Spot plates (Autoimmune Diagnostika) precoated with capture monoclonal antibody to IFN-γ (4 μg/ml) and blocked with Dulbecco's modified Eagle's medium containing 10% fetal calf serum. Plates were washed at 24 hours and probed with a sandwich biotinylated antibody to IFN-γ. Spots were detected with an anti-biotin alkaline phosphatase (AP) (murine ElisaSpot) or streptavidin–horseradish peroxidase (human ElisaSpot) with detection enzyme reactions of either NBT/BCIP (Pierce) or 3-amino-9-ethylcarbzole and *N,N*-dimethylformamide (Pierce/Fisher), generating purple or red spots, respectively. Scores were determined by the Series I T-Spot Image analyzer (Autoimmune Diagnostika) as the difference between the number of spots produced with and without antigen. OspA protein was a kind gift from B. Lade and J. Dunn (Brookhaven National Lab) and purified hLFA-1 was a kind gift from D. Staunton (ICOS Corporation). Human spinal chord extract was prepared according to standard procedures. The following antibodies were used for murine in vitro assays: 145.2C11 (murine antibody) or OKT3 (human antibody), CD3 antibody (hybridoma supernatant); R4-6A2, coat, IFN-γ antibody and XMG1.2, capture, biotinylated IFN-γ antibody (PharMingen); biotin-AP antibody (Vector). The following antibodies were used for human in vitro assays: OKT3, CD3 antibody (hybridoma supernatant); coat, IFN-γ antibody, and capture, biotinylated IFN-γ antibody (Endogen); streptavidin–horseradish peroxidase (Zymed).

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12. B10.m/Sn mice transgenic for DRB1*0101 were a kind gift from D. Zaller (Merck Research Laboratories); S. Feng, S. W. Barthold, L. K. Bockenstedt, D. M. Zaller, E. Fikrig, *J. Infect. Dis.* **172**, 286 (1995).
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15. We studied 11 patients (7 male, 4 female; between 12 and 40 years old) with treatment-resistant Lyme arthritis and 9 control patients (4 male, 5 female; between 17 and 78 years old) with RA or other forms of chronic inflammatory arthritis. All Lyme patients met the case definition of the U.S. Centers for Disease Control and Prevention for diagnosis of Lyme disease. They had arthritis affecting the knee and serologic reactivity with *B. burgdorferi* by ELISA and protein blotting. The 11 Lyme arthritis patients and 5 of the control patients were evaluated in the Lyme Disease Clinic at New England Medical Center (NEMC). The remaining 3 RA (patients 12, 13, and 15) and 1 psoriatic (patient 19) control patients' samples were a generous gift from R. Schumacher (Department of Medicine, University of Pennsylvania Medical School). The protocol was approved by the Human Investigations Committee, and informed consent was obtained from each subject. Patients with Lyme arthritis were treated with both oral and intravenous antibiotic regimens. The duration of arthritis after antibiotic therapy ranged from 2 to 33 months. High-resolution HLA-DR typing with sequence-specific amplification was performed by the Clinical Laboratory of Immunology (NEMC) and by Lee Ann Baxter-Lowe (University of South Carolina, Columbia, SC). Patient *DRB1* alleles are as follows: 10, *0102* and *1501*; 6, *0102* and *1501*; 5, *0401* and *1501*; 7, *0701* and *1601*; 2, *0301* and *1201*; 1, *1* and *11*; 4, *14* and *15*; 11, *0401* and *0401*; 8, *0402* and *7*; 9, *0301* and *1302*; 3, *0404* and *13*, 12, *0401* and *1*; 13, *15* and *7*; 16, *4*; 15, *0401* and *7*; 17, *4* and *17*; 18, *11*, *3*, or *13*; 20, *1* and *13*. Insufficient DNA was available from patients 14 and 19, so *DR* typing was not performed on them.
16. Patient SF cells were plated in 96-well U-bottomed plates (Costar) at a density of 2×10^5 cells per 200 μl in complete RPMI medium (Sigma). Cells were stimulated for 5 days with antigen (2 days with phytohemagglutinin), pulsed with 0.5 μCi of [^{3}H]thymidine during the final 16 to18 hours, and harvested for scintillation counting. Insufficient cells were available from patients 5 and 11; therefore proliferation assays were not performed. All Lyme arthritis patients' cells responded to OspA (except for patient 1) and $OspA_{164-183}$ (except for patients 1 and 2). Responses ranged from 254 to 2552 cpm (background) and from 2275 to 56,725 cpm (antigen).
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9 March 1998; accepted 8 June 1998

