Neural Maintenance Roles for the Matrix Receptor Dystroglycan and the Nuclear Anchorage Complex in *Caenorhabditis elegans*

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ABSTRACT Recent studies in *Caenorhabditis elegans* have revealed specific neural maintenance mechanisms that protect soma and neurites against mispositioning due to displacement stresses, such as muscle contraction. We report that *C. elegans* dystroglycan (DG) DGN-1 functions to maintain the position of lumbar neurons during late embryonic and larval development. In the absence of DGN-1 the cell bodies of multiple lumbar neuron classes are frequently displaced anterior of their normal positions. Early but not later embryonic panneuronal expression of DGN-1 rescues positional maintenance, suggesting that dystroglycan is required for establishment of a critical maintenance pathway that persists throughout later developmental stages. Lumbar neural maintenance requires only a membrane-tethered N-terminal domain of DGN-1 and may involve a novel extracellular partner for dystroglycan. A genetic screen for similar lumbar maintenance mutants revealed a role for the nesprin/SYNE family protein ANC-1 as well as for the extracellular protein DIG-1, previously implicated in lumbar neuron maintenance. The involvement of ANC-1 reveals a previously unknown role for nucleus–cytoskeleton interactions in neural maintenance. Genetic analysis indicates that lumbar neuron position is maintained in late embryos by parallel DGN-1/DIG-1 and ANC-1–dependent pathways, and in larvae by separate DGN-1 and ANC-1 pathways. The effect of muscle paralysis on late embryonic- or larval-stage maintenance defects in mutants indicates that lumbar neurons are subject to both muscle contraction-dependent and contraction-independent displacement stresses, and that different maintenance pathways may protect against specific types of displacement stress.

AN emerging theme in neurobiology is the importance of systems dedicated to the maintenance of the intricate architecture of the nervous system (Benard and Hobert 2009). The locations of neuronal soma, axons, and dendrites must be maintained to ensure proper nervous system function during the addition and removal of neurons and synapses and in response to mechanical stresses associated with body growth and movement. The factors maintaining nervous system architecture are often distinct from those involved in its establishment during development, a division of labor which likely allows flexibility to cope with the stresses involved in remodeling, growth, and movement.

The involvement of extracellular matrix components, cell adhesion molecules, and cytoskeletal proteins in previously reported neural maintenance activities demonstrate that ad-

hesive cell–matrix and possibly cell–cell interactions play a critical role (Aurelio *et al.* 2002; Bulow *et al.* 2004; Sasakura *et al.* 2005; Wang *et al.* 2005; Benard *et al.* 2006, 2009; Burket *et al.* 2006; Pocock *et al.* 2008; Woo *et al.* 2008; Zhou *et al.* 2008). Although previously unreported, factors controlling nuclear position in neurons may also be predicted to play key roles in positional maintenance of neuronal soma. Regulated movement of the cell nucleus is important in neuronal migration in the vertebrate cerebral cortex and retina (Nadarajah and Parnavelas 2002; Baye and Link 2008), and nucleus–cytoskeletal linkages have effects on cell rigidity and adhesion (Stewart-Hutchinson *et al.* 2008; Olins *et al.* 2009; Chancellor *et al.* 2010).

We report a genetic approach to identify and explore the role of two novel maintenance factors in stabilizing neuron cell body position in *C. elegans*. We find that the dystroglycan (DG) ortholog DGN-1 plays an important role in maintenance of cell body position in posterior lumbar ganglion neurons in *C. elegans*. In vertebrates, DG is a critical basement membrane receptor in skeletal muscle, in glia of both the central and peripheral nervous systems, and possibly in epithelia (Cohn *et al.* 2002; Michele *et al.* 2002; Moore *et al.*

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2002; Muschler *et al.* 2002; Saito *et al.* 2003; Satz *et al.* 2009, 2010). Dystroglycan is expressed in neurons throughout the mouse brain (Zaccaria *et al.* 2001), where it has no significant role in histogenesis but is important in synaptic function, *e.g.*, during long-term potentiation in the hippocampus (Moore *et al.* 2002; Satz *et al.* 2010).

