*p*NPP at 30°C for 1 hour. Absorbance of the reaction mixture was measured at 410 nm.
27. S. M. Schoenwaelder *et al.*, *Curr. Biol.* **10**, 1523 (2000).
28. D. M. Spencer, T. J. Wandless, S. L. Schreiber, G. R. Crabtree, *Science* **262**, 1019 (1993).
29. T. M. Saxton *et al.*, *EMBO J.* **16**, 2352 (1997).
30. S. Manes *et al.*, *Mol. Cell. Biol.* **19**, 3125 (1999).
31. E. S. Oh *et al.*, *Mol. Cell. Biol.* **19**, 3205 (1999).
32. D. H. Yu, C. K. Qu, O. Henegariu, X. Lu, G. S. Feng, *J. Biol. Chem.* **273**, 21125 (1998).
33. We thank R. A. Weinberg for reading of the manuscript. We also thank T. Matozaki for human SHP-2 cDNA. Supported by a Grant-in Aid for Scientific Research on Priority Areas from the Ministry of Education, Culture, Sports, Science and Technology of Japan; a Research Grant from the Human Frontier Science Program Organization; and a Research Grant from the Nippon Boehringer Ingelheim Co., Ltd.

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Immunoglobulin-Domain Proteins Required for Maintenance of Ventral Nerve Cord Organization

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During development, neurons extend axons along defined routes to specific target cells. We show that additional mechanisms ensure that axons maintain their correct positioning in defined axonal tracts. After termination of axonal outgrowth and target recognition, axons in the ventral nerve cord (VNC) of *Caenorhabditis elegans* require the presence of a specific VNC neuron, PVT, to maintain their correct positioning in the left and right fascicles of the VNC. PVT may exert its stabilizing function by the temporally tightly controlled secretion of 2-immunoglobulin (Ig)–domain proteins encoded by the *zig* genes. Dedicated axon maintenance mechanisms may be widely used to ensure the preservation of functional neuronal circuitries.

The development of axonal tracts in the nervous system depends on defined and tightly regulated interactions between individual cell types that ultimately guide axons to their specific targets. In the nematode *C. elegans*, the PVT interneuron plays a prominent role during the development of the ventral nerve cord (VNC). The cell body of PVT is located at the posterior end of the VNC and its axon, which is one of the first to pioneer the VNC (*1*), extends along the entire length of the right VNC from the posterior of the animal into the anteriorly located nerve ring (*2*). PVT transiently expresses the *unc-6*/netrin guidance cue during embryonic development (*3*) and embryonic ablation of PVT as well as mutations in *unc-6*/netrin cause defects in dorso-ventral axon attraction into the VNC (*4*).

Various aspects of axon pathfinding and target recognition are regulated by members of the immunoglobulin superfamily (IgSF) of secreted and cell surface bound proteins (*5*). In a comprehensive and genome-wide analysis of expression patterns of IgSF proteins in *C. elegans* (*6*), we noted that six members of a novel family of secreted 2-Ig domain proteins, termed *zig-1*, *zig-2*, *zig-3*, *zig-4*, *zig-5*, and *zig-8* (Fig. 1A), are co-expressed in the PVT neuron of larval and adult stage animals (Fig. 1, B and C). Given the previously established embryonic role of PVT in axon guidance in mid-embryonic stages, we were intrigued to find that the onset of expression of all six PVT-expressed *zig* reporter gene constructs occurred significantly later than the stages of embryonic VNC axon outgrowth; this is particularly apparent with *zig-1::gfp*, *zig-2::gfp*, *zig-3::gfp*, *zig-4::gfp*, and *zig-8::gfp*, whose expression is activated postembryonically in the first larval (L1) stage (Fig. 1, D and E). This expression profile prompted us to investigate a potential postembryonic role of PVT by assessing the effect of its microsurgical removal in the L1 stage. Using a reporter gene that labels the complete VNC, we noted that 30% of animals in which PVT was laser ablated in the L1 stage contained axons that were aberrantly placed across the ventral midline (Fig. 2, B through D). By ablating PVT in the L1 stage of animals in which various subsets of neurons in the VNC are labeled with *gfp* (*7*), we determined that the misplaced axons derived from the AVKL/R, PVQL/R, HSNL/R, and RMEV neuron classes (Fig. 2, A, C, and D). With the exception of HSNL/R, the axons of all these neuron classes have completed their outgrowth in mid- to late embryonic stages, i.e., long before we ablated PVT in the first larval stage. The observation that PVT ablation in the L1 stage disrupts the correct positioning of embryonically generated axons thus reveals an unexpected nonautonomous role for a neuron in maintaining VNC architecture past the stage of its initial patterning.

