

The *Caenorhabditis elegans* gene *unc-76* and its human homologs define a new gene family involved in axonal outgrowth and fasciculation

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ABSTRACT The gene *unc-76* (*unc*, uncoordinated) is necessary for normal axonal bundling and elongation within axon bundles in the nematode *Caenorhabditis elegans*. The UNC-76 protein and two human homologs identified as expressed sequence tags are not similar to previously characterized proteins and thus represent a new protein family. At least one of these human homologs can function in *C. elegans*, suggesting that it, like UNC-76, acts in axonal outgrowth. We propose that the UNC-76 protein, which is found in cell bodies and processes of all neurons throughout development, either has a structural role in the formation and maintenance of axonal bundles or transduces signals to the intracellular machinery that regulates axonal extension and adhesion.

Axons in developing nervous systems navigate through a varied set of extracellular environments to reach their targets. Most axons grow along other axons for much of their lengths, and the association of axons in specific bundles, or fascicles, is likely to play a major role in nervous system assembly. Although many cell-surface proteins involved in fascicle formation have been identified (1), relatively little is known about the intracellular mechanisms by which surface interactions lead to the elongation of axons specifically along other axonal surfaces.

Genetic screens for fasciculation-defective mutants can, in principle, identify molecules necessary for fasciculation without presuppositions as to their biochemical nature or subcellular localization. Analysis of strains of the nematode *Caenorhabditis elegans* with mutations causing locomotory defects (uncoordinated or *unc* mutants) has revealed a group of three genes that, when mutant, affect the growth of axons in fascicles, but not along nonneuronal substrates (cells of the lateral hypodermis and the overlying basement membrane; refs. 2–4). Mutations in this fascicle-specific group of genes, *unc-34*, *unc-71*, and *unc-76*, cause two types of defects: many axons fail to extend fully within the axon bundles of the dorsal and ventral nerve cords, and many fail to remain in their normal fascicles (2–4). The best-characterized example of these defects is provided by the axons of the left and right hermaphrodite-specific neuron (HSN) motor neurons. In fascicle-specific mutants, these axons end prematurely within the left and right fascicles of the ventral nerve cord, respectively, and they often fail to remain on opposite sides of the cord (refs. 3, 4; L.B. and H.R.H., unpublished observations). If a second mutation causes the HSN axons to be rerouted along a lateral process tract instead of the ventral nerve cord, the axons in these mutants grow to their normal lengths, indicating that

unc-34 and *unc-76* affect the interaction of these axons with the ventral cord environment rather than the ability of the HSN axons to grow beyond a certain length (4).

Among the mutants with fascicle-specific defects, *unc-76* mutant animals have the most severe abnormalities in locomotion and HSN outgrowth (3, 4). To understand the basis of these defects, we have undertaken a genetic and molecular analysis of the *unc-76* gene.

MATERIALS AND METHODS

Genetic Methods and Strains. Strains were constructed and maintained according to standard methods (5). Most mutant strains used in this paper were described in refs. 5–7; J. Culotti (Mt. Sinai Hospital, Toronto) and S. Siddiqui (Toyoashi University) provided *unc-76(ev424)*, and E. Hedgecock (Johns Hopkins University) provided *unc-76(rh116)*. Mutations that failed to complement *unc-76(e911)* were obtained by treating wild-type (N2) or *egl-1(n986dm)* males with ethyl methane-sulfonate (5), mating them with + *sdsc-3(y52) unc-76(e911)/unc-61(e228) ++*; *dpy-3(e27)* hermaphrodites, and isolating severely Unc non-Dpy F₁ hermaphrodites. After three generations of backcrossing to N2, mutants were stained with antiserotonin antisera (3), and HSN axon length was estimated to the nearest tenth of the distance between the vulva and the posterior end of the pharynx. Because a large-scale screen for suppressors of the Unc phenotype of *unc-76(e911)* produced only *smg* suppressors (L.B. and H.R.H., unpublished observations; ref. 8), the effects of *smg-1* on other *unc-76* alleles were tested in strains of genotype *smg-1(e1228) him-2(e1065); unc-76*.

Molecular Analysis of *unc-76*. Molecular biological and immunological procedures were performed according to standard methods (9–11). Cosmids obtained as part of the *C. elegans* genome project (12, 13) and plasmids containing fragments of the rescuing cosmid C56C4 were injected at 50 µg/ml into the gonads of *unc-76(e911)* mutant hermaphrodites (14), and the Unc phenotype was scored in the F₁ and F₂ generations. *unc-76* cDNA clones were obtained by screening 220,000 plaques from a mixed-stage *C. elegans* cDNA library (15) with the ³²P-labeled insert from p76–8. DNA from exons and splice junctions of each mutant *unc-76* strain was amplified by PCR (16) for sequence determination. Database searches were performed at the National Center for Biotechnology Information with the BLAST program (17).