In *C. elegans*, the dystroglycan ortholog DGN-1 is not present in muscle but is expressed in epithelia and neurons and is involved in epithelial morphogenesis and neural guidance (Johnson *et al.* 2006; Johnson and Kramer 2012). We find that *dgn-1* null mutants display a progressive displacement of lumbar neuron cell bodies, indicative of a defect in neuroanatomical maintenance. This neural role of dystroglycan likely involves interaction with extracellular partners, including the matrix component DIG-1 previously implicated in neural maintenance (Benard *et al.* 2006; Burket *et al.* 2006).

A forward genetic screen for other mutations producing *dgn-1*-like lumbar neuron maintenance defects identified a second novel maintenance factor: the KASH domain protein ANC-1, a nesprin/Syne protein known to mediate anchorage of nuclei and mitochondria (Hedgecock and Thomson 1982; Starr and Han 2002). Mutants in the KASH protein UNC-83 (Starr *et al.* 2001) and in the ANC-1- and UNC-83-binding SUN domain protein UNC-84 (Malone *et al.* 1999) also display lumbar maintenance defects, supporting the prediction that factors controlling nuclear position and migration are indeed critical in maintenance of neuron cell body location. These studies begin to elucidate the interplay between pathways mediating cell-matrix adhesion and nuclear position and migration in the maintenance of neural architecture.

Materials and Methods

Strains used

Culture and manipulation of *C. elegans* were performed according to standard methods (Brenner 1974). Strains were maintained at 20° for phenotype analysis. The wild-type strain N2 var. Bristol and the following mutant alleles were used: *anc-1(e1753)* I; *anc-1(e1873)* I; *unc-54(e1092)* I; *dys-1(cx18)* I; *dyb-1(cx36)* I; *sgn-1(ok2432)* II; *T07D3.4(ok664)* II; *dig-1(n1321)* III; *nob-1(ct223)* III; *unc-83(e1408)* V; *unc-84(e1410)* X; *dgn-1(cg121)* X; *stn-2(ok2417)* X; *sgca-1(ok1529)* X; and *lge-1(tm1051)* X. The *dgn-1(cg121)* allele was maintained as a heterozygote balanced by the visible marker *qls54* [*myo-2p::GFP*, *pes-10p::GFP*, *gut promoter::GFP*] X, as previously described (Johnson *et al.* 2006). The following reporter transgenes were used for phenotype analysis of the indicated neuron(s): PHA, PHB, and PHC, *inIs179[ida-1p::GFP]* II; PVC, *rhIs4[glr-1p::GFP, dpy-20(+)]* III; and PVQ, *oyIs14[sra-6p::GFP]* V. The following integrated GFP markers were used for initial mapping in the genetic screen described below: *evIs82[unc-129p::GFP]* I; *julIs76[unc-25p::GFP]* II; *ruIs38[myo-2p::GFP]* III; *mls11[myo-2p::GFP]* IV; and *qls54*, X. All

strains, except for *dgn-1(cg121)*, were obtained from the *Caenorhabditis* Genetics Center (University of Minnesota, Minneapolis) or from the National Bioresource Project for *C. elegans* (Japan).

dgn-1(+) and deletion mutant transgenes

The following heterologous promoter regions were used (basepair numbers are relative to ATG start codon as identified in WormBase): *unc-119p*, −1671 bp to −6 bp (Maduro and Pilgrim 1995; Altun-Gultekin *et al.* 2001; Hardin *et al.* 2008); *unc-33p*, −2746 bp (Altun-Gultekin *et al.* 2001); *unc-14p*, −1398 bp (Ogura *et al.* 1997); *rgef-1p*, −3440 bp (Altun-Gultekin *et al.* 2001); *aex-3p*, −1320 bp (Iwasaki *et al.* 1997); *rab-3p*, −1343 bp (Nonet *et al.* 1997); *sra-6p*, −2987 bp (Troemel *et al.* 1985); *dpy-7p*, −303 bp (Gilleard *et al.* 1997); *lin-26FGHip*, −4000 bp (Landmann *et al.* 2004); *ajm-1p*, −6325 bp to −830 bp of C25A11.4e form ATG (Koppen *et al.* 2001; Hardin *et al.* 2008). Descriptions of sites and timing of expression can be found in the references cited and/or in WormBase (www.wormbase.org). We confirmed the activity of the promoters in transgenic animals generated by germline injection into N2 (Mello *et al.* 1991) using a GFP cassette under their control at 25 µg/ml plus 75 µg/ml pRF4 as coinjection marker. The timing of embryonic expression of the *aex-3* and *rab-3* panneural promoters was not clearly defined in the citations above, but we first detected their activity at the comma stage (*aex-3*) or the 1.5-fold stage (*rab-3*) of embryogenesis, *i.e.*, in postmitotic neurons (not shown).