PVT is required to exert its maintenance function during a narrowly defined temporal window. Laser ablation of PVT at the L1, L2, and adult stages revealed that PVT function is only required during the L1 stage (Fig. 2D). Moreover, individual PVT-ablated animals that were scored at any stage later than the L1 stage as either "wild type" or "defective," still showed the same phenotype when scored 2 days after the initial scoring (n = 14). Thus, axons that have drifted in the L1 stage, will remain in the inappropriate track, while axons that managed to remain in the correct position, will not flip over at later stages. One explanation for the stability of the phenotype past the L1 stage could be the growth of the physical barrier presented by the hypodermal ridge, which significantly increases its size during larval development (*8*). Another, not mutually exclusive possibility for the stability of the phenotype is that a drifted axon is kept in the opposite cord by homophilic interaction with the axon of its contralateral analog (*9*). Double laser ablations revealed this to be indeed the case. In the absence of PVT, the aberrant axon flip-over of PVQL into the right VNC is suppressed if PVQR is ablated (Fig. 2D).

We also considered the possibility that axon flip-over into the opposite fascicle in PVT-ablated animals may be facilitated by the mechanical force provided by the constant bending of animals along their longitudinal axis during sinusoidal locomotion of the animals. We tested this hypothesis by culturing PVT-ablated L1 animals on plates that contain the cholinergic agonist levamisole, which immobilizes animals due to hypercontraction of their body wall muscles (*10*). In an independent approach, we ablated PVT in muscle-defective *unc-97* animals (*11*). Both immobilization protocols caused a complete suppression of the axon flip-over phenotype upon PVT ablation (Fig. 2D), suggesting that movement and hence mechanical force is a contributing factor for the axon flip-over.

Taken together, our data suggest that specifically at the L1 stage the left and right VNC are intrinsically unstable structures that require the presence of a specific neuron to maintain the integrity of individual axonal tracts (Fig. 2E). PVT presumably supplies stabilizing cues that prevent axons from drifting into the opposite fascicle, an event that is facilitated by mechanical force and homophilic attraction of bilaterally analogous axons in the opposite fascicle. Stabilizing cues provided by PVT either directly help to anchor axons in the left or right

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VNC or specifically antagonize homo-attractive forces between left/right contralateral analogs. Such a stabilizing impact of PVT may be specifically required in the L1 stage for several reasons. In contrast to later larval and adult stages, the hypodermal ridge that separates the left from the right VNC fascicle is comparatively shallow in the L1 stage (*8*), thus potentially not providing enough of a physical barrier to separate the left and right VNC and to prevent homophilic cues to attract axons into the opposite fascicle. Moreover, in spite of the persistence of the overall shape of the hypodermal ridge, the cellular environment at the ventral midline is very dynamic at the L1 stage due to positional rearrangements, cell shape changes, and fusions of several hypodermal cells (*12*) and due to the addition of several motor neurons into the VNC (*13*). These dynamic changes in the cellular and presumably also molecular environment may necessitate the employment of a stabilization mechanism to ensure the maintenance of the correct positioning of embryonically generated axonal tracts.

What are the molecular mechanisms by which PVT affects VNC axon maintenance? Because we ablated PVT at the developmental stage in which expression of five of the *zig* genes is normally activated, it was conceivable that secreted ZIG proteins mediate the role of PVT in maintaining VNC organization. To test this hypothesis, we analyzed the effect of a deletion in the *zig-4* locus, *zig-4(gk34)*, which was retrieved through PCR screening of a deletion library (Fig. 3A). PVT shows an intact axon morphology and adopts its correct cellular fate

