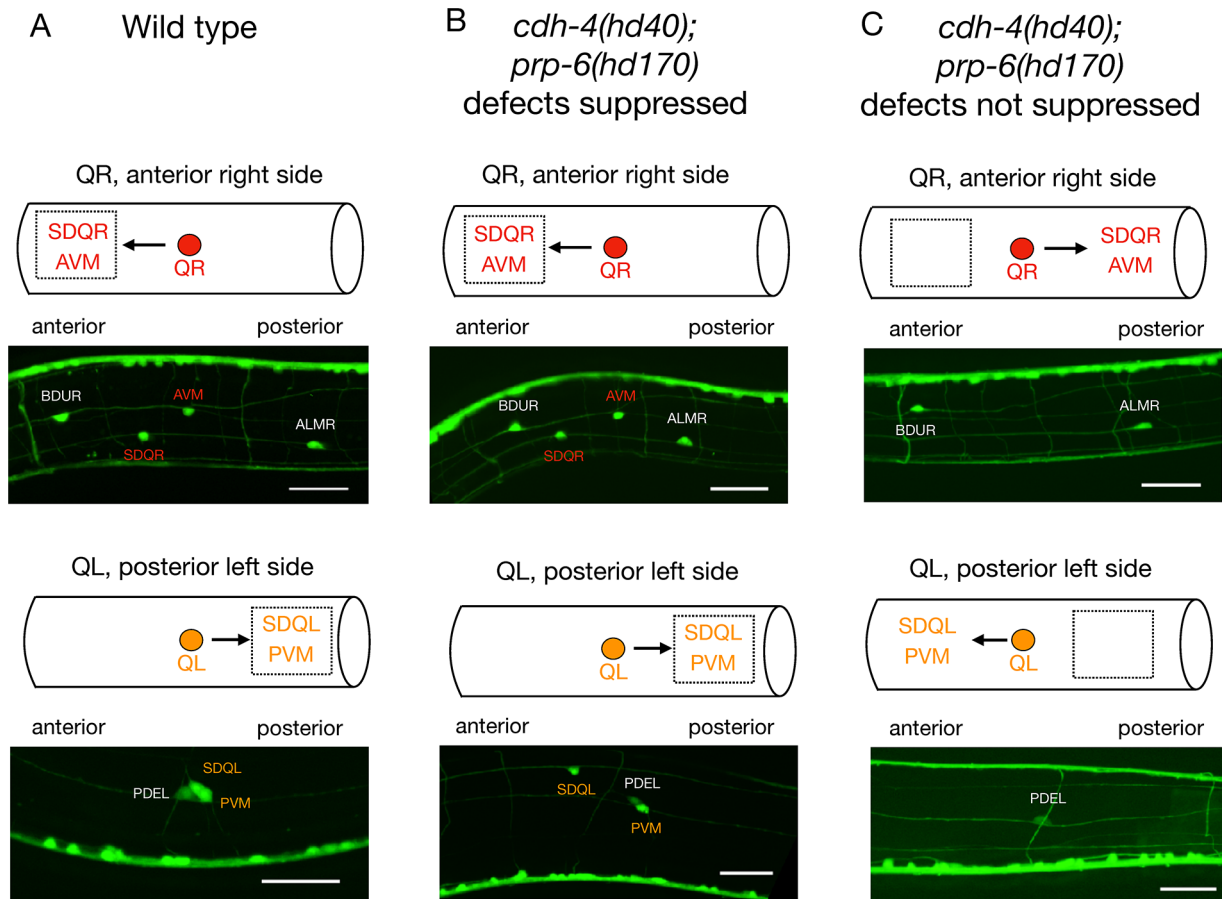
VNC crossover defects were also partially suppressed in all suppressors (Table 3, Figure 3), ranging from 19% to 33% penetrance compared to 50% in *cdh-4(hd40)*. In 5 suppressors, *sup(hd162)*, *sup(hd168)*, *sup(hd169)*, *prp-6(hd170)*, *and sup(hd172)*, DNC and VNC defects were suppressed to a similar extent. For the other suppressors, there was no clear correlation between suppression of DNC and VNC defects. For example, VNC defects are strongly suppressed in *sup(hd174)* but DNC defects are not. Conversely, DNC defects are strongly suppressed in *sup(hd161)* but VNC defects are not. This suggests that *cdh-4* might act through different pathways in the DNC and VNC and that some suppressors only affect one of the 2 pathways strongly.