Transgene constructs of *dgn-1(+)* and *dgn-1* deletion mutants under heterologous promoters were generated in the plasmid pBJ230, which was derived from pPD30.38 (A. Fire lab 1995 vector kit) by replacing the *unc-54* promoter region (nt 25–985 of pPD30.38) with tandem *NotI* and *AscI* sites by mutagenic PCR. Heterologous promoter regions (except for *rgef-1p*) were amplified from N2 genomic DNA using primers that introduced 5' *NotI* and 3' *AscI* sites, A/T cloned into pGEM-T (Promega), and subcloned as *NotI*–*AscI* fragments into *NotI*/*AscI*-digested pBJ230. The *rgef-1* promoter was amplified from the plasmid pCB101.2 (a generous gift of C. Benard, Columbia University, New York) using the same approach. The coding region of the *dgn-1(+)* genomic DNA was amplified from plasmid pJK600 using sense primer *NheIdgn1F* (5'-GCTAGCATGCGTCTCA TTTCTGGT-3'), introducing a 5' *NheI* site immediately before the ATG start codon in *dgn-1* exon 2, and antisense primer *KpnIdgn1R* (5'-GGTACCTTAAGGAGGAATGAATG GAG-3'), introducing a 3' *KpnI* site immediately after the TAA stop codon in exon 6. This PCR product was A/T cloned into pGEM-T and subcloned as a *NheI*–*KpnI* fragment into pBJ230-derived plasmids carrying heterologous promoters.

Deletion mutations of *dgn-1* were generated in pBJ195, a plasmid containing the coding region of *dgn-1(+)*, by mutagenic PCR. For DGN-1pat-3TM, mutagenic primers were used which replaced the coding sequence for the DGN-1 transmembrane domain (amino acid residues 474–496) with

the coding sequence for the *PAT-3* transmembrane domain (amino acid residues 738–760 of *PAT-3*). Deletion mutant coding regions flanked by 5' *NheI* and 3' *KpnI* sites were amplified from pBJ195-derived deletions, A/T cloned into pGEM-T and subcloned as *NheI*–*KpnI* fragments into pBJ236, a pBJ230-derived plasmid carrying *unc-119p*. The sense primer Nhdgn1F was used for all deletions. The following antisense primers were used: for DGN-1ΔCyto and DGN-1ΔCoreΔCyto, *KpnIdgn1delR* (5'-AAGGTACCTTATTCTTGATACAAGCAC-3'); for DGN-1Nterm, *KpnIdgn1delTMcytoR* (5'-AAGGTACCTTAGTCAGCTTCTTGAATTGA-3'); for all other constructs, primer *KpnIdgn1R*. The complete coding regions of wild-type and deletion mutant constructs were verified by DNA sequencing. Further details will be provided upon request.

Transgene plasmids were introduced by germline injection (Mello *et al.* 1991) into *N2* animals and transferred as extrachromosomal arrays by mating into the *dgn-1(cg121)* background. All transgene plasmids were injected at 25 μg/ml with 75 μg/ml pRF4 (Mello *et al.* 1991) and 10 μg/ml pPD122.45 [*myo-3p::GFP-NLS* (nuclear localization signal)] (A. Fire lab 1999 vector kit).