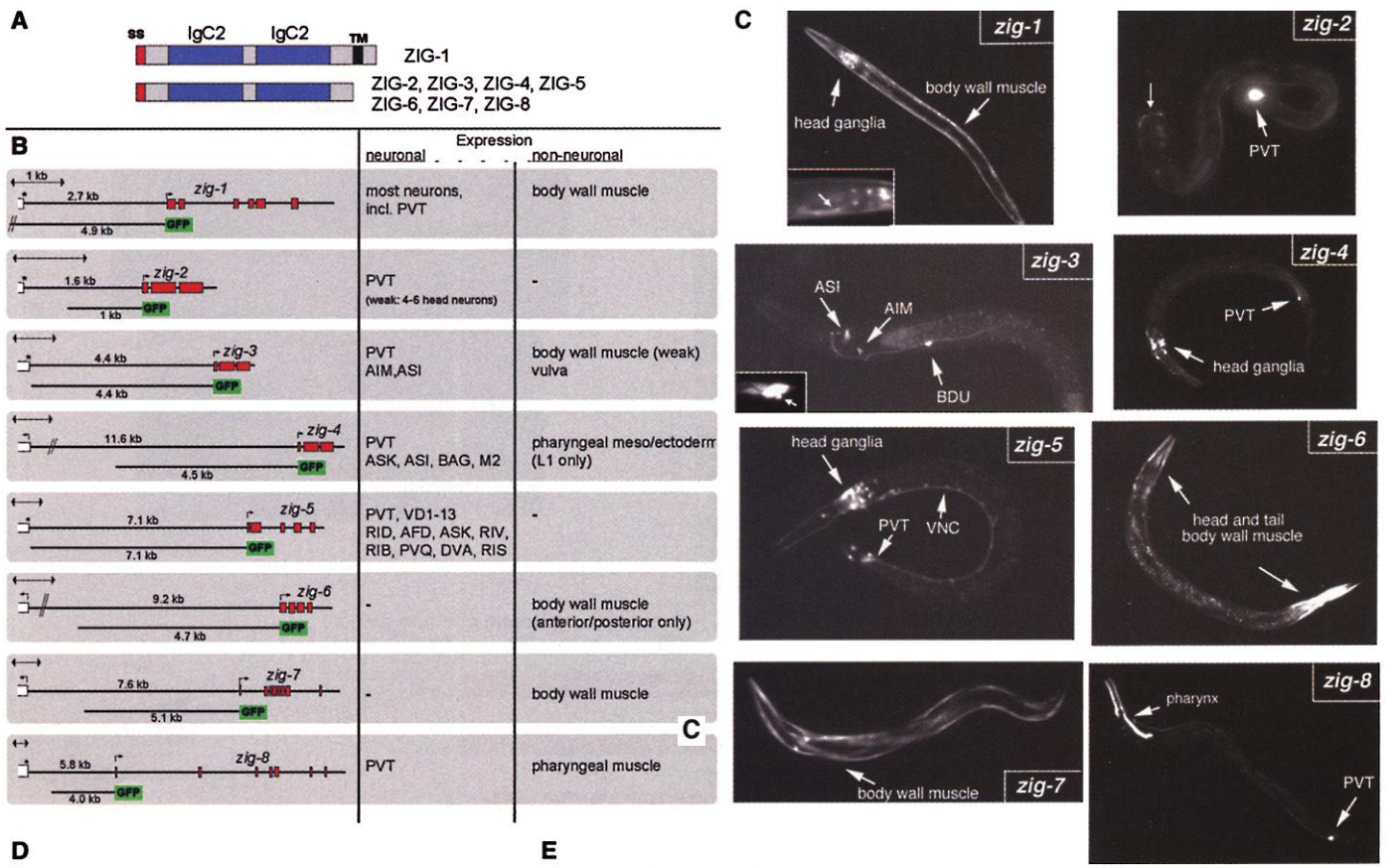
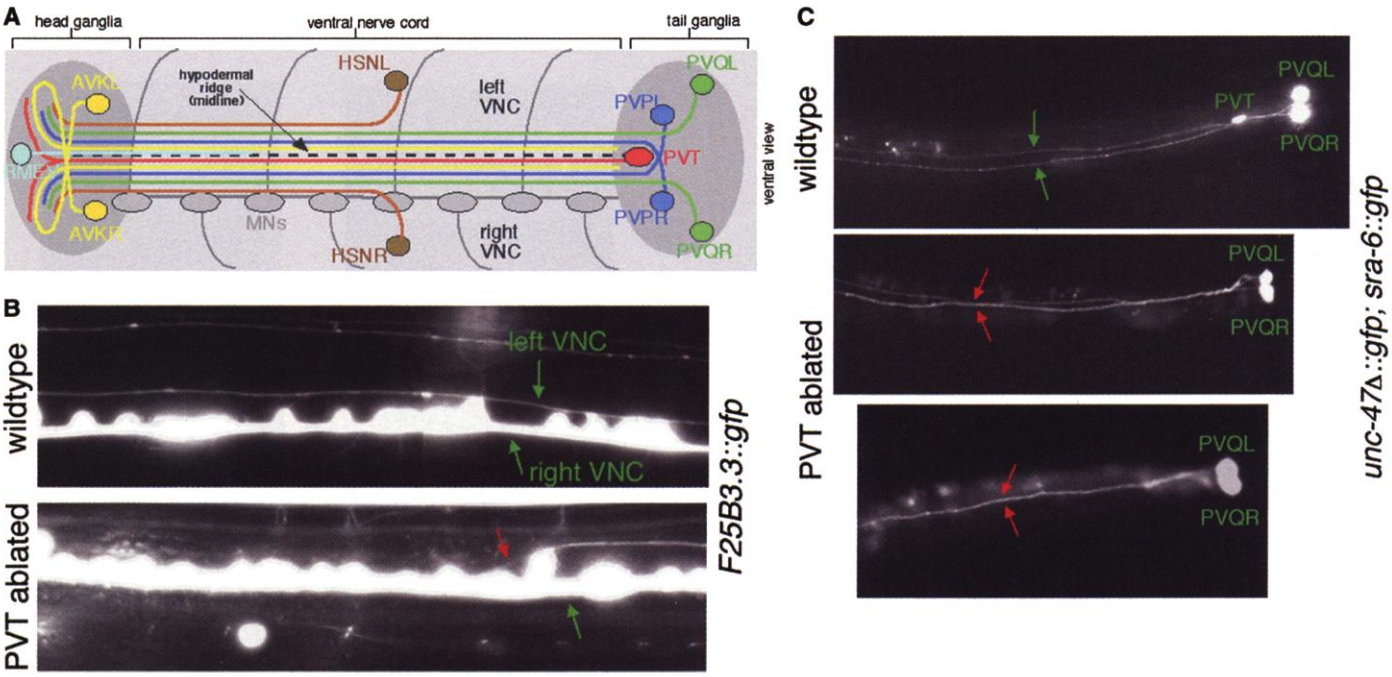