cdh-4(hd40) mutants also show movement defects. These could be the consequence of axonal defects[4] or defects seen in synapse formation in D-type motoneurons.[15] When put in liquid media, wildtype animals bend regularly and rapidly by alternate and coordinated contractions of dorsal and ventral body wall muscles with a frequency of around 180 bends per minute. This frequency is reduced to about 40 bends per minute in *cdh-4(hd40)* mutant animals. Furthermore, while all wildtype animals show regular and complete bending in both directions, only about 30% of *cdh-4(hd40)* mutant animals show normal movement behavior. We tested 5 suppressors that strongly suppressed axon guidance defects for a potential suppression of movement defects by qualitatively and quantitatively evaluating movement in liquid. Two of the suppressors, *sup(hd158)* and *prp-6(hd170)*, showed partial improvement of movement both qualitatively and quantitatively (Figure 4). The three other suppressors, *sup(hd160)*, *sup(hd161)*, and *sup(hd163)* showed movement defects similar to *cdh-4(hd40)* (Figure 4). Since all five suppressors show comparable suppression of axonal defects, this indicates that suppression of movement defects is likely not due to suppression of axonal defects.

Overexpression of cdh-5 partially suppresses axonal defects in cdh-4 mutants

prp-6(hd170), as a component of the spliceosome, could suppress *cdh-4(hd40)* defects by directly affecting splicing of the *cdh-4* mRNA. Since there is only one known splice variant of *cdh-4*, *prp-6* is unlikely to affect alternative splicing of the *cdh-4* mRNA. *cdh-4(hd40)* is a deletion eliminating the first exon and is unlikely to produce any mRNA. To test whether the *prp-6* suppression effects are specific for the *cdh-4(hd40)* allele, we evaluated defects in a second *cdh-4* allele, *rh310*. *cdh-4(rh310)* is a point mutation creating a stop codon in exon 5 and causes defects like those seen in *cdh-4(hd40)* mutants. We found that *prp-6(hd170)* suppressed DNC defects of both *cdh-4* alleles equally well (Table 4). This suggests that suppression of axonal defects by *prp-6(hd170)* is not specific to the *hd40* allele of *cdh-4*.

Partial inactivation of *prp-6* by RNAi leads to changes in the expression levels of 475 genes; 407 of these are upregulated and the rest are downregulated.[11] Among the upregulated

A   Wild type

QR, anterior right side

QL, posterior left side

B   *cdh-4(hd40); prp-6(hd170)* defects suppressed

QR, anterior right side

QL, posterior left side

C   *cdh-4(hd40); prp-6(hd170)* defects not suppressed

QR, anterior right side

QL, posterior left side

Figure 2. Q cell migration defects in *cdh-4(hd40); prp-6(hd170)*. (A) In wild type animals QR migrates anteriorly and its descendants (SDQR and AVM) are located in the anterior part of the animal. QL migrates posteriorly and its descendants (SDQL and PVM) are in the posterior part of the animal. (B) In some *cdh-4(hd40); prp-6(hd170)* animals Q cell migration defects are suppressed, that is, the location of the Q cell descendants is as in wild type. (C) In some *cdh-4(hd40); prp-6(hd170)* animals Q cell migration defects are not suppressed, that is, either QR descendants are not in their normal anterior location or QL descendants are not in their normal posterior location. Scale bar is 20 μm. Marker used: *evIs111*.

genes is *cdh-5*, encoding a functionally uncharacterized member of the cadherin superfamily.[16] The domain structure of CDH-5 is similar to that of CDH-4 (Figure 1); however, CDH-5 is much smaller (1449 amino acids) than CDH-4 (4328 amino acids). This raises the possibility that overexpression of *cdh-5* caused by a partial inactivation of *prp-6* in the *prp-6(hd170)* mutant might be the cause for the suppression of *cdh-4* defects. This would suggest that *cdh-5* can partially replace *cdh-4* for its axon guidance function.