PVQ displacement screen

A partially synchronized population of *oyIs14* animals enriched in early L4-stage worms was mutagenized with 50 mM ethylmethylsulfonate (EMS) in M9 buffer for 4 hr at room temperature. Mutagenized animals were washed extensively with M9 and allowed to recover on an NGM plate for 30 min. Late L4-stage animals were transferred to a new plate and incubated for 24 hr at 20°. The resulting gravid early adults were picked to 20 fresh plates (5 adults/plate). These P0 animals were serially transferred twice to fresh plates after 3 hr of egg laying at 20° and removed from the final plates. F₁ animals resulting from these eggs were grown to gravid adults at 20° and allowed to lay eggs for 2 days before being counted and removed by aspiration. The total number of F₁ animals represented 20,050 mutagenized haploid genomes. When F₂ animals had developed to late L4/young adult stage, plates were screened for individuals with anteriorly displaced PVQs (Pva phenotype), which were transferred singly to new plates. Only a single viable Pva mutant from one original plate was retained. The resulting 10 Pva mutants were mated to *him-5(e1467)* males carrying visible GFP transgenic markers integrated on specific chromosomes (chromosome I, *evIs82*; chromosome II, *juls76*; chromosome III, *ruls38*; chromosome IV, *mls11*; and chromosome X, *qls54*). Segregation of the Pva phenotype against the marked chromosome was assessed in the offspring of cross-progeny to assign linkage. Pva mutants showing linkage to the same chromosome were mated to assign complementation groups. Six mutants were assigned to a single complementation group on chromosome I and three to a complementation group on chromosome III. A single mutant from each of these groups was mapped by bulked segregant snip-SNP analysis using the *C. elegans* Hawaiian isolate CB4856 (Wicks *et al.*

2001) and by three-point recombination analysis with visible markers. Tentative identification of the chromosome I complementation group as *anc-1* and the chromosome III group as *dig-1* was confirmed by complementation tests of the Pva mutants with the strong loss-of-function alleles *anc-1(e1753)* or *dig-1(n1321)*. Two previously characterized *anc-1* alleles, *anc-1(e1753)* and *anc-1(e1873)*, were found to have a PVQ anteriorization phenotype using the *oyIs14* marker. The PVQ displacement phenotype of *dig-1* mutants was reported previously (Benard *et al.* 2006). The 10th Pva mutant, constituting a separate complementation group on chromosome III, was determined to be a mutation in *nob-1* on the basis of its abnormal tail morphology and its failure to complement the standard strong loss-of-function allele *nob-1(ct223)*. The *nob-1* gene encodes a Hox family transcription factor involved in patterning the tail region (Van Auken *et al.* 2000), and we did not characterize whether it has a specific role in lumbar neural maintenance, independent of its patterning role.

Phenotype analysis

Position of lumbar ganglion neuron cell bodies was scored on a Zeiss Axiophot epifluorescence microscope, using animals immobilized by 10 mM sodium azide or 200 μM levamisole mounted on a 2% agarose pad. In wild-type *C. elegans*, positions of neuron cell bodies within the lumbar ganglion can vary slightly from animal to animal, but are all located at or posterior to the line of the rectal canal when viewed laterally. A lumbar neuron was therefore scored as mispositioned if its cell body was completely anterior to the rectal canal visualized by DIC optics. Specific lumbar neurons were visualized using the integrated transgene markers *oyIs14* (PVQ), *rhIs4* (PVC), and *inIs179* (PHA/PHB/PHC). Data for the PHA, PHB, and PHC were pooled since all three neurons are marked by *inIs179* and could not always be unambiguously distinguished when one or more was displaced on the same side of the animal. We note that *inIs179* is also active in the PVP neurons of the preanal ganglion, which are readily distinguished by their lower intensity signal compared to PHA/B/C. We also note that in threefold embryos *rhIs4* is also active in PVQ, but PVC can be readily distinguished by the lower intensity signal at this stage; by the L1 stage, the PVQ signal is much lower than the PVC signal and is undetectable in adults (please also see Supporting Information, File S1, Table S1, Table S2, Table S3, Table S4, Table S5, Table S6, Table S7, Table S8).