Fig. 1. Structure and expression of *zig* genes. (**A**) Schematic structure of the predicted ZIG proteins. Protein domain, signal sequence (SS), transmembrane (TM), and GPI-anchor-predictions were performed as described in Web fig. 1 and based on PCR-amplified cDNAs. ZIG proteins are solely defined by the presence of exclusively two Ig domains (hence "*zig*" for "zwei Ig"); otherwise the ZIG proteins are highly divergent among themselves both in primary sequence (Web fig. 2) and exon/intron organization [panel (B)], suggesting an ancient origin of this gene family. Database searches reveal that other metazoan genomes also contain a variety of secreted and transmembrane 2-Ig domain-only proteins whose role in neural development has not been explored (Web fig. 1B). (**B**) Structure of *zig*-promoter::*gfp* fusion constructs and summary of expression patterns (*21*). (**C**) Transgenic animals expressing *zig::gfp* fusion constructs. Arrows in insets point to PVT (*21*). (**D**) Temporal dynamics of *zig-4::gfp* expression from the integrated array *otIs20*. Note the absence of *zig-4::gfp* expression in PVT at embryonic and early L1 stages (upper left panel). Similar temporal expression profiles in PVT are observed with chromosomally integrated *zig-1*, *zig-2*, *zig-3*, and *zig-8::gfp* reporter genes. *zig-5::gfp* expression can first be observed at late (>threefold) stages of embryonic development. (**E**) Timeline of *zig* gene expression in relation to *C. elegans* development, timing of pioneer axon outgrowth (*1*) and *unc-6/netrin* expression (*3*). Expression of *zig* genes in PVT persists in adults, yet becomes less penetrant.

in *zig-4(gk34)* animals (*14*). As suggested by the PVT ablation data, visualization of individual motor-neuron classes with specific GFP markers demonstrated that loss of *zig-4* also does not affect the generation, positioning, or axonal path of either embryonically or postembryonically generated VNC motor neurons (*7*). However, *zig-4* mutant animals display defects in VNC axon positioning that strongly resemble those seen in PVT laser ablated animals, such that axons of embryonically generated neurons from the left and right VNC drift into the opposite cord. These defects could be observed on the light microscopical (Fig. 3, B through D) as well as on the electron microscopical level (Fig. 3, G and H). In contrast to the PVT ablation, the VNC axonal organization defects in *zig-4* mutant animals show a striking cellular specificity. The AVKL/R and PVQL/R axons are as strongly affected in *zig-4* null mutant animals as in PVT-ablated animals, while the HSNL/R and RMEV axons are unaffected (Fig. 3D). It is conceivable that other *zig* genes are required to ensure the structural integrity of these axons.

The axonal defects of *zig-4* mutant animals can be rescued by expressing *zig-4* under its own promoter, which within the VNC is only