Pioneer Axon Guidance by UNC-129, a *C. elegans* TGF-β

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The *unc-129* gene, like the *unc-6* netrin gene, is required to guide pioneer motoraxons along the dorsoventral axis of *Caenorhabditis elegans*. *unc-129* encodes a member of the transforming growth factor–β (TGF-β) superfamily of secreted signaling molecules and is expressed in dorsal, but not ventral, rows of body wall muscles. Ectopic expression of UNC-129 from ventral body wall muscle disrupts growth cone and cell migrations that normally occur along the dorsoventral axis. Thus, UNC-129 mediates expression of dorsoventral polarity information required for axon guidance and guided cell migrations in *C. elegans*.

Axon guidance along the dorsoventral (D/V) axis of animals of diverse phyla involves secreted, laminin-related, UNC-6/netrin guidance cues (*1*). The signaling pathways activated by these molecules require the UNC-5 and UNC-40/DCC transmembrane receptor families (*2*–*4*). In *C. elegans*, mutations in *unc-129* (*5*) cause defects in the dorsally oriented trajectories of motoraxons that resemble those present in *unc-5*, *unc-6*, and *unc-40* mutants (*5*, *6*).

A 6.5-kb genomic subclone of cosmid C53D6 was able to rescue the uncoordinated phenotype of *unc-129* mutants after germline transformation (*7*, *8*) (Fig. 1A). Sequence analysis by the *C. elegans* genome-sequencing consortium (*9*) revealed a single open reading frame on this fragment that encodes a protein related to the TGF-β superfamily. The corresponding 1.5-kb cDNA (*10*) includes 5 exons, 34 base pairs (bp) of 5′ untranslated region (UTR), and 281 bp of 3′ UTR and is predicted to encode a protein of 407 amino acids (Fig. 1B). Northern (RNA) analysis of wild-type mRNA revealed a single transcript (*11*) consistent with the size of the cDNA. The 6.5-kb rescuing genomic fragment includes 3 kb of 5′ promoter sequence. A minigene containing 4.5 kb of 5′ promoter sequence fused to the *unc-129* cDNA was able to rescue the phenotype of *unc-129* mutants, indicating that there are no essential regulatory elements in introns or the 3′ sequence (*12*).

UNC-129 shares features with the TGF-β superfamily, including a signal sequence, a prodomain, and a COOH-terminal region that contains seven conserved cysteines (*13*). The UNC-129 COOH-terminal sequence identity ranges from 33% with human BMP-7 to 24% with TGF-β2. Thus, *unc-129* likely represents a subfamily of the TGF-β superfamily.

Sequence analysis revealed the absence of residues in UNC-129 that would be expected between the α-helical region and β sheet of TGF-β molecules (Fig. 1C) (*14*). This interdomain region forms a β turn with a protruding loop accessible to solvent. The three-dimensional structures of TGF-β1 and TGF-β2 differ at this site, which may promote their differing receptor-binding affinities (*15*). Deletion of the loop in TGF-β1 abolishes certain TGF-β1–mediated responses (*16*). Without knowledge of the crystal structure of UNC-129, it remains unclear whether the missing residues form the COOH-terminal end of the long α-helix or affect receptor specificity.