Results

Dystroglycan null mutants are defective in maintenance of lumbar neuron cell body position

Null mutants of *dgn-1* display a defect in the positioning of the cell bodies of lumbar ganglion neurons such as PVQ, PVC, and PHA, PHB, and PHC. In *dgn-1(cg121)*, the soma of one or more of these neurons is displaced anterior or

anteroventral to their normal positions (Figure 1, A–E). In the extreme, lumbar ganglion cell bodies can be displaced into or near the preanal ganglion. We refer to this lumbar neural positioning defect as the Pva (PVQ/lumbar neuron anteriorization) phenotype.

The Pva phenotype in dystroglycan mutants represents a failure of maintenance of cell body position. Lumbar ganglion neurons are born close to their final position in the developing embryo and do not undergo significant cell migrations (Sulston *et al.* 1983). In *dgn-1* embryos, the position of the PVQ cell body is largely normal in threefold embryos, the earliest point at which the *oyls14* cell marker is detectable (Figure 1F). The frequency of PVQ anteriorization increases approximately fourfold between threefold embryos and newly hatched (≤ 2 hr) L1 larvae, and increases a further twofold between L1 larvae and young (1 day) adult animals. The cell bodies of PVC and PHA/B/C are likewise positioned normally in three- to fourfold *dgn-1* embryos, when marker signals become detectable, but L1 animals show displacement defects that increase significantly between L1 and adult stages (Figure 1F).

Lumbar cell body maintenance role of DGN-1 requires early neural expression

The *dgn-1* gene is expressed in many neuroblasts and neurons throughout embryogenesis, and in particular is expressed in lumbar ganglion neurons from at least the comma stage until the first larval stage. Neural expression of *dgn-1* subsides after the L1 stage, with the exception of the PVP neurons in the preanal ganglion (Johnson *et al.* 2006; Johnson and Kramer, 2012). We reexpressed *dgn-1(+)* in *dgn-1* null animals from transgenes under the control of various tissue-restricted heterologous gene control regions (“promoters”) to determine the site of DGN-1 function in lumbar neuron maintenance (references in *Materials and Methods*). Expression of *dgn-1(+)* under control of panepithelial (*ajm-1*) or hypodermis-specific (*dpy-7*, *lin-26FGHi*) promoters yields little to no rescue (Figure 2). In contrast, *dgn-1(+)* expression by the early-expressing panneural *unc-119* or *unc-33* promoters shows significant rescue of the PVQ Pva phenotype (Figure 2). Expression by the early-expressing panneural *unc-14* promoter may also yield weak partial rescue in *dgn-1* null animals, but it is obscured by high background displacement caused by this transgene even in wild-type animals. We conclude that *dgn-1* is required cell autonomously in neurons for its maintenance function.

Surprisingly, later expressing (postmitotic) panneural promoters from genes that are required for synaptic function in mature neurons, such as *rgef-1*, *aex-3*, and *rab-3*, provide little to no rescue (Figure 2). The postmitotic *sra-6* promoter, which in the lumbar ganglion is expressed only in PVQ, is detectable after the threefold stage of embryogenesis, but also provides little or no rescue. These nonrescuing panneural or PVQ-specific promoters begin expressing in postmitotic neurons (comma stage or later) but are active throughout the period of late embryogenesis and larval development when the Pva