To test whether overexpression of *cdh-5* can suppress axonal defects in *cdh-4* mutants, we generated transgenic animals overexpressing *cdh-5* from an extrachromosomal array containing multiple copies of the wild type *cdh-5* gene. *cdh-5* overexpression in a wild type background has no significant DNC or VNC defects (Table 4). We crossed the transgene into *cdh-4(hd40)* mutants and scored DNC and VNC defects. We found that VNC crossover defects in this strain are reduced to 28% (n = 101) and DNC defects are reduced to 34% (n = 117). The suppression effects resemble those seen in *cdh-4(hd40); prp-6(hd170)* with 27% DNC and 24% VNC defects (Table 4). This suggests that *prp-6* mediated overexpression of *cdh-5* accounts for the suppression seen in the *prp-6(hd170)* mutant.

If overexpression of *cdh-5* causes the suppression, introducing a *cdh-5* mutation into the *cdh-4(hd40); prp-6(hd170)* strain should abolish suppression. All our attempts to generate a *cdh-4(hd40); cdh-5(ok1977); prp-6(hd170)* triple mutant strain failed. We concluded that this mutant combination is not viable. This is an indication that a mutation in *cdh-5* enhances *cdh-4* associated lethality, supporting the idea that overexpression of *cdh-5* ameliorates defects of *cdh-4* mutants.

Discussion

Suppressors of axon guidance defects of cdh-4 mutants

To identify genes that act together with *cdh-4* to guide axons we performed a suppressor screen. In the primary screen, we selected mutants that grew substantially better than *cdh-4* mutants, since it is difficult to directly screen for suppression of axon guidance defects. Two factors contribute to the slow growth of *cdh-4* mutants. First, only 24% of *cdh-4(hd40)* mutant animals survive to adulthood. Second, the number of progeny per individual is substantially reduced in *cdh-4(hd40)* mutants compared to wild type. Lethality is mainly caused by hypodermal defects leading to morphogenetic defects and embryonic

Table 3. DNC and VNC axonal defects in the suppressors.

GENOTYPE	% DNC FASCICULATION DEFECTS (N)	% VNC CROSS-OVER DEFECTS (N)
cdh-4(hd40)	53 (100)	50 (100)
cdh-4(hd40); prp-6(hd170)	27*** (109)	24*** (100)
cdh-4(hd40); sup(hd158)	23*** (134)	29** (112)
cdh-4(hd40); sup(hd160)	26*** (135)	29** (101)
cdh-4(hd40); sup(hd161)	18*** (101)	30** (100)
cdh-4(hd40); sup(hd162)	28*** (120)	25*** (106)
cdh-4(hd40); sup(hd163)	25*** (106)	33* (100)
cdh-4(hd40); sup(hd167)	32** (105)	23*** (128)
cdh-4(hd40); sup(hd168)	19*** (105)	21*** (109)
cdh-4(hd40); sup(hd169)	24*** (105)	19*** (109)
cdh-4(hd40); sup(hd171)	35** (114)	25** (100)
cdh-4(hd40); sup(hd172)	29*** (103)	25** (107)
cdh-4(hd40); sup(hd173)	37* (124)	28** (109)
cdh-4(hd40); sup(hd174)	39 (120)	21*** (113)