In *C. elegans*, TGF-β signaling pathways

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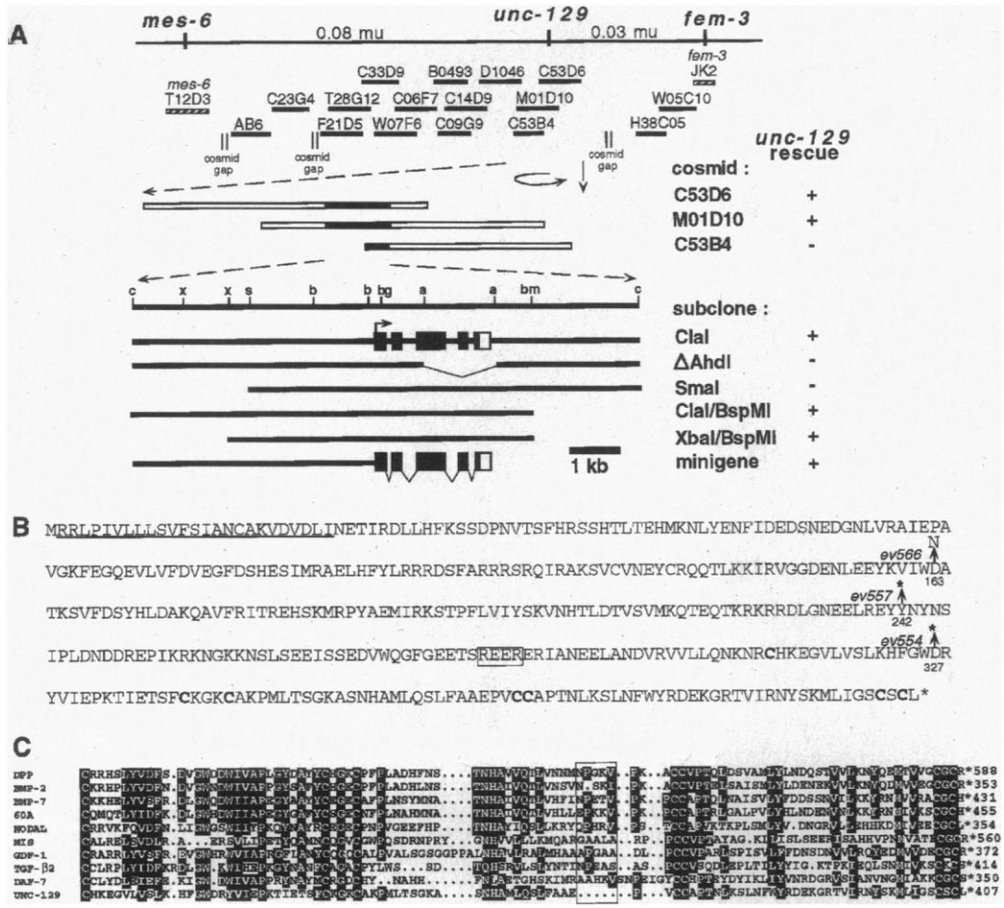


Fig. 1. Positional cloning and primary sequence of *unc-129*. (**A**) Cosmids spanning the *mes-6–fem-3* interval were assayed for *unc-129* rescuing activity (+ or −). The rescuing region was delimited by testing genomic subclones and an *unc-129* minigene. Genomic structure is indicated by boxed regions, black (coding), white (3′ UTR). Restriction sites: c, Cla I; x, Xba I; s, Sma I; b, Bst EII; bg, Bgl II; a, Ahd I; bm, Bsp MI. (**B**) Peptide sequence of UNC-129 with putative signal sequence (underlined), putative cleavage site (boxed), and conserved cysteines (highlighted in bold). Amino acid substitutions in *unc-129* alleles are indicated above the sequence (arrows). Asterisks indicate stop codons. (**C**) Comparison of the mature region of UNC-129 with members of the TGF-β superfamily; DPP, 60A (*Drosophila*); DAF-7 (*C. elegans*); BMP-2 (chicken); Nodal (mouse); BMP-7, MIS, GDF-1, TGF-β2 (human). The interdomain region predicted to be absent from UNC-129 is boxed (*unc-129* is gene C53D6.2, accession number AF029887). Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