Figure 1 Neural maintenance defects in *dgn-1* mutants. (A) In wild-type *C. elegans*, neural cell bodies in the bilateral lumbar ganglia are clustered at a lateral subventral position posterior to the line of the rectal canal (left). A subset of neurons in the left lumbar ganglion is shown. In *dgn-1* (*cg121*) null mutants one or more lumbar neuron cell bodies on either side of the animal are displaced anterior to their normal position (right). (B and C) PVQ cell body position (black arrowhead) in wild-type animals carrying the *oyls14* GFP marker, shown in fluorescence image in C and fluorescence/differential interference contrast (DIC) overlay in B. Fluorescence image (E) and fluorescence/DIC (D) showing anteriorized PVQ cell body (black arrowhead) in *dgn-1*(*cg121*). The axon of a displaced PVQ extends back to the lumbar commissure, where it takes a normal ventralward trajectory through the lumbar commissure (white arrows); due to orientation the further extension of the PVQ axons along the ventral midline is not visualized. (F) Progressive increase in level of lumbar neuron cell body displacement in *dgn-1*(*cg121*) between three- to fourfold embryos, newly hatched (≤ 2 hr) L1-stage larvae, and young (≤ 1 day) adult animals. The PVQ marker (*oyls14*) and the PVC (*rhl54*) markers are first detectable in threefold embryos, while the PHA/B/C marker becomes detectable in 3.5- to 4-fold embryos. The percentage of displaced neuron cell bodies and the standard error of the proportion (error bars) are shown for each stage ($N \geq 100$ neurons). $**P < 0.005$ (Z test).

phenotype in *dgn-1* animals first becomes manifest and subsequently increases in penetrance. In contrast, the rescuing *unc-119* and *unc-33* genes are expressed early, in neuroblasts as well as postmitotic neurons. This difference suggests that the late embryonic/larval maintenance role of *dgn-1* may require its expression in neuroblasts or young postmitotic neurons to establish a cell body positional maintenance mechanism that continues to function throughout later development.

Figure 2 Transgenic rescue of PVQ displacement in *dgn-1(cg121)* by early neural expression of *dgn-1(+)*. PVQ displacement defects were scored in young adult *dgn-1(cg121)* or wild-type animals carrying transgenic extrachromosomal arrays expressing the wild-type *dgn-1* coding region under the control of heterologous tissue-restricted promoters from the indicated genes. Average percentage of displaced neuron cell bodies and range (error bars) of two independent transgene arrays is shown ($N \geq 100$ PVQs scored for each array). The early panneural promoters from *unc-119* and *unc-33* afford almost complete rescue of PVQ displacement in *dgn-1(cg121)*. Hatched area indicates region of no statistically significant difference ($P > 0.05$, Z test) from *dgn-1* (no transgene) control. *** $p < 0.005$ (Z test).

The extracellular N-terminal domain of DGN-1 mediates lumbar neuron cell body maintenance

The family of dystroglycan-like proteins is defined by the presence of one or more DG core motifs (Johnson *et al.* 2006) composed of an amino-terminal cadherin-repeat like CADG domain (Dickens *et al.* 2002) and a carboxy-terminal SEA domain (Akhavan *et al.* 2008). The central DG core motif is flanked by variable N-terminal and cytoplasmic domains (Johnson *et al.* 2006). In *DGN-1* the single DG core motif is accompanied by an N-terminal region composed of immunoglobulin-like (Ig-like) and ribosomal RNA binding protein-like (rRBP-like) domains (Bozic *et al.* 2004), and C-terminal transmembrane and cytoplasmic domains, all of which are homologous to the corresponding portions of vertebrate dystroglycan (Johnson *et al.* 2006).

We expressed a series of domain deletion mutants of *DGN-1* under control of the *unc-119* promoter in *dgn-1(cg121)* animals to determine the structural requirements of dystroglycan function in lumbar soma positional maintenance (Figure 3). Robust rescue of the Pva phenotype requires the N-terminal domain tethered to a transmembrane anchor. The specific sequence of the transmembrane anchor is not critical, since the transmembrane domain of *PAT-3/β-integrin* (Gettner *et al.* 1995) can substitute for the native *DGN-1* transmembrane region. The Ig-like and rRBP-like subdomains of the N terminus are both required for rescue, but the small threonine-rich region at the end of the N-terminal domain (Johnson *et al.* 2006) is dispensable.

Expression of a secreted N-terminal domain did not rescue displacement and indeed caused high levels of PVQ and PVC displacement in a wild-type background, suggesting the free N terminus competes with endogenous *DGN-1*. The requirement of the N-terminal domain implies that *DGN-1*/dystroglycan mediates critical interactions with one or more extracellular partners important to maintenance of lumbar neuron position.