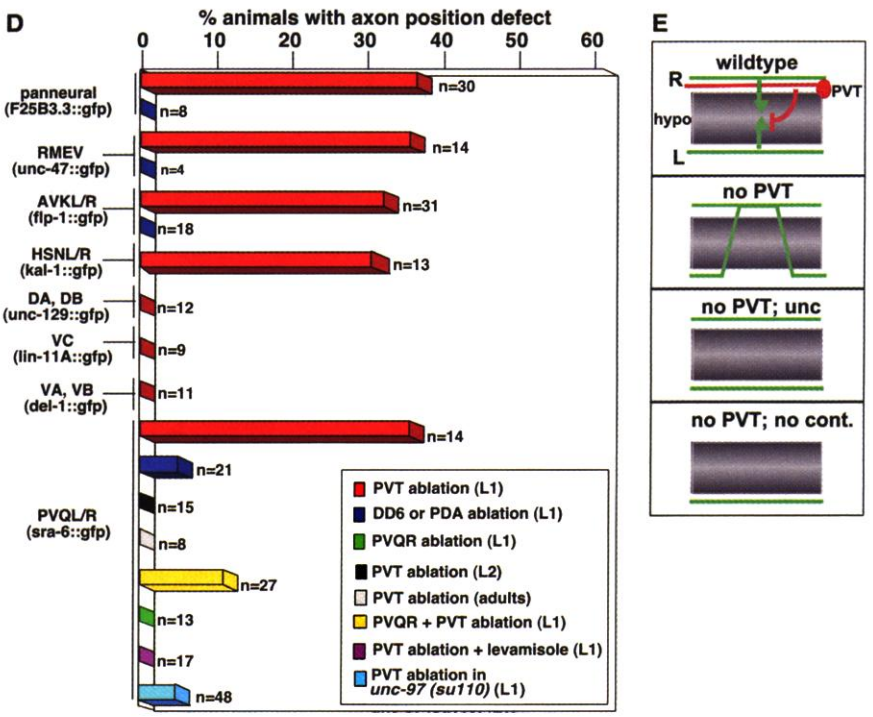


Fig. 2. Postembryonic laser ablation of PVT in first larval stage animals causes mispositioning of axons in the VNC. (**A**) Schematic of VNC structure. The right VNC consists of axons from more than 40 neurons (including all motorneurons; shown schematically in gray) while the left VNC contains only AVKR, PVQL, PVPR (*2*) in the posterior half of the VNC, and in addition, HSNL and RMEV in the anterior half of the VNC (all shown in color). Other than RMEV, each of these has contralateral analogs in the right VNC. All these neurons are embryonically born and with the exception of HSNL/R initiate and terminate axon outgrowth in mid- to late embryonic stages. With the exception of PVPR, we examined the anatomy of every individual left VNC axon. (**B** and **C**) Representative examples of VNC defects in adult animals in which PVT was ablated early in the L1 stage. Red arrows indicate aberrantly positioned axons. Axons flip from the left into the right fascicle and vice versa. Laser ablation was performed as previously described (*22*), using a LSI VSL-337 laser. In (B), the whole VNC is labeled with the panneural marker *F25B3.3::gfp*; in (C), two classes of VNC neurons are labeled simultaneously with two different *gfp* markers, *unc47Δ::gfp* for PVT and *sra-6::gfp* for PVQL/R (*7*). Successful ablation of PVT can be observed via absence of a *gfp* signal. (**D**) Quantification of defects caused by PVT-ablation in the L1 stage. Animals were transgenic for *gfp* markers as indicated (*7*) and scored as adults, with the exception of levamisole-treated (220 mM) animals, which were scored at the late L1/early L2 stage, i.e., the earliest stage in which the mutant phenotype is observed in non-levamisole treated animals. Whereas levamisole treatment ultimately led to a developmental arrest, animals nevertheless developed well into the L2 stage allowing scoring of the phenotype. (**E**) Schematic summary of VNC defects observed in laser-operated animals. Green axons exemplify any pair of left/right bilaterally analogous axons in the VNC. "*unc*" = immobilized animals, "*no cont.*" = contralaterally analogous axon removed.

active in the PVT neuron in postembryonic stages (Fig. 3E). Inserting a synthetic transmembrane domain to this rescuing construct abolishes rescuing activity (Fig. 3E). Furthermore, expression of *zig-4* under control of the *unc-4* promoter, which within the VNC is exclusively active in several motor neurons in the right VNC (DA, VA, VC) (*15*) is capable of substituting for loss of *zig-4* in PVT (Fig. 3E). Together with the nonautonomy of the defects observed in PVT-ablated and *zig-4* mutant animals, we conclude that the ZIG-4 protein does not act directly on neighboring cells but may act as a long-range cue.

The appearance of *zig-4* defects correlates with the onset of *zig-4* reporter gene expression in the mid- to late L1 stage; *zig-4* null mutant animals show no defects in axon positioning shortly after hatching, while the defects are visible at any stage after the late L1 stage (Fig. 3F). Consistent with the PVT ablation data, the defect is remarkably stable once it is manifested in late L1/early L2 stage, such that individual animals that were scored as "defective" or "wild type" at a stage data, the *zig-4* gene appears to be specifically required during the L1 stage to stabilize the positioning of a specific subset of axonal tracts in the VNC.