*$P < .05$ compared to *cdh-4(hd40)*.
**$P < .01$ compared to *cdh-4(hd40)*.
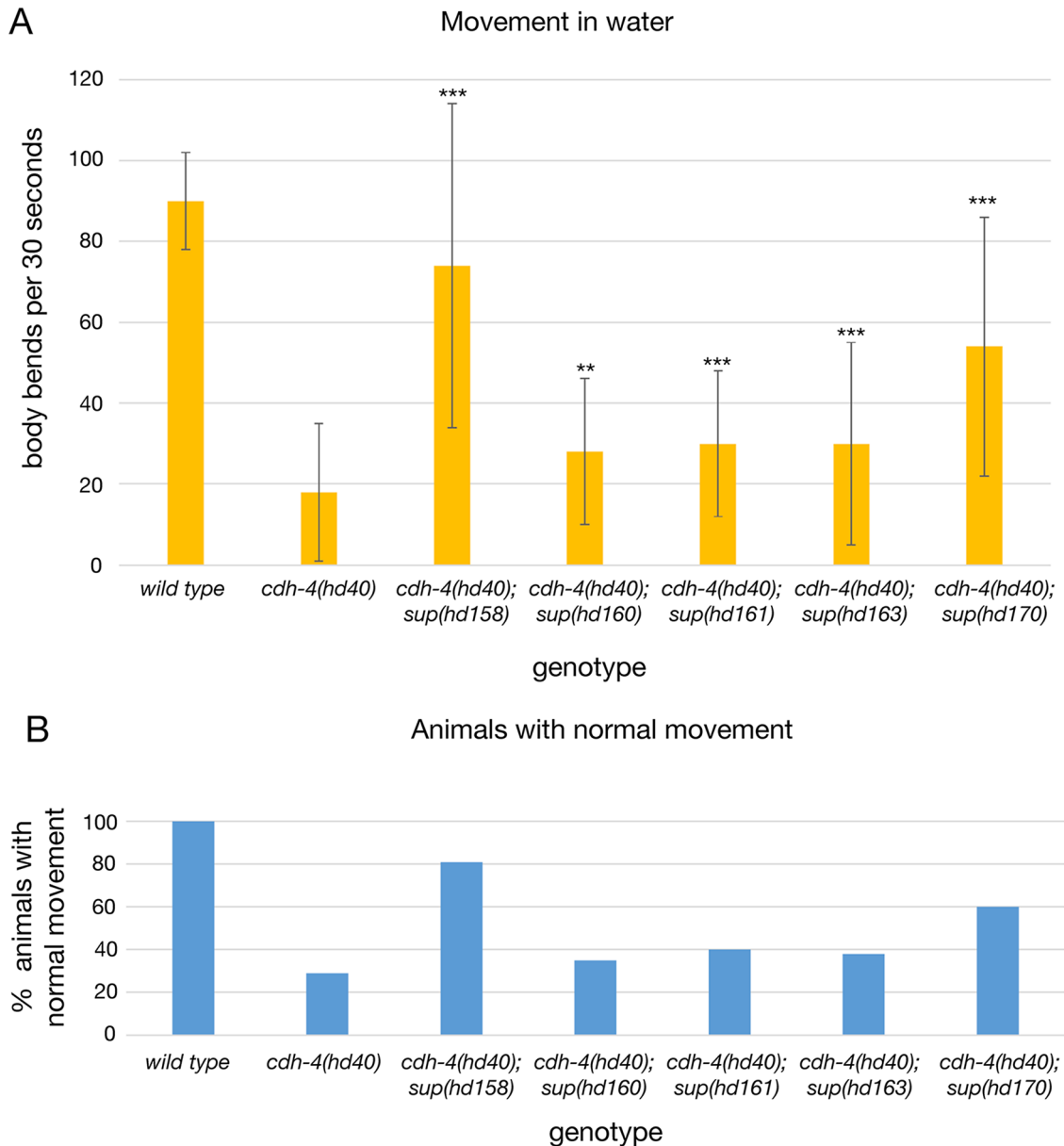***$P < .001$ compared to *cdh-4(hd40)*; (χ^2 test).
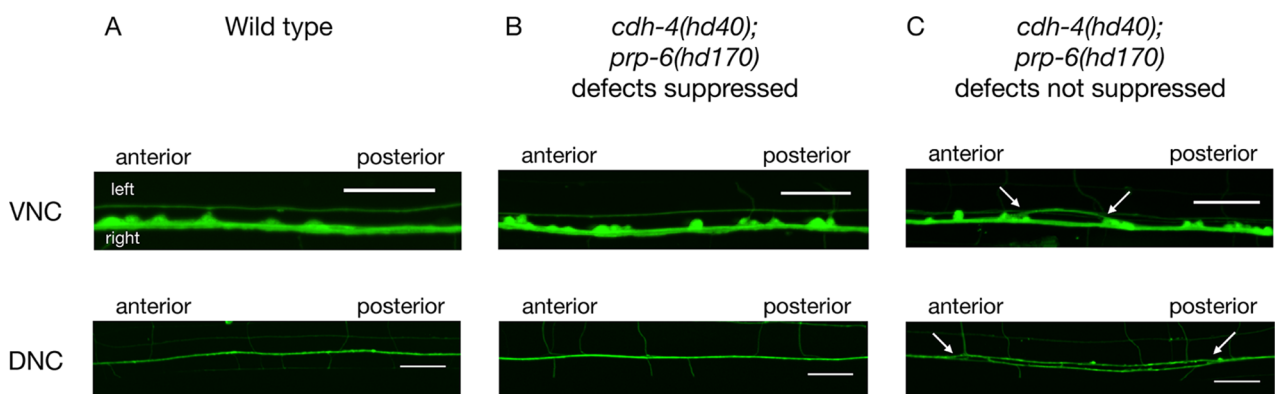
Figure 3. Ventral and dorsal nerve cord phenotypes in *cdh-4(hd40); prp-6(hd170)*. (A) In wild type animals axons do not cross between the right and left VNC axon tracts. The DNC consists of a tightly fasciculated axon bundle. (B) In some *cdh-4(hd40); prp-6(hd170)* animals VNC or DNC defects are suppressed, that is, VNC and DNC look like wild type. (C) In some *cdh-4(hd40); prp-6(hd170)* animals VNC or DNC defects are not suppressed, that is, axons cross the midline in the VNC (arrows) or DNC axons are defasciculated (arrows). Scale bar is 20 μm. Marker used: *evIs111*.