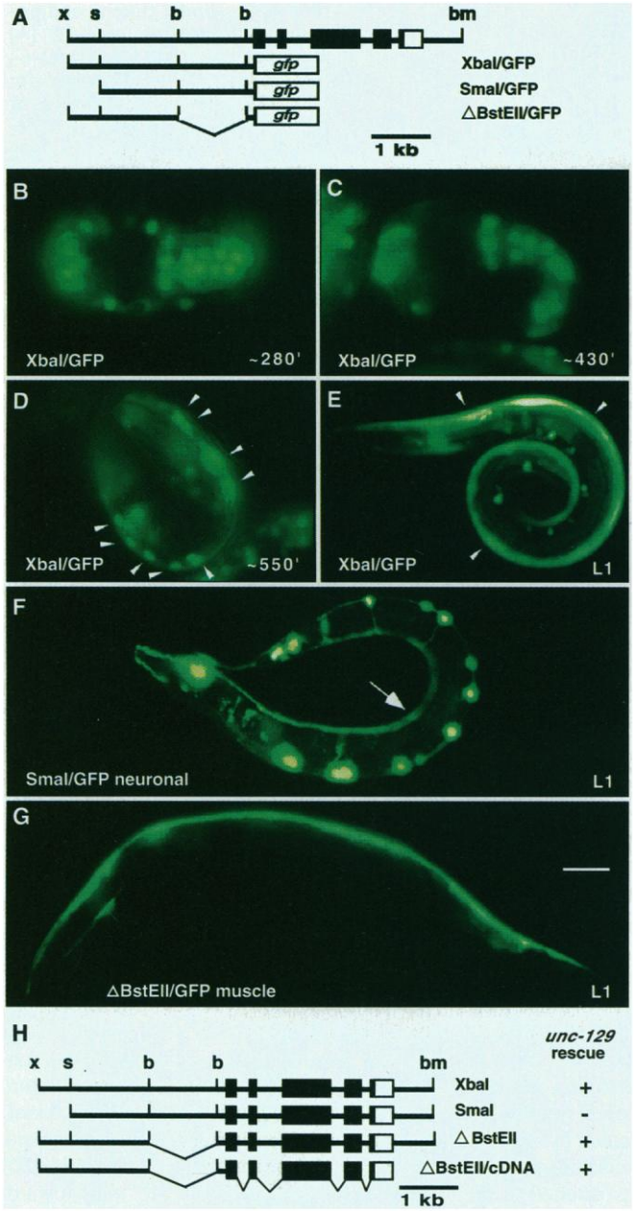
have been implicated in control of dauer larva formation, male tail patterning, and body size (*17–19*). These functions appear unaffected in *unc-129* null mutants. Rather, *unc-129* mutations disrupt axon guidance. No other patterning or morphological defects were identified. *ev557* and *ev554* mutations, which introduce stop codons (Fig. 1B) (*20*), cause axon guidance defects with higher penetrance than the hypomorph, *ev566* (*5*). Mutations in known components of TGF-β signaling pathways did not cause any axon guidance defects in the DA (n = 120) or DB (n = 120) classes of motorneurons (*21*). Alleles tested were *daf-1(m213*ts*)* and *daf-4(e1364*ts*)*, which are genes encoding type I and type II serine-threonine kinase receptors, respectively (*17*, *18*).

We assessed the expression pattern of *unc-129* using *unc-129::gfp* transcriptional reporter genes (*22*) expressed from transgenic arrays (Fig. 2, A to E). Promoter activity was first detected at late gastrulation stage in cells that include descendants of the AB and E lineages. Expression continues through embryonic elongation in some of these cells. About 450 to 520 min after first cleavage, expression was observed in a subset of cells in the head, including one ventral muscle, and in all dorsal body wall muscles. Between 520 min and hatching, green fluorescent protein (GFP) expression was detected in the DA and DB classes of motorneurons, excluding DA8 and DA9. This pattern of expression persisted into the adult stage. In addition, expression was detected in a subset of cells in the head and in pharyngeal neurons and muscle. Of these, only interneuron I4 and muscle m8 were unambiguously identified. In late larval stages, expression was detected in the spermatheca, seam cells, CAN, PDE socket, and four cells that encircle the vulva. Among cells that express *unc-129* promoter activity, only DA and DB motorneurons display morphological or axon guidance defects.

Deletion analysis revealed portions of the *unc-129* 5′ regulatory region that promoted predominantly muscle- or motorneuron-specific GFP expression (*22*, *23*) (Fig. 2, F and G). These truncated regulatory regions were used to express wild-type *unc-129* coding sequence in either the dorsal muscle or the DA and DB motorneurons to test for its ability to rescue the *unc-129* mutant phenotype. UNC-129 expression from the dorsal body wall muscle-specific promoter, but not from the motorneuron-specific promoter, rescued the uncoordinated movement and axon guidance defects of *unc-129* mutants (*23*) (Fig. 2H). This result suggests that wild-type UNC-129 expressed by dorsal muscle acts cell nonautonomously to guide the circumferential migrations of pioneer axons.