Because proteins with Ig domains often interact in various heteromeric configurations

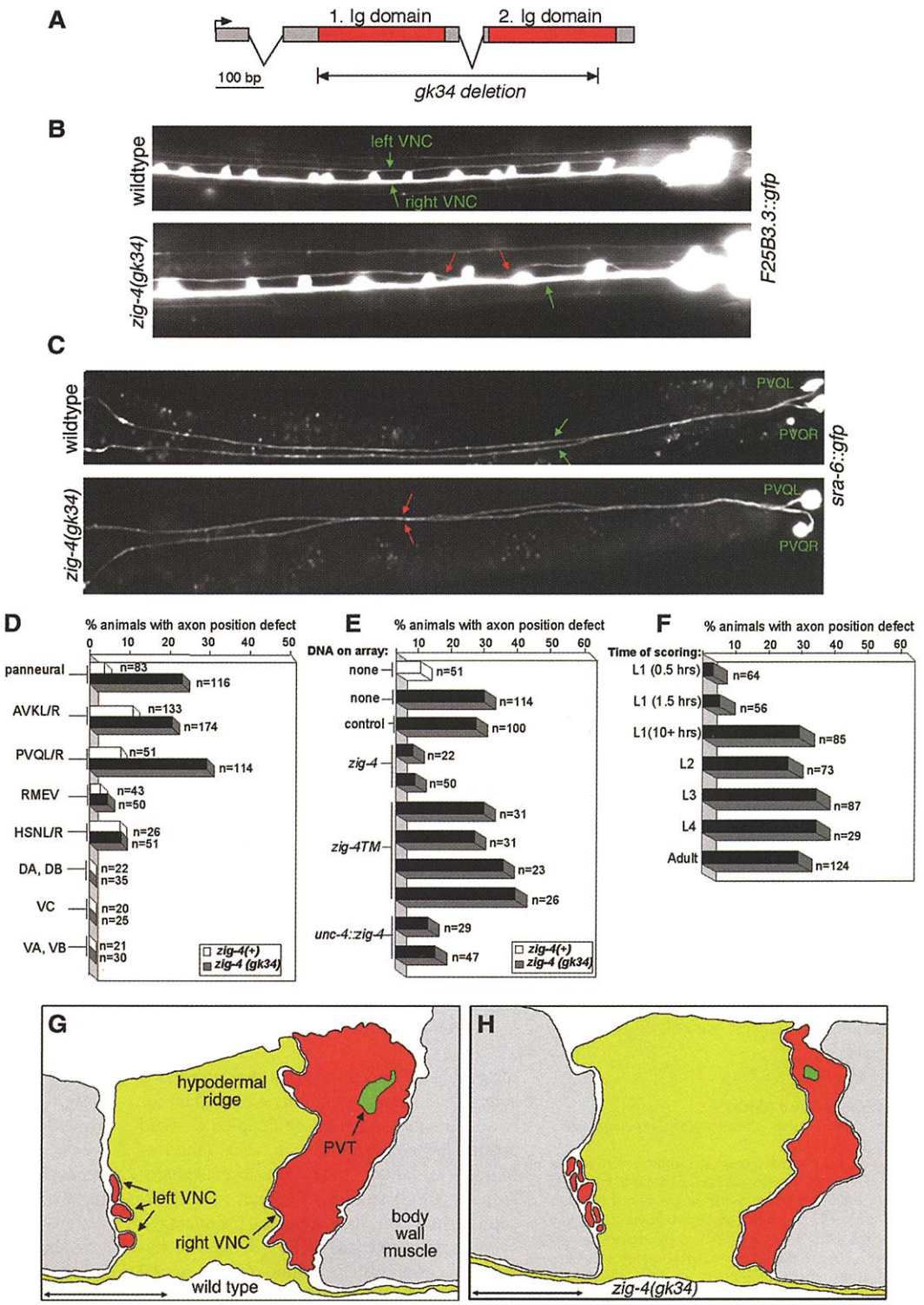


Fig. 3. A *zig-4* null mutation causes defects in axon positioning in the VNC. (**A**) Schematic presentation of the *zig-4* deletion allele *gk34*, provided by the Vancouver Knock-out Consortium. (**B** and **C**) Representative example of VNC defect in *zig-4(gk34)* using the panneural *F25B3.3::gfp* and PVQL/R-expressed *sra-6::gfp* marker. Red arrows point to aberrantly positioned axons. Similar to PVT-ablated animals, there is no directionality in the phenotype, meaning axons flip from the left into the right fascicle and vice versa. (**D**) Quantification of axonal defects caused by *zig-4(gk34)* detected with the *gfp* reporters indicated in Fig. 2D and rescue (**E**) of defects with various constructs, expressed from several independent extrachromosomal arrays (*21*). In (E), all strains express *sra-6::gfp* from a chromosomally integrated array (*21*). "*control*": Bluescript vector; "*zig-4*": 7.4 kb of genomic regions encompassing the *zig-4* gene, cloned into Bluescript. "*zig-4TM*": same as before with synthetic transmembrane domain added 10aa before the stop codon. "*unc-4::zig-4*": 2.9 kB of *unc-4* upstream regulatory region (*15*) fused to the *zig-4* genomic coding region. (**F**) Time course of occurrence of PVQL/R axon placement defects in *zig-4(gk34); oyIs14*. (**G** and **H**) Representative electron micrograph tracings of the VNC in wild-type and *zig-4* mutant animals, showing an aberrant number of axons in the left VNC of *zig-4* mutant animals. Three of 10 sectioned animals contain an aberrant number of axons in the left and right fascicles. The EM analysis also reveals that the gross anatomy of the VNC, and specifically the hypodermal ridge, are roughly intact in *zig-4* mutant animals. More samples, original EM images, and details on EM methods are shown in Web figs. 3 and 4.