lethality. In addition, in some larvae the pharynx becomes detached from the mouth region leading to an inability to feed and larval lethality.[4] The basis for the reduced number of progeny is unknown. We expected the suppressors to improve survival rates, but this was not the case for most of the suppressors. Instead, the improved growth rate was predominantly due to an increased number of progeny. This suggests that most suppressors do not suppress the defects that cause lethality.

Q neuroblasts and their descendants migrate to defined positions along the anterior-posterior axis of the animal, with Q-cells on the right side (QR) migrating anteriorly and Q-cells on the left side (QL) migrating posteriorly.[17]

cdh-4(hd40) mutants show prominent Q-cell migration defects, with most QL neuroblasts migrating anteriorly. Only three of the six suppressors we tested reduced QL migration defects. Four of the six suppressors showed increased QR migration defects when compared to *cdh-4(hd40)* mutants. Suppression of QL migration defects means more QL cells migrating posteriorly. Likewise, increased QR migration defects mean more QR cells migrating posteriorly. This suggests that some of the suppressors support posterior migration of Q cells in general.

All 13 suppressors partially suppressed DNC fasciculation as well as VNC midline crossing defects. Most suppressors

A

B

Figure 4. Movement defects in suppressor mutants. (A) Movement in water: Shown are body bends per 30 seconds (n = 31-100). **P < .01 compared to *cdh-4(hd40)*; ***P < .001 compared to *cdh-4(hd40)*; unpaired *t*-test. (B) Animals with normal movement: shown is the fraction of animals showing only normal body bends within 30 seconds of scoring (n = 31-100).

suppressed DNC and VNC defects to different degrees. The lack of correlation between suppression of DNC and VNC defects in many suppressors may imply that separate pathways control guidance and fasciculation of axons in VNC and DNC, and that CDH-4 activates multiple pathways. DNC fasciculation defects could affect motoneurons that innervate dorsal body wall muscles. However, since muscle cells send muscle arm processes toward the motoneurons and are able to locate even misguided motoneuron axons,[18] the fasciculation defects in the DNC are not expected to cause significant problems in the innervation of dorsal muscle cells. Midline crossing defects in the VNC affecting interneuron axons synapsing with motoneurons in the VNC may lead to a loss of motor circuit synapses, with potentially negative consequences for the functioning of

the motor circuit. *cdh-4(hd40)* mutants have prominent movement defects including uncoordinated and slow movement in a swimming assay. These behavioral phenotypes are partially suppressed in some but not all *cdh-4* suppressing alleles analyzed for movement defects. As all suppressors show partial suppression of axonal defects, the lack of suppression of movement defects in some of these lines suggests that defects other than those affecting axon guidance are responsible for at least some of the behavioral phenotypes. *cdh-4* is implicated together with *fmi-1*, a Flamingo-like cadherin, in the patterning of GABAergic neuromuscular junctions.[15] It is possible that synaptic defects are a major cause for the movement defects observed, and that some but not all suppressors improve synaptic development leading to a partial suppression of movement defects.

Table 4. DNC and VNC axonal defects in *prp-6* mutants.