The timing of *unc-129::gfp* expression also suggests that *unc-129* mediates axon guidance. *unc-129* is expressed in dorsal muscle at the twofold stage of embryogenesis (450 to 520 min) when DA, DB, and DD motoraxons grow (480 to 515 min) toward the dorsal midline (*24*). *unc-129* is also expressed in dorsal muscle postembryonically as VD motoraxons grow toward the dorsal midline.

To further investigate whether dorsal-specific expression of *unc-129* is important for its function, we expressed a functional hemagglutinin (HA)-tagged UNC-129 (*25*) in both dorsal and ventral rows of body wall muscles (verified by immunostaining) under the control of the muscle-specific *myo-3* (myosin) promoter (*26*). We examined axon morphologies of the DA and DB neurons in independent lines of ectopic UNC-129–expressing animals using the neuron-specific GFP fusion to the *unc-129* promoter. Wild-type worms transgenic for *myo-3::unc-129HA* displayed uncoordinated locomotory defects and axon guidance defects in the DA and DB neurons that



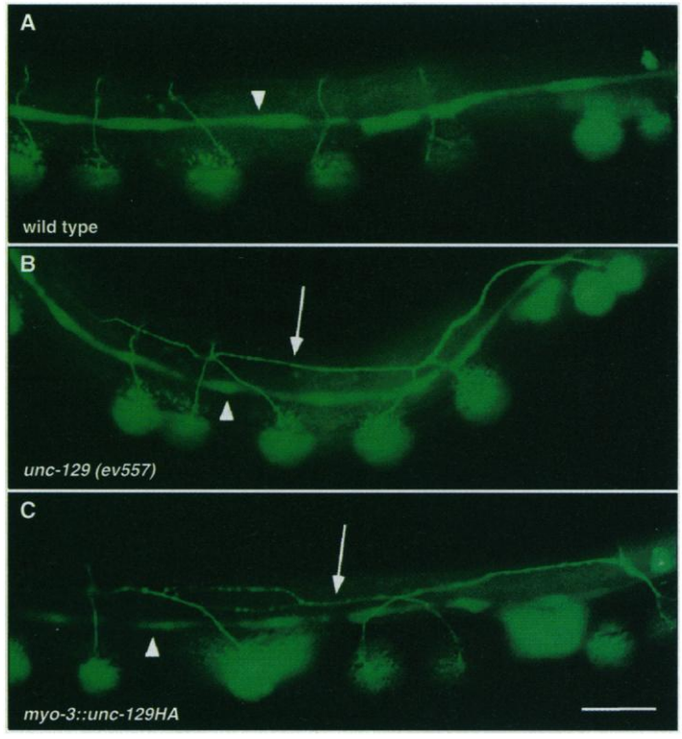


Fig. 2 (left). Expression pattern and cell nonautonomous activity of *unc-129*. (**A**) Comparison of *unc-129::gfp* transcriptional reporters used in this study with *unc-129* rescuing genomic DNA. (**B** to **E**) *unc-129::gfp* expression pattern at ~280 min (B), ~430 min (C), ~550 min (D) postcleavage, and in L1 larvae (E). Expression in dorsal body wall muscle [arrowheads in (D) and (E)] is observed before DA and DB expression. GFP expression vectors used in (B) to (D) contain a nuclear localization signal. (**F** and **G**) *unc129::gfp* reporter derivatives expressed predominantly in DA and DB motorneurons (F) (arrow indicates dorsal nerve cord) and dorsal body wall muscle in L1 larvae (G). (**H**) *unc-129* expression from the dorsal muscle–specific promoter is sufficient to completely rescue the *unc-129* phenotype. Restriction sites as in Fig. 1. Bar, 10 μm. **Fig. 3 (right).** Ectopic expression of *unc-129* in dorsal and ventral body wall muscle phenocopies the axon guidance defects of an *unc-129* null mutant. DA and DB neurons are visualized in L4 stage animals through use of the neuronal *unc-129::gfp* reporter. Motoraxons are misrouted longitudinally at lateral positions (arrows). (**A**) Wild type; (**B**) *unc-129(ev557)*; and (**C**) *unc-129(+)* animal carrying an integrated *myo-3::unc-129HA* transgene. Arrowheads indicate row of lateral seam cells. Bar, 50 μm.

resembled the defects observed in *unc-129* mutants (Fig. 3), but with lower penetrance for the DA than for the DB class (Fig. 4A).