(*5*), we investigated whether *zig-4* is a possible interaction partner of the Robo/SAX-3 receptor, mutations in which cause VNC axon cross-over defects similar to those observed in *zig-4* mutants (*16*). We found, however, that unlike in *zig-4* mutant animals, the *sax-3* mutant phenotype is already manifested in freshly hatched L1 animals (*17*), and thus presumably represents an axon outgrowth defect rather than a maintenance defect. Consistent with such a notion, a *sax-3(0)zig-4(0)* double mutant shows additive defects in VNC disorganization (*17*). Taken together, *C. elegans* employs separate mechanisms for axon outgrowth and axon maintenance.

Several distinct models can be envisioned for how ZIG-4 and possibly other ZIG proteins mediate the maintenance function of PVT. ZIG-4 may "anchor" axons in the left and right VNC and thus prevent their flipping into the opposite fascicle by being an essential component of a ternary complex that ensures axon attachment to its environment in the left and right VNC. Because axon flip-overs in the absence of PVT-secreted ZIG cues require the presence of the contralateral analog of an axon, it could also be envisioned that an intrinsic attractive force of left/right analogous axons is directly antagonized by ZIG proteins, for example by ZIG proteins binding and inhibiting the activity of homophilic molecules presented by left/right contralaterally analogous axons. In a more indirect model, ZIG proteins could induce hypodermal cells to present specific stabilizing cues on the surface of the hypodermal ridge. Ultimately, the identification of the receptors for the ZIG proteins as well as their localization will illuminate the molecular mechanisms of ZIG protein action.

In summary, we have uncovered the existence as well as the cellular and molecular basis of a novel mechanism required to maintain correct axon positioning during specific stages of postembryonic growth and development. Axon fibers in any organism are subjected to various forms of mechanical stress and changing molecular and cellular environments during embryonic and postembryonic growth phases. The need to preserve the functional and structural integrity of the nervous system thus makes it likely that similar maintenance mechanisms, possibly also mediated by as yet uncharacterized 2-Ig domain proteins present in other metazoan genome sequences, are conserved across phylogeny.