Clones for *FEZ1*, *FEZ1-T*, and *FEZ2* (accession numbers R61145, R25079, and R21583, respectively) were obtained by

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Abbreviations: GABA, γ-aminobutyric acid; HSN, hermaphrodite-specific neuron.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database [accession nos.: *unc-76* genomic DNA, U28153; *unc-76* cDNA clones, U60058 (p76-c7) and U60059 (p76-c4); *FEZ1*, U60060; *FEZ1-T*, U60062; and *FEZ2*, U60061].

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the Washington University–Merck EST Project (unpublished results) and provided to us by the I.M.A.G.E. consortium (18). p76HsA-5 contained a 1.5-kb *HindIII*-*BsrBI* *FEZ1* cDNA fragment driven by a 1.05-kb *unc-76* promoter fragment (Y. Jin, personal communication) in pPD49.26 (19). p76HsA-5 was injected into *unc-76(e911)* animals, or, together with a *dpy-20*-rescuing plasmid (20), into *dpy-20(e1282ts)*; *unc-76(e911)* animals, and rescued lines were stained with anti-GABA (γ -aminobutyric acid) antisera (3). p86/76-1 contained a 5-kb *SpeI*-*StyI* fragment from the *unc-86::lacZ* fusion SA2 (provided by G. Ruvkun; Massachusetts General Hospital) fused to a *BglIII*-*BclI* *unc-76* fragment, which was fused in turn to *lacZ*. In p86-L1, the same *unc-86* fragment was fused directly to *lacZ*. N2-derived lines carrying either fusion plasmid with the *rol-6* plasmid pRF4 (21) were stained with a monoclonal anti- β -galactosidase antibody (Promega; ref. 22).

Anti-UNC-76 Antibodies. Three rabbits were immunized with the following series of UNC-76 fusion proteins produced in *Escherichia coli*: a maltose binding protein fusion to amino acids 13–385, a glutathione *S*-transferase fusion to amino acids 48–385, and a fusion of amino acids 12–385 to His₆. Sera were affinity-purified on either UNC-76-His₆ or UNC-76::TrpE fusion proteins bound to nitrocellulose. Worms were fixed 16 h with 4% paraformaldehyde in PBS at 4°C. Half of the worms were frozen and thawed 2–3 times in liquid nitrogen, and then all worms were partially broken in a Dounce homogenizer, washed in PBS, stained as described for anti-serotonin staining (3), and washed in 1 μ g/ml diaminophenolindole (Sigma). Serum from one rabbit (no. 275) was used for all experiments shown.

RESULTS

Genetic Analysis of *unc-76*. To determine the effects of a complete loss of *unc-76* function, we obtained five new *unc-76* alleles in a screen for mutations that failed to complement *unc-76(e911)*. The new mutations, *n2367*, *n2397*, *n2398*, *n2399*, and *n2457*, arose at a frequency (1/3,300 mutagenized genomes) close to the average frequency (1/2,000) of inducing a loss-of-function mutation using the same ethyl methanesulfonate mutagenesis procedure (5, 23–24). The defects caused by all *unc-76* alleles appeared to be restricted to fascicles; HSN cell body migration and ventral axonal outgrowth along the lateral hypodermis were nearly normal (data not shown). All

Table 1. HSN axon length in *unc-76* mutants

Genotype	HSN length, % wild type	<i>n</i>
N2	100	>1000
<i>e911</i>	66 \pm 3	55
<i>e911/yDf8</i>	73 \pm 3	46
<i>ev424</i>	64 \pm 4	47
<i>ev424/yDf8</i>	73 \pm 4	64
<i>rh116</i>	65 \pm 5	56
<i>n2367</i>	79 \pm 4	57
<i>n2367/yDf8</i>	77 \pm 7	31
<i>n2397</i>	77 \pm 3	90
<i>n2397/yDf8</i>	76 \pm 6	42
<i>n2398</i>	89 \pm 2	75
<i>n2399</i>	73 \pm 4	68
<i>n2457</i>	72 \pm 5	40
<i>n2457/yDf8</i>	77 \pm 5	44

HSN axon length (\pm 95% confidence limits) in *unc-76* mutants. *n*, number of axons scored. The small number of HSNs with cell bodies in an abnormal posterior position or with axons in a lateral position were not included because these displacements affect outgrowth (4). The genotypes of the deficiency heterozygotes are *dpy-11(e224)unc-76(e911)/unc-42(e270)yDf8* and for *ev424*, *n2367*, *n2397* and *n2398*, *sma-1(e30)unc-76/yDf8*.

unc-76 alleles, except *n2398*, caused similar HSN axonal outgrowth defects (Table 1), with HSN axons extending an average of 64–79% of the distance from the vulva to the head (wild type = 100%). Animals carrying the allele *n2398* had a mean HSN length of 89% and were slightly less uncoordinated than the rest. The mean HSN lengths in animals carrying *e911*, *n2367*, *n2397*, *n2457*, or *ev424* in *trans* to the deficiency *yDf8* were all similar to one another (73–77%) and to those observed in animals homozygous for each mutant allele. The mutation *smg-1(e1228)*, which is believed to elevate the levels of unstable RNA species (25), suppressed the effects of two of the eight alleles, *n2398* and *e911*. These observations are consistent with a model in which *n2398* is a weak allele, *ev424*, *rh116*, *n2397*, *n2367*, *n2399*, and *n2457* are severe loss-of-function or null alleles, and *e911* is similar in its consequences to the strong alleles but not fully null, because *smg* suppression can restore partial function. Analyses of DNA and protein from *unc-76* mutants support this model (see below).