Mutants of potential cytoplasmic and transmembrane partners of DGN-1/dystroglycan show no lumbar soma maintenance phenotype

We examined mutants of the *C. elegans* genes for dystrophin (*dys-1*), the dystrophin-associated protein complex components dystrobrevin (*dyb-1*) and syntrophin (*stn-2*), and the components of the transmembrane sarcoglycan complex α/ϵ -sarcoglycan (*sgca-1*) and γ/δ -sarcoglycan (*sgn-1*) for evidence of a Pva phenotype like that found in *dgn-1* mutants. Homologs of these proteins are important in dystroglycan function in vertebrate muscle (Durbeej and Campbell 2002; Lapidus *et al.* 2004). No significant defects were observed in PVQ, PVC, or PHA/B/C position in any of these mutants (Table S4, Table S5, Table S6). This finding is consistent with the apparent lack of involvement of the transmembrane and cytoplasmic domains of *DGN-1* in mediating maintenance of lumbar neuron cell body position. We also examined mutants in the *C. elegans* homologs of LARGE (*lge-1*) and fukutin-related protein (T07D3.4), factors which in vertebrates are involved in the glycosylation of α -dystroglycan to generate a high-affinity binding site for laminin and other matrix proteins (Michele and Campbell 2003), but found no significant defects in lumbar neuron soma positioning in these mutants (Table S4, Table S5, and Table S6).

A forward genetic screen for additional Pva mutants reveals a role for the extracellular matrix and for nuclear anchoring in neuronal cell body position maintenance

To identify other genes potentially involved with *DGN-1* in neural maintenance, we screened >20,000 EMS-mutagenized haploid genomes for viable mutants producing a Pva phenotype in the PVQ neurons (see Materials and Methods). Three of 10 new Pva mutations proved to be alleles of *dig-1*, which encodes a putative extracellular matrix component previously reported to be involved in position maintenance of axons and neuronal cell bodies (Benard *et al.* 2006; Burket *et al.* 2006). Six other new Pva mutations were identified as alleles of *anc-1*, which encodes a member of the nesprin/SYNE family of outer nuclear membrane proteins that link the cytoskeleton to the inner nuclear membrane (Starr and Fridolfsson 2010). The *anc-1* gene has been characterized previously for its role in anchoring hypodermal and pharyngeal nuclei and controlling mitochondrial morphology (Hedgecock and Thomson 1982; Starr and Han 2002), but its neural functions have not been reported.

We examined *dig-1(n1321)* and *anc-1(e1753)*, well-characterized strong loss-of-function alleles (Hedgecock and

Figure 3 The level of PVQ or PVC cell body displacement in young adult *dgn-1(cg121)* or wild-type animals carrying transgenic extrachromosomal arrays expressing a series of deletion (Δ) mutants or a transmembrane (tm) domain-swap (with the PAT-3 tm domain) mutant of DGN-1 under the control of the *unc-119* promoter. DGN-1 function in neural maintenance requires a membrane-anchored N-terminal region (Nterm) of the extracellular (EC) portion of DGN-1, including the Ig-like (IgL) and rRBP-like (rRBP) domains, but not the threonine-rich region (Trich). Neither the cadherin-like (CADG) or SEA domains of the dystroglycan core motif nor the cytoplasmic domain (Cyto) are required. The free, secreted N-terminal region induces PVQ and PVC displacement defects in the wild-type background. Average percentage of displaced neuron cell bodies and range (error bars) of two independent transgene arrays are shown ($N \geq 100$ PVQs or PVCs scored for each array). Hatched areas indicate regions of no statistically significant difference ($P > 0.05$, Z test) from *dgn-1* (no transgene) control.