References and Notes

1. R. M. Durbin, thesis, University of Cambridge, Cambridge, UK (1987).
2. In their electron microscopical reconstruction of the nervous system, White *et al.* had lost the tracking of the PVT axon in the posterior half of the animal (*18, 19*). Using several independent *gfp* reporters, we could show that the axon of PVT extends into the nerve ring. Another update to the White *et al.* report (*18*) is Durbin's finding that PVPR and not PVPL extends along the left side of the VNC (*1*).
3. W. G. Wadsworth, H. Bhatt, E. M. Hedgecock, *Neuron* **16**, 35 (1996).
4. X. C. Ren, S. Kim, E. Fox, E. M. Hedgecock, W. G. Wadsworth, *J. Neurobiol.* **39**, 107 (1999).
5. P. Sonderegger, Ed., *Ig Superfamily Molecules in the Nervous System* (Harwood Academic, Amsterdam, 1998).
6. Using standard sequence analysis tools, we identified the complete set of IgSF-only proteins in the *C. elegans* genome and examined their presumptive expression pattern using *gfp* reporter technology. The sequence and expression pattern analysis are shown in Web figs. 1 and 2, and Web table 1 (*20*).
7. Transgenic *gfp* reporter strains used to analyze VNC neuroanatomy are described in the Supplementary Methods (*20*).
8. See Web fig. 5 for electron micrographs of the hypodermal ridge of L1 animals (*20*).
9. An intrinsic homophilic affinity of bilateral analogs is suggested by the fact that the left and right axons of many bilaterally symmetric neurons, including AVKL/R, PVQL/R, PVPL/R, and HSNL/R, make direct contact with their contralateral analog in the nerve ring; moreover, axons of contralateral analogs that in wild-type animals both extend along the right VNC in tandem (e.g., AVBL/R, AVHL/R, AVAL/R, etc.) directly fasciculate with one another (*18*).
10. J. A. Lewis, C. H. Wu, H. Berg, J. H. Levine, *Genetics* **95**, 905 (1980).
11. O. Hobert, D. G. Moerman, K. A. Clark, M. C. Beckerle, G. Ruvkun, *J. Cell Biol.* **144**, 45 (1999).
12. B. Podbilewicz, J. G. White, *Dev. Biol.* **161**, 408 (1994).
13. J. E. Sulston, *Philos. Trans. R. Soc. London Ser. B* **275**, 287 (1976).
14. PVT cell fate and axon morphology were assessed using *unc-47Δ::gfp*, *zig-2::gfp*, and *zig-8::gfp*. *zig-4(0)* animals also appear indistinguishable from wild-type animals in regard to gross body morphology, locomotion, and the execution of simple behaviors.
15. D. M. Miller III, C. J. Niemeyer, *Development* **121**, 2877 (1995).
16. J. A. Zallen, S. A. Kirch, C. I. Bargmann, *Development* **126**, 3679 (1999).
17. Axon mispositioning defects were scored with the PVQL/R marker *oyIs14*. Newly hatched *sax-3(ky123)* L1s: 28.6% crossed-over, 25.7% laterally displaced (n = 35). Newly hatched *zig-4(gk34)* L1s: see Fig. 3D. In adults: *sax-3(ky123)*: 40.3% crossed-over (n = 57), *zig-4(gk34)*: 29% (n = 114), *sax-3(ky123)zig-4(gk34)*: 69.1% (n = 55).
18. J. G. White, E. Southgate, J. N. Thomson, S. Brenner, *Philos. Trans. R. Soc. London Ser. B* **314**, 1 (1986).
19. D. H. Hall, R. L. Russell, *J. Neurosci.* **11**, 1 (1991).
20. Supplementary material is available on *Science* Online at www.sciencemag.org/cgi/content/full/295/5555/686/DC1
21. For generation of constructs and transgenic animals see Supplementary Methods (*20*).
22. C. I. Bargmann, L. Avery, in Caenorhabditis elegans: *Modern Biological Analysis of an Organism*, H. F. Epstein, D. Shakes, Eds. (Academic Press, New York, 1995), vol. 48, pp. 225–250.

23. We are grateful to the Vancouver Knock-out consortium for providing the *gk34* allele, J. White and J. Hodgkin for supplying the raw EM data of several *C. elegans* strains from MRC/LMB files to the Center for *C. elegans* Anatomy, members of the worm community for providing transgenic *gfp* reporter strains, W. Raich for communicating results on *F02G3.1::gfp* expression, C. Bargmann for comments on bilaterality, T. Stephney for help with EM, J. Zhu and L. Diamond for expert technical assistance, the Hobert and Greenwald labs for support and discussion, and P. Sengupta, T. Jessell, R. Axel, I. Greenwald, and members of the Hobert lab for comments on the manuscript. Several strains were provided by the *C. elegans* Genetics Center, which is funded by the NIH. This work was funded by the Whitehall Foundation, the March of Dimes Foundation, an NIH postdoctoral fellowship to O.A. (5F32NS11107-02), and an NIH grant RR12596 to the Center for *C. elegans* Anatomy (D.H.H.). O.H. is a Searle and Irma T Hirschl Scholar, a Sloan Research Fellow, and a Klingenstein Fellow.

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Dynamic Brain Sources of Visual Evoked Responses

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It has been long debated whether averaged electrical responses recorded from the scalp result from stimulus-evoked brain events or stimulus-induced changes in ongoing brain dynamics. In a human visual selective attention task, we show that nontarget event-related potentials were mainly generated by partial stimulus-induced phase resetting of multiple electroencephalographic processes. Independent component analysis applied to the single-trial data identified at least eight classes of contributing components, including those producing central and lateral posterior alpha, left and right mu, and frontal midline theta rhythms. Scalp topographies of these components were consistent with their generation in compact cortical domains.

Increasing evidence suggests that correlated activity in neural populations is important for brain function and may be coupled to field potential oscillations over a wide range of frequencies (*1*). Most electroencephalographic (EEG) studies of human visual perception, however, have assumed the averaged event-related potential (ERP) evoked in humans by brief visual stimuli reflects neural activity within discrete, functionally defined visual cortical processing regions. In this view, response averaging removes background EEG activity (considered to be noise), whose time course is presumed to be independent of experimental events, as well as most artifacts produced by eye and muscle activity. Other researchers, by contrast, suggest that ERP features arise from alterations in the dynamics of ongoing neural synchrony generating the scalp EEG (*2–4*). By these