Isolation of *unc-76* Genomic and cDNA Clones. We cloned the *unc-76* gene by identifying cosmid clones able to restore wild-type locomotion to uncoordinated *unc-76(e911)* animals after germ-line transformation. *unc-76* is located less than 0.1 map units to the right of *sdc-3* (26). Any of five overlapping cosmids, C56C4, T25A9, C08C1, C01G10, and C13G10, located to the right of *sdc-3* on the *C. elegans* physical map (12–13) rescued the Unc phenotype of *unc-76(e911)* animals, while cosmids flanking this group (C25D7 to the left and T06H10, T01G5, and C28G7 to the right) did not. A 10.7-kb *XbaI* fragment from C56C4, represented in p76-16B, completely restored normal locomotion of *unc-76(e911)* animals,

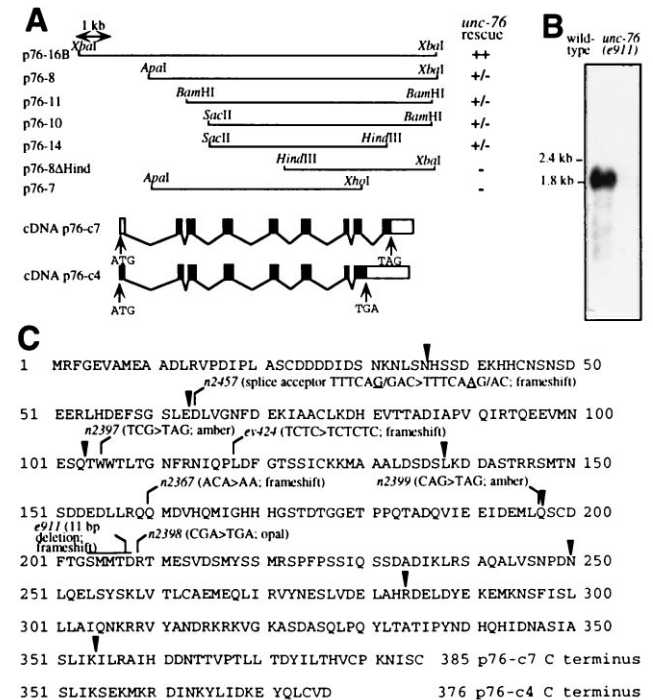


FIG. 1. The *unc-76* gene. (A) Structures of *unc-76* genomic and cDNA clones. ++, restoration of locomotion of *unc-76(e911)* animals to that of the wild type; -, no effect on locomotion; \pm , rescue in young larvae but poorer locomotion in older animals. Exons (black bars) and introns (thin lines) of the cDNA clones p76-c4 and p76-c7 are aligned with the genomic map. (B) *unc-76* RNA. The ³²P-labeled insert from p76-c4 was hybridized to 10 μ g of poly(A)⁺ RNA from wild-type and *unc-76(e911)* mutant embryos. (C) UNC-76 protein sequences deduced from p76-c4 and p76-c7. The sequences are identical through amino acid 354, and the alternative C termini are shown beginning at amino acid 351. Exon boundaries (arrowheads) and positions affected by *unc-76* mutations are indicated. The sequence deleted from *e911* is ATCCGTCATCA.