Thomson 1982; Starr and Han 2002; Benard *et al.* 2006; Burket *et al.* 2006), and compared their effect on lumbar neuron positioning to that of *dgn-1*. Both single *dig-1* and *anc-1* mutations show an increase in PVQ cell bodies anteriorized between threefold embryos and L1 larvae (Figure 4). Like *dgn-1(cg121)*, *anc-1(e1753)* shows a further increase in the penetrance of PVQ soma displacement between L1 and adult stages, indicating a continued requirement for both *anc-1* and *dgn-1* over the course of larval development. In contrast, the level of PVQ soma anteriorization in *dig-1(n1321)* plateaus at L1, suggesting that the temporal requirement for *dig-1* function in PVQ maintenance is limited to late embryogenesis. The displacement phenotypes of *anc-1* and *dig-1* are qualitatively distinct from that of *dgn-1*. In *anc-1* and *dig-1* PVQ cell bodies are exclusively displaced directly anterior along the lateral body wall, while in *dgn-1* PVQ cell bodies are displaced anteriorly or anteroventrally toward the preanal ganglion (not shown). In *dgn-1* adults, only 0.6% ($N = 176$) of displaced PVQ cell bodies are located anterior of the most posterior intestinal cell pair *int9L/R*, whereas in *dig-1* adults 2.6% ($N = 78$) and in *anc-1* adults 24.3% ($N = 185$) of displaced PVQ soma are located anterior of *int9L/R*. Although noteworthy, these differences may reflect idiosyncrasies of the interaction of an unanchored PVQ soma with its microenvironment in each mutant background, rather than specific aspects of the loss of maintenance gene function beyond the failure of PVQ anchorage.

Analyses using markers for the lumbar ganglion neurons PVC (*rhIs4*) and PHA/PHB/PHC (*inIs179*) indicate that *anc-1* function is required moderately for PVC but only weakly for PHA/B/C soma positional maintenance. In contrast, positional maintenance of these other lumbar ganglion neurons is only weakly dependent on *dig-1* (Figure 4). Thus, DGN-1/dystroglycan appears to be broadly required for lumbar neuron soma maintenance, whereas ANC-1 and DIG-1 may be more cell specific. Maintenance of the PVQ soma, which lies anteriormost in the lumbar ganglion, is dependent on all three proteins and provides a locus to assess genetic interactions between these maintenance factors.

Analysis of *dgn-1*, *dig-1*, and *anc-1* double and triple mutants suggests the existence of distinct neuronal soma maintenance mechanisms throughout development

The positions of the PVQ neurons in double and triple mutants of *dgn-1*, *dig-1*, and *anc-1* were assessed in threefold embryos, newly hatched L1 larvae, and young adults (Figure 5A), and changes in penetrance of the PVQ soma displacement defect between stages were calculated (Figure 5B). The *dig-1*; *dgn-1* double mutant shows the same level of defects in threefold embryos and L1 larvae as both single mutants, indicating that *dig-1* and *dgn-1* likely act in the same PVQ maintenance pathway during late embryogenesis (Figure 5). In contrast, *anc-1*; *dig-1* and *anc-1*; *dgn-1* double

Figure 4 Lumbar neuron displacement defects in *dig-1* and *anc-1* mutants. Progressive defects in displacement of PVQ, PVC, and PHA/B/C neuron cell bodies between embryonic and adult stages in *dig-1* (*n1321*) and *anc-1* (*e1753*) mutants. Data for *dgn-1* (*cg121*) from Figure 1F is included for comparison. Percentage of displaced neuron cell bodies and standard error of the proportion (error bars) are shown for each mutant at each stage ($N \geq 100$ neurons). $**P < 0.005$; NS, not significant: $P > 0.2$ (Z test).

mutants and *anc-1; dig-1; dgn-1* triple mutants show a greater increase in PVQ mispositioning between embryos and L1 larvae than the three single mutants and the *dig-1; dgn-1* double mutant (Figure 5). This result suggests that during late embryogenesis *anc-1* acts in a parallel maintenance pathway to that involving *dgn-1* and *dig-1*.

In adult-stage animals, *anc-1; dgn-1* double mutants show the same level of defects as *anc-1* mutants alone. Moreover, the level of defects in *dgn-1* adults is comparable to that in *anc-1* and *anc-1; dgn-1* double mutant adults (Figure 5A). These facts may indicate that *anc-1* and *dgn-1* work in the same maintenance pathway during larval development. However, the level of defects in *anc-1; dgn-1