Ectopic *unc-129* expression also causes axon guidance and cell migration errors that are not found in *unc-129* mutants. We assessed ventral axon guidance by examining AVM and PVM mechanosensory neurons using a *mec-4::gfp* reporter. In both wild-type and *unc-129* mutants, these neurons are located at lateral positions and send a single process toward the ventral nerve cord. In all *myo-3::unc-129HA* transgenic lines, however, many AVM and PVM axons are misrouted along longitudinal trajectories (Fig. 4B). Similar ventral guidance defects are also found in *unc-6* and *unc-40* mutants (*6*). Therefore, *unc-129* expression from all body wall muscle perturbs guidance of axon growth cones in both dorsal and ventral directions. We also observed defects in the dorsalward migrations of the distal tip cells (DTCs), which are mesodermal cells that normally follow a U-shaped trajectory along the body wall (Fig. 4C). Similar DTC migration defects are present in *unc-5*, *unc-6*, and *unc-40* mutants (*6*). Thus, spatially restricted expression of *unc-129* promotes normal axon guidance and DTC migration (*27*).

Any model of UNC-129 function must take into account its genetic interactions with the UNC-6/netrin pathway, particularly UNC-5. The identification of *unc-129* mutations as suppressors of ectopic UNC-5–induced growth cone guidance is consistent with either a direct role in the UNC-6/netrin pathway or a role in a parallel pathway of related function. The nearly complete penetrance of axon guidance defects exhibited by *unc-5* and *unc-6* null mutants compared to the similar but milder defects in *unc-129* null mutants (*5*) precludes the use of double-mutant analysis to distinguish between these possibilities.

UNC-129 may act directly as a guidance cue that provides polarity information to migrating growth cones, or indirectly, by inducing neighboring cells to form a guidance cue. In principle, UNC-129 could affect expression of components of UNC-5 signaling. However, UNC-129 does not affect transcription of *unc-5*, *unc-6*, or *unc-40* as judged by examining expression of *unc-5::gfp* (*28*), *unc-40::gfp* (*3*), and *unc-6::HA* (*29*) reporter genes in wild-type

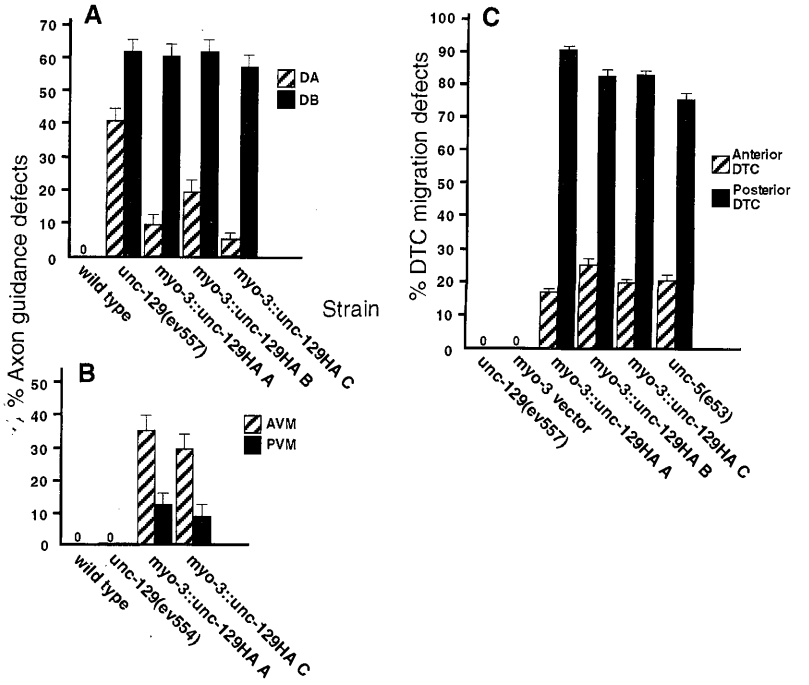


Fig. 4. Ectopic expression of *unc-129* in all body wall muscle causes axon guidance and cell migration defects. (**A**) DA and DB axon guidance defects in wild type, an *unc-129* mutant, and three independent lines carrying integrated *myo-3::unc-129HA* transgenes. All strains are transgenic for the neuronal *unc-129::gfp* reporter. (**B**) Ventral guidance defects in the AVM and PVM neurons. Axons were scored as ventral guidance defective if AVM and PVM axons were misrouted longitudinally along the lateral hypodermis. All strains are transgenic for a *mec-4::gfp* reporter. (**C**) DTC dorsal migration defects in wild-type and *unc-5* mutants expressing *myo-3::unc-129HA* transgenes. The *myo-3* vector strain carries an extrachromosomal array containing the *myo-3* expression vector alone. Bars represent ±SD of a binomial distribution of the same sample size and observed mean.