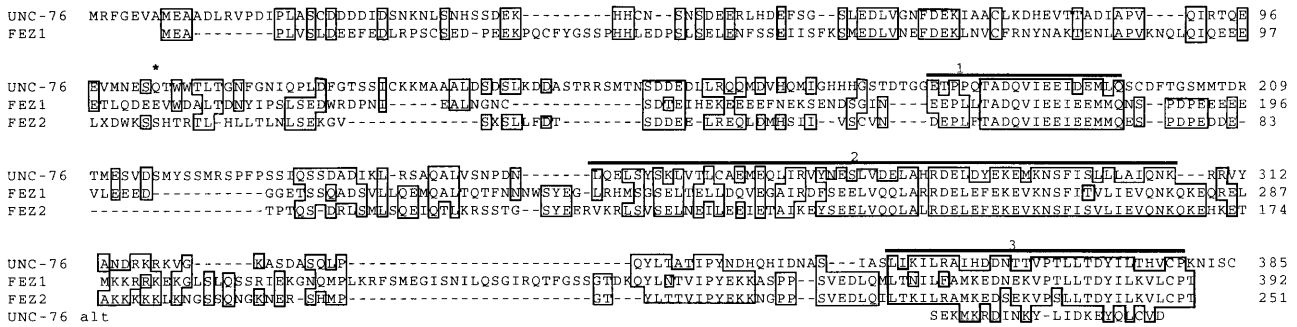


FIG. 2. Alignment of *C. elegans* UNC-76 protein with two human homologs. Identical amino acids are boxed. Amino acids 1–98 of FEZ2 were inferred from the overlapping clone DY1C1TG01 (accession number F15259), which probably lacks sequences encoding the N terminus of FEZ2. Longer FEZ2 clones were not present in dbEST. The alternatively spliced C terminus from p76-c4 is labeled UNC-76 alt. X, amino acid was ambiguous in the reported DY1C1TG01 sequence. *, C terminus of FEZ1-T. Bars labeled 1–3, regions of highest conservation.

while transformed lines carrying any of several subclones as small as 5.5 kb (p76–14) showed wild-type movement among young larvae but increasing uncoordination of older animals (Fig. 1A).

Inserts from two clones isolated from a mixed-stage *C. elegans* cDNA library, p76-c4 (2.6 kb) and p76-c7 (1.7 kb), hybridized to sequences throughout the rescuing genomic DNA (data not shown) and to a major transcript of 1.8 kb in RNA from wild-type embryos (Fig. 1B). This transcript was reduced in abundance in *unc-76(e911)* embryos. A low-abundance 2.4-kb transcript that hybridized to the p76-c4 and the p76-c7 inserts was observed in long exposures of blots of wild-type, but not mutant, RNA (data not shown).

Analysis of *unc-76* Sequence and RNA Structure. Comparison of genomic and cDNA sequences showed that the two cDNA clones, each of which contained a single large open reading frame (Fig. 1C), differed primarily in their 5' ends (p76-c4 extended 119 nucleotides further 5' than p76-c7) and in the presence or absence of an intron near the 3' end. RNase protection experiments showed that p76-c7 corresponds to the major splice form (intron 8 removed) in mixed-stage RNA (data not shown). Primer extension and reverse transcriptase-PCR experiments with mixed-stage RNA indicated that the two major *unc-76* transcript types contain the leader sequence SL1 (27) trans-spliced 5 or 23 nucleotides upstream of the 5' end of p76-c7 (data not shown), consistent with protein products starting at amino acids 1 or 8 in the sequences shown in Fig. 1C. These experiments also indicated that at least one other class of transcripts begins approximately 500 bp further 5' and extends at least to the beginning of exon 2. Such transcripts would not alter the predicted UNC-76 protein products.

We identified mutations in the coding regions or splice junctions in seven of the eight *unc-76* alleles (Fig. 1C). All are predicted to generate truncated protein products; three mutations introduced premature stop codons (*n2397*, *n2398*, and *n2399*), and four caused frameshifts (*e911*, *ev424*, *n2367*, and *n2457*). The mutant allele encoding the longest truncated protein, *n2398*, caused the least severe HSN and locomotory defects.

UNC-76 and Its Human Homologs Define a New Protein Family. The 376 and 385 amino acid forms of the UNC-76 protein (encoded by p76-c4 and p76-c7, respectively) showed no strong similarity to any previously characterized proteins, but three groups of similar human sequences were identified in the expressed sequence tag database, dbEST (28). We determined the sequences of the longest available cDNA clones from each group and found that they are probably derived from two genes. Two other laboratories recently have identified rat *unc-76* homologs in independent yeast two-hybrid screens (see Discussion). To reflect the names given by these groups, this family of UNC-76-like proteins has been

called FEZ, for fasciculation and elongation protein; zygin/zeta-1, and we have named the two human genes we identified FEZ1 and FEZ2.

Alignment of the 251 amino acid FEZ2 fragment inferred from partial cDNA clones with the complete 392 amino acid FEZ1 protein and the *C. elegans* UNC-76 proteins (Fig. 2) showed that UNC-76 is 35% identical (46% similar) to FEZ1 and 34% identical (45% similar) to FEZ2 in the region of overlap. The two human proteins are 49% identical (56% similar) to each other in the region represented in both proteins. Three regions in the C-terminal halves are most similar, with amino acids 179–197, 251–307, and 354–381 (numbered according to the *C. elegans* protein), showing 68%, 46%, and 54% identity, respectively, among all three proteins. The N-terminal halves are more divergent but still show substantial similarity.