GENOTYPE	% DNC FASCICULATION DEFECTS (N)	% VNC CROSS-OVER DEFECTS (N)
cdh-4(hd40)[#]	53 (100)	50 (100)
cdh-4(rh310)	50 (101)	45 (100)
prp-6(hd170)	1*** (100)	2*** (100)
cdh-4(hd40); prp-6(hd170)[#]	27*** (109)	24*** (100)
cdh-4(rh310); prp-6(hd170)	30** (100)	22*** (100)
cdh-4(hd40); prp-6(gk527875)	25*** (107)	25*** (100)
cdh-4(hd40); cdh-5(+)	34** (117)	28** (101)
cdh-5(+)	1** (100)	7** (91)

[#]Data taken from Table 3.
*$P < .05$ compared to *cdh-4(hd40)*.
**$P < .01$ compared to *cdh-4(hd40)*.
***$P < .001$ compared to *cdh-4(hd40)*; (χ^2 test).

prp-6 as suppressor of axonal defects of cdh-4 mutants

We identified *prp-6* as one of the suppressors. PRP-6 is an evolutionarily conserved component of the spliceosome.[19-21] The spliceosome removes introns from pre-processed mRNA. PRP-6 is a U5 component, which is part of the active core of the spliceosome that catalyzes the splicing reactions.[21] Since complete loss-of function mutations in *prp-6* are lethal,[11] the missense mutation in *prp-6(hd170)* most likely results in a partial loss-of-function allele. A partially dysfunctional spliceosome causes inefficiencies in splicing and other associated transcriptional activities.[22,23] In addition, a dysfunctional spliceosome may lead to a shift in the prevalence of specific splice variants of genes that have more than one splice variant. In mammalian cells, PRP6 is implicated in affecting alternative splice site choices when depleted.[24,25] Partially inactivating *prp-6* by RNAi in *C. elegans* also leads to changes in the expression levels of 475 genes; 407 of these are upregulated and the rest are downregulated.[11] Among the upregulated genes is *cdh-5*, encoding a functionally uncharacterized member of the cadherin superfamily.[16] We found that overexpression of *cdh-5* in a *cdh-4(hd40)* mutant background suppresses DNC and VNC defects similar to those observed in *prp-6* mutants. This suggests that *cdh-5* can partially replace *cdh-4* for its axon guidance function, and that overexpression of *cdh-5* is responsible for the suppression effect rather than changes in splicing patterns.

In this study, we identified 13 alleles that partially suppress VNC and DNC axon guidance defects of *cdh-4* mutants. Non-neuronal defects such as embryonic and larval lethality as well as Q cell migration defects were not, or only marginally, suppressed by these suppressors. This suggests that the different functions of *cdh-4* might be mediated through different pathways, and that the suppressors we identified interact with only a few of those pathways. We identified *prp-6*, a key component of the spliceosome, as one of the suppressors. We found evidence that upregulation of *cdh-5* in *prp-6* mutants is likely responsible for this suppression effect, suggesting that *cdh-5* can partially replace *cdh-4* to ensure correct navigation of axons in the ventral and dorsal nerve cords.

Author Contributions
Concept/Design: HH, JP. Data acquisition: JP, ZA. Data processing/analysis: ZA, JP. Interpretation of results: ZA, JP, HH. Writing: HH.

Significance Statement
The Fat-like cadherin *cdh-4* controls axonal navigation and fasciculation as well as epidermal and pharyngeal cell attachment in *Caenorhabditis elegans*. Genes acting together with *cdh-4* controlling these processes are currently unknown. In a suppressor screen we identified 13 suppressors that partially suppress the axonal defects of *cdh-4* mutants. One of the suppressor genes, *prp-6*, encodes a core spliceosome component. In addition, we show that overexpression of the cadherin *cdh-5* can suppress axonal defects in *cdh-4* mutants, suggesting that CDH-5 can partially compensate for loss of CDH-4.

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Supplemental Material

Supplemental material for this article is available online.