and *unc-129(ev554)* animals (*30*). Because none of the known TGF-β receptors (including DAF-1 and DAF-4) affect motoraxon guidance and TGF-β or Smad mutants do not show uncoordinated phenotypes (*17–19*), we suggest that UNC-129 uses an unconventional TGF-β–based mechanism to guide axons on the D/V axis in *C. elegans*. One possibility, suggested by the finding that TGF-β molecules can bind to TSP type I domains (*31*), is that UNC-129 acts directly on UNC-5 to enhance the ability of UNC-5 to mediate UNC-6–dependent growth cone repulsion (away from the ventral midline). Or, UNC-129 may function in a separate signaling pathway that mediates motoraxon attraction to the dorsal midline. The latter model predicts that motoraxons would be simultaneously repelled and attracted dorsally by opposite gradients of UNC-6 and UNC-129, respectively (*17*, *18*). The identification and localization of additional UNC-129 signaling pathway components should help to distinguish between these models.

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21. Axons of DA and DB motorneurons were examined by fluorescence microscopy with the *unc-129::gfp* reporter pAC12 (*5*, *23*) passed genetically into *daf-1* and *daf-4* mutants and a newly identified TGF-β receptor mutant (*18*).
22. A fragment of the *unc-129* 5′ regulatory region extending 4.8 kb upstream from the initiator methionine codon was amplified by polymerase chain reaction (PCR) from a genomic clone (pAC3) with the primers 5′-CATTTTCTTGCTTGCTCTTCC and T7. Transcriptional reporters were made by inserting the 4.8-kb regulatory region upstream of, and in frame with, the GFP coding sequence contained in vectors pPD95.79 and pPD95.70 (+NLS) to create pAC9 and pAC10, respectively. pAC9 was cut with either Xba I, Sma I, or Bst EII Xba I and religated to generate GFP reporters containing 3 kb (pAC11), 2.5 kb (pAC12), or 3 kb with an internal deletion of 1.2 kb (pAC13) of *unc-129* regulatory sequence, respectively (Fig. 3). Extrachromosomal arrays carrying *unc-129::gfp* reporters were created by standard germline transformation (*7*) and chromosomally integrated with the protocol described by M. Hamelin, I. M. Scott, J. C. Way, and J. G. Culotti [*EMBO J.* **11**, 2885 (1992)].
23. The neuron- and muscle-specific promoter derivatives express in essentially the same set of cells as full-length promoter derivatives (see text), except for muscle and neurons (plus seam cells), respectively. Neither derivative appears to disrupt embryonic expression. Expression of *unc-129(+)* from the *unc-129* neuron-specific promoter does not rescue motoraxon guidance defects of *unc-129 (ev554)* (45% DA and DB defects collectively, $n = 96$), but expression from the muscle-specific promoter does rescue (3% DA and DB defects collectively, $n = 88$). VD and DD motoraxons are also visibly rescued by expression of *unc-129(+)* from the muscle-specific promoter (A. Colavita, S. Krishna, H. Zheng, R. W. Padgett, J. G. Culotti, unpublished data).
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32. We thank A. Coulson for providing cosmids; A. Fire, M. Driscoll, and D. Pilgrim for reporter constructs; R. Barstead for a cDNA library; R. Steven for the Northern blot; and D. Merz, M. T. Killeen, J. Segall, A. Spence, and H. D. Lipshitz for reviewing the manuscript. The Caenorhabditis Genetics Center, supported by the NIH National Center for Research Resources, provided strains for this work. This work was supported by grants from the NIH (R.W.P.) and from the Canadian Medical Research Council (J.G.C.).

31 March 1998; accepted 19 June 1998