A third class of human cDNA clones contained sequence identical to that of FEZ1 through codon 104, followed by a TAA stop codon and 1.3 kb of unrelated sequence. The truncated protein encoded by this clone, which we have named FEZ1-T, is probably a product of alternative splicing, because

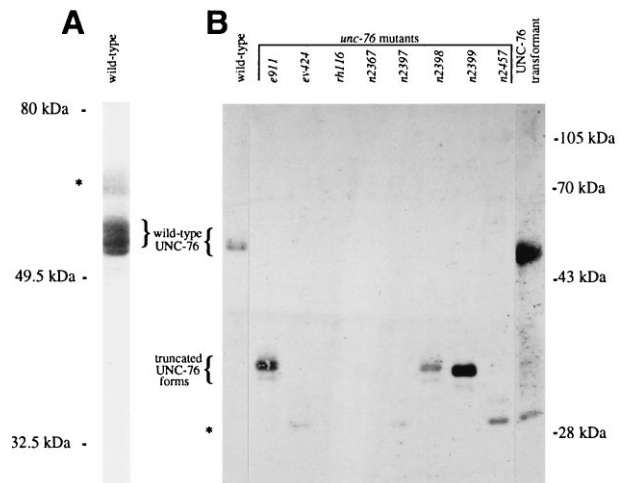


FIG. 3. Immunoblots of UNC-76 protein. Proteins from mixed-stage populations of worms were separated on (A) 7.5% and (B) 10% polyacrylamide gels, blotted with affinity-purified anti-UNC-76 serum and reacted with a peroxidase-conjugated anti-rabbit antiserum and the ECL chemiluminescent reagent (Amersham). The UNC-76 transformant carried the rescuing plasmid p76-16B in an *unc-76(e911)* background and contained the *e911* mutant form of UNC-76 protein, not visible in this exposure, in addition to the wild-type form. Bands indicated by asterisks probably do not represent UNC-76 protein, because they appeared in only a subset of samples from wild-type and *unc-76* mutant animals in other experiments (data not shown) and were unaffected by *unc-76* mutations.

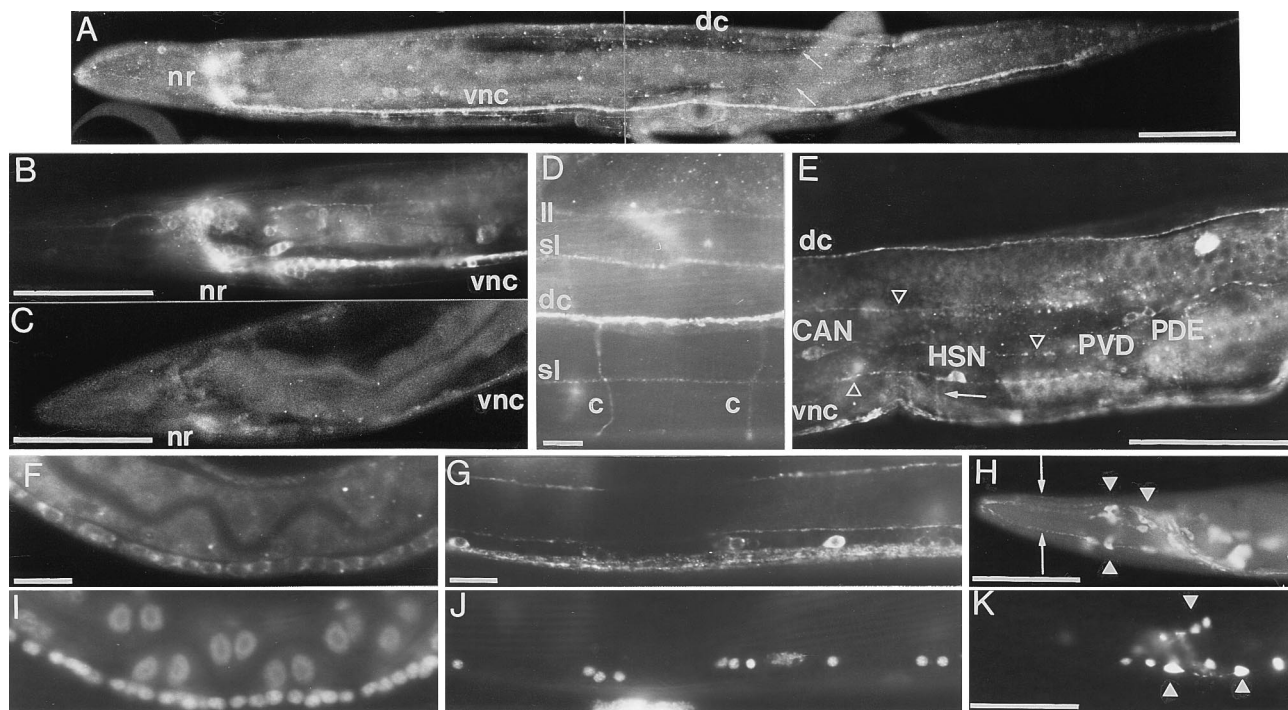


FIG. 4. Localization of UNC-76 and UNC-76:: β -galactosidase fusion proteins. Anterior is to the left, and dorsal is at the top. (A) UNC-76 protein in a wild-type adult is visible in the nerve ring (nr), ventral cord (vnc), dorsal cord (dc), and dorsal and ventral sublateral process tracts (arrows). UNC-76 protein in a wild-type adult (B) is more abundant than in an *unc-76(ev424)* mutant adult (C). (D) Dorsal region of a wild-type adult just anterior to the vulva. Staining is visible in the dorsal nerve cord (dc), dorsal sublateral tracts (sl), a left lateral tract (ll), and two motor commissures (c). (E) Midbody region of a wild-type adult. Staining is visible in cell bodies of PVDR, PDER, HSNR, and CANR as well as the dorsal (dc) and ventral (vnc) nerve cords, several longitudinal process tracts (arrowheads), and the circumferentially directed HSN axon (arrow). UNC-76 staining near the midbody region of L2 (F) and adult (G) ventral nerve cords and the corresponding nuclei visualized by diamidinophenolindole (I and J). (H) An UNC-86::UNC-76:: β -galactosidase fusion protein encoded by p86/76-1 is localized to axons (arrow) as well as cell bodies (arrowheads) in a small set of neurons in the head of an adult stained with anti- β -galactosidase antibodies. (K) The equivalent UNC-86:: β -galactosidase fusion protein lacking UNC-76 sequences (encoded by p86-L1) is confined primarily to cell bodies (arrowheads). Cell identities in H and K were not determined. [Bars = 50 μ m (A–C, E, H, and K) and 10 μ m (D, F, G, I, and J).]

the cDNA sequences diverge after an AG dinucleotide. The nematode *unc-76* gene is spliced at the equivalent position.

None of the FEZ family proteins appeared by Kyte-Doolittle hydrophobicity analysis (29) to have a hydrophobic region sufficiently long to serve as a signal sequence or transmembrane domain (data not shown), suggesting that the proteins are intracellular. Secondary structure predictions suggested that nine conserved regions (UNC-76 amino acids 48–61, 66–83, 105–121, 182–196, 237–248, 257–282, 292–307, 351–360, and 369–378) could form amphipathic helices. No other structural or functional motifs were identified.

UNC-76 Protein Is Present in All Axons Throughout Development. On immunoblots, affinity-purified UNC-76 antisera from each of three rabbits (see *Materials and Methods*) recognized a set of at least three 51- to 54-kDa proteins (Fig. 3) that were not recognized by preimmune sera (data not shown). These proteins were absent from all eight *unc-76* mutants and were restored by transformation of *unc-76(e911)* mutant animals with the rescuing clone p76-16B. Three mutants (*e911*, *n2398*, and *n2399*) showed novel truncated forms 19–20 kDa smaller than the wild-type proteins, as predicted from the mutant DNA sequences (Fig. 3B).

Indirect immunofluorescence microscopy of wild-type worms stained with affinity-purified sera from each rabbit showed intense staining in all major nerve bundles (the nerve ring and dorsal and ventral nerve cords) and all minor longitudinal and circumferential process tracts and bundles of sensory processes (Fig. 4). Staining was visible in neuronal cell bodies but not in nuclei (Fig. 4 B and E–G). No neuronal fluorescence was observed in worms stained with any of the three preimmune sera (data not shown), and staining was

reduced or nearly eliminated in all eight mutant *unc-76* strains (Fig. 4C). The residual staining in *unc-76(e911)* animals was eliminated by preincubation of antisera with maltose binding protein::UNC-76 or UNC-76::His₆ fusion proteins (data not shown), indicating that this staining represented mutant UNC-76 protein.

Outside the pharynx, all neurons, but no other cells, stained with anti-UNC-76 antisera. Specifically, no staining was observed in cells that flank the ventral or dorsal nerve cords, such as body wall muscles or hypodermal cells. Pharyngeal UNC-76 expression was not visible, but pharyngeal cells were probably not made accessible to antibodies during fixation because they lacked the background stain seen in the rest of the animal.

Axonal segments that are not fasciculated appear normal in *unc-76* mutant animals, in contrast to the segments of the same axons that grow in fascicles (2–4). Nonetheless, we observed UNC-76 protein throughout the axons of wild-type animals, including some nonfasciculated axon segments known to be normal in *unc-76* mutant animals (all motor neuron commissures and the ventral-directed segments of the HSN, PDE, and PVD axons; Fig. 4 D–E). UNC-76 protein-containing commissural and longitudinal axons on the lateral body wall usually crossed without apparent changes of direction (Fig. 4D), indicating that the simple presence of UNC-76 protein does not cause bundling.

UNC-76 staining was visible throughout the nervous system of animals at all developmental stages from newly hatched larvae through adults. Embryos were not sufficiently permeabilized to allow anti-UNC-76 antibody staining, but *unc-76::lacZ* fusions that appeared to be expressed normally in larvae and adults were expressed in a few cells, the identities

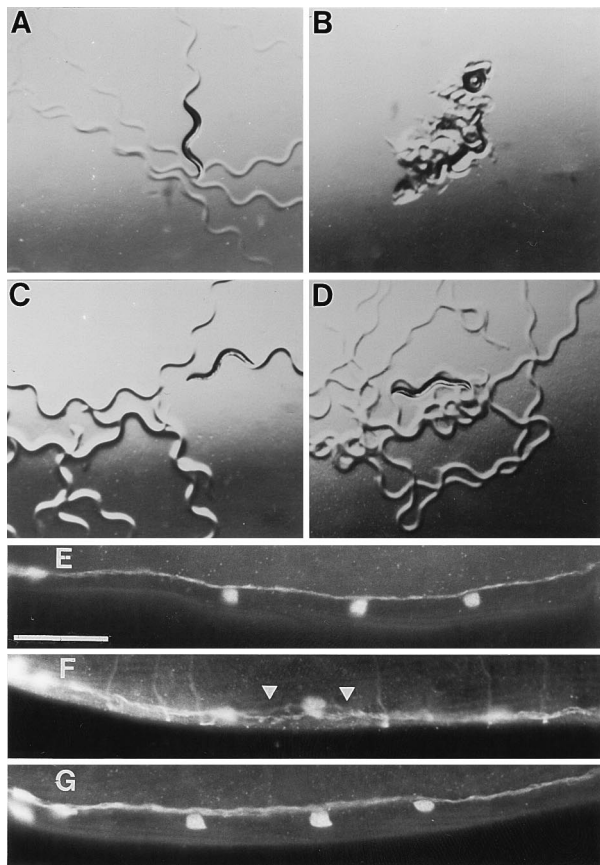


FIG. 5. Restoration of locomotion and ventral cord fasciculation in *unc-76(e911)* worms by *FEZ1*. (A–D) Animals were photographed after 1 h on slightly dried agar plates without bacteria. (A) Wild type; (B) *unc-76(e911)*; (C) *unc-76(e911)* carrying the *C. elegans unc-76* gene in p76–16B; (D) *unc-76(e911)* carrying the human *FEZ1* gene in p76-HsA-5. (E–G) Ventral nerve cords just posterior to the heads of adults stained with anti-GABA antisera. Anterior is at the left. (E) Wild type; (F) *unc-76(e911)*; (G) *dpy-20(e1292ts); unc-76(e911)* carrying the human *FEZ1* gene in p76-HsA-5 and the *C. elegans dpy-20* gene. Arrowheads, regions of defasciculation. Axons perpendicular to ventral nerve cord in F are motor commissures, out of the plane of focus in E and G. (Bar = 5 μ m.)

of which were not determined, in embryos of about 200 cells, before the outgrowth of the first axons (data not shown). UNC-76 staining in axons was strong throughout development, whereas cell body staining was strong in young larvae, but weaker in adults except for cell bodies in the head and tail ganglia (Figs. 4 B and F–G). Of neurons with laterally positioned cell bodies, only the CAN and HSN cell bodies consistently contained UNC-76 protein in adults (Fig. 4E).

The presence of C terminally truncated UNC-76 proteins in axons of mutant worms (Fig. 4C) suggested that axon-targeting activity of UNC-76 resides in the N-terminal third of the protein. Consistent with these observations, we found that amino acids 13–186 of UNC-76 can confer axonal localization to an otherwise nuclear-localized fusion of amino acids 1–83 of the *C. elegans* UNC-86 protein (22) to β -galactosidase (Fig. 4H and K). Amino acids 1–197 of UNC-76 have been used with several promoters to direct the green fluorescent protein (30) or β -galactosidase to axons in *C. elegans* (L.B., Y. Jin and H.R.H., unpublished observations; refs. 31–34) and in zebrafish (J. Dynes and J. Ngai, personal communication).

Rescue of *unc-76(e911)* by *FEZ1*. To determine whether the human *FEZ1* protein can function in *C. elegans*, we transformed *unc-76(e911)* and *dpy-20(e1282ts); unc-76(e911)* worms with the *FEZ1* cDNA under the control of a 1-kb fragment of the *C. elegans unc-76* promoter. *FEZ1* partially

rescued the *unc-76(e911)* locomotory defect (Fig. 5 A–D): *FEZ1*-carrying animals moved with an irregular sinusoidal waveform and were somewhat sluggish compared with wild-type animals, but were considerably more active and coordinated than *unc-76* mutant animals. Fasciculation of GABA-containing motor axons in the ventral nerve cord was also subtly improved in *FEZ1*-rescued *dpy-20(e1282ts); unc-76(e911)* animals (Fig. 5 E–G). Although axons aberrantly exited and re-entered bundles (data not shown), axons in the rescued worms seemed to remain in closer proximity than did axons in the mutants, suggesting improved overall fasciculation in the ventral nerve cord. The severely defective outgrowth of the axons of the HSNs and of the sensory neurons PHA and PHB observed in *unc-76(e911)* animals was not improved by *FEZ1* (data not shown).

DISCUSSION

***unc-76* Function Is Necessary for Normal Fascicle Structure.** *C. elegans unc-76* mutants have two types of axonal defects: axons in fascicles often do not reach their full lengths, and many axons that grow in fascicles fail to bundle tightly together (2–4). In *unc-76* mutants, many of the axons with abnormal growth in fascicles nonetheless extend normally around the body wall, unaccompanied by other axons, suggesting that *unc-76* is required specifically for axon-axon interactions. We have shown that these defects are caused by *unc-76* alleles that encode severely truncated proteins and are not substantially different in *unc-76/unc-76* homozygotes and *unc-76/deficiency* heterozygotes, indicating that this phenotype is likely to result from an absence of *unc-76* function.

***unc-76* Encodes a New Class of Axonal Protein.** We cloned the *unc-76* gene by rescue of the uncoordinated phenotype of *unc-76(e911)* animals after germ-line transformation. Evidence that the 10.7-kb rescuing clone contains the *unc-76* gene includes the observations that mutant *unc-76* strains contain alterations in DNA sequence, mRNA levels, and size and abundance of the protein predicted from analysis of cDNA clones that correspond to this genomic DNA fragment.

The major predicted UNC-76 protein is highly similar to two human proteins, identified as expressed sequence tags, which we have called FEZ1 and FEZ2, for fasciculation and elongation proteins; zygin/zeta-1 (see below). The *FEZ1* gene was able to restore partial locomotion and axonal fasciculation to *C. elegans unc-76* mutants in germ-line transformation experiments, indicating that both the function and the structure of the FEZ proteins have been conserved in evolution. Amino acids 1–197 of the *C. elegans* UNC-76 protein can target reporter proteins to axons in both *C. elegans* and zebrafish, suggesting that proteins that interact with this region in FEZ family members likewise have been conserved.

The proteins of the FEZ family have little similarity to previously characterized proteins and thus define a new class of proteins involved in axonal outgrowth. The N-terminal halves of the FEZ proteins are highly acidic, and large regions of the proteins are predicted to form amphipathic helices. However, the proteins lack motifs indicating specific functions. The absence of a potential signal sequence or transmembrane domain suggests that the FEZ proteins are intracellular. We observed *C. elegans* UNC-76 protein in the cell bodies and axons of nearly all neurons but not in nuclei or in nonneuronal cells. Membrane and cytoplasmic staining could not be distinguished in these experiments.

While most *FEZ1*-related clones in dbEST were derived from brain libraries (15/17 *FEZ1* and 4/5 *FEZ1-T* clones), all but two of the 15 *FEZ2* clones were derived from nonneuronal tissues (placenta, melanocytes, spleen, lung, heart, testis, and muscle). This distribution indicates that the FEZ family functions in nonneuronal cell types as well as in neurons.

Possible Functions of the FEZ Family Proteins. The severe defects in newly hatched *unc-76* mutant larvae suggest a function for UNC-76 early during nervous system development, whereas the persistence of UNC-76 in axons through adulthood suggests a continuing role. We propose two possible UNC-76 functions consistent with its likely intracellular localization and the fascicle-specific defects of *unc-76* mutants. First, the UNC-76 protein could play a structural role in the formation and/or maintenance of fascicles, e.g., by its intracellular association with a cell-surface adhesion molecule, the axonal membrane, or the cytoskeleton. Second, UNC-76 could transduce signals from cell-surface molecules to the intracellular machinery that regulates axonal extension and adhesion. In either case, the requirement for UNC-76 protein only in regions of axon-axon contact despite its presence throughout the axon could reflect the restricted distribution of cell-surface adhesion molecules or their ligands.

Consistent with both roles, two interactions of FEZ family members recently have been suggested from the results of two-hybrid screens in yeast. A rat homolog of FEZ1, called zeta 1, was found to bind to the C1 regulatory domains of protein kinase C types ζ and ϵ (S. Kuroda and U. Kikkawa, personal communication), and rat FEZ1 and FEZ2 homologs, named zygins I and II, were identified as synaptotagmin-binding proteins (T. Sudhof, personal communication). The protein kinase C family has been implicated in a wide range of cellular functions, including modulation of membrane structure (35). Synaptotagmins are membrane proteins required for synaptic vesicle exocytosis and are believed to function in other membrane-fusion events in nonneuronal cells (36). If the interactions of these proteins with FEZ family members are confirmed for endogenous proteins *in vivo*, the UNC-76 protein and other FEZ family members could provide points of convergence between signaling pathways and assembly of the cell membrane, perhaps as regulators of membrane insertion in growing axons or in motile organelles of nonneuronal cells.

An understanding of the biochemical functions of FEZ family members awaits further characterization of their interactions with other proteins, both those discussed above and others that might interact with the axon-targeting N-terminal region of the UNC-76 protein and the corresponding portion of the putative nonneuronal FEZ2. Our finding that the function of FEZ family members has been conserved between *C. elegans* and humans suggests that this novel protein family and the proteins with which its members interact are important components of the network of molecules that regulate cellular morphology.

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