REFERENCES

1. Sulston JE, Schierenberg E, White JG, Thomson JN. The embryonic cell lineage of the nematode Caenorhabditis elegans. *Dev Biol*. 1983;100:64-119.
2. White JG, Southgate E, Thomson JN, Brenner S. The structure of the nervous system of the nematode *Caenorhabditis elegans. Philos Trans R Soc Lond B Biol Sci*. 1986;314:1-340.
3. White JG, Southgate E, Thomson JN, Brenner S. The structure of the ventral nerve cord of Caenorhabditis elegans. *Philos Trans R Soc Lond B Biol Sci*. 1976;275:327-348.
4. Schmitz C, Wacker I, Hutter H. The Fat-like cadherin CDH-4 controls axon fasciculation, cell migration and hypodermis and pharynx development in Caenorhabditis elegans. *Dev Biol*. 2008;316:249-259.
5. Tanoue T, Takeichi M. New insights into fat cadherins. *J Cell Sci*. 2005;118:2347-2353.
6. Mahoney PA, Weber U, Onofrechuk P, Biessmann H, Bryant PJ, Goodman CS. The fat tumor suppressor gene in Drosophila encodes a novel member of the cadherin gene superfamily. *Cell*. 1991;67:853-868.
7. Yang CH, Axelrod JD, Simon MA. Regulation of frizzled by fat-like cadherins during planar polarity signaling in the Drosophila compound eye. *Cell*. 2002;108:675-688.
8. Cho E, Irvine KD. Action of fat, four-jointed, dachsous and dachs in distal-to-proximal wing signaling. *Development*. 2004;131:4489-4500.
9. Fanto M, McNeill H. Planar polarity from flies to vertebrates. *J Cell Sci*. 2004;117:527-533.
10. Cho E, Feng Y, Rauskolb C, Maitra S, Fehon R, Irvine KD. Delineation of a fat tumor suppressor pathway. *Nat Genet*. 2006;38:1142-1150.
11. Rubio-Peña K, Fontrodona L, Aristizábal-Corrales D, et al. Modeling of autosomal-dominant retinitis pigmentosa in Caenorhabditis elegans uncovers a nexus between global impaired functioning of certain splicing factors and cell type-specific apoptosis. *RNA N Y N*. 2015;21:2119-2131.
12. Brenner S. The genetics of Caenorhabditis elegans. *Genetics*. 1974;77:71-94.
13. Thompson O, Edgley M, Strasbourger P, et al. The million mutation project: a new approach to genetics in Caenorhabditis elegans. *Genome Res*. 2013;23: 1749-1762.
14. Wicks SR, Yeh RT, Gish WR, Waterston RH, Plasterk RH. Rapid gene mapping in Caenorhabditis elegans using a high density polymorphism map. *Nat Genet*. 2001;28:160-164.
15. Najarro EH, Wong L, Zhen M, et al. Caenorhabditis elegans flamingo cadherin fmi-1 regulates GABAergic neuronal development. *J Neurosci*. 2012;32: 4196-4211.
16. Pettitt J. The cadherin superfamily. In: The C. elegans Research Community, ed. *WormBook*. Published online December 29, 2005. doi:10.1895/wormbook. 1.50.1
17. Sulston JE, Horvitz HR. Post-embryonic cell lineages of the nematode, Caenorhabditis elegans. *Dev Biol*. 1977;56:110-156.
18. Hedgecock EM, Culotti JG, Hall DH. The unc-5, unc-6, and unc-40 genes guide circumferential migrations of pioneer axons and mesodermal cells on the epidermis in C. elegans. *Neuron*. 1990;4:61-85.
19. Maita H, Kitaura H, Ariga H, Iguchi-Ariga SM. Association of PAP-1 and prp3p, the products of causative genes of dominant retinitis pigmentosa, in the tri-snrnp complex. *Exp Cell Res*. 2005;302:61-68.
20. Liu S, Rauhut R, Vornlocher HP, Lührmann R. The network of protein–protein interactions within the human U4/U6.U5 tri-snRNP. *RNA N Y N*. 2006;12: 1418-1430.
21. Fabrizio P, Dannenberg J, Dube P, et al. The evolutionarily conserved core design of the catalytic activation step of the yeast spliceosome. *Mol Cell*. 2009;36: 593-608.
22. Das R, Yu J, Zhang Z, et al. SR proteins function in coupling RNAP II transcription to pre-mRNA splicing. *Mol Cell*. 2007;26:867-881.
23. Lee KM, Tarn WY. Coupling pre-mRNA processing to transcription on the RNA factory assembly line. *RNA Biol*. 2013;10:380-390.
24. Moore MJ, Wang Q,, Kennedy CJ, Silver PA. An alternative splicing network links cell-cycle control to apoptosis. *Cell*. 2010;142:625-636.
25. Tanackovic G, Ransijn A, Thibault P, et al. PRPF mutations are associated with generalized defects in spliceosome formation and pre-mRNA splicing in patients with retinitis pigmentosa. *Hum Mol Genet*. 2011;20:2116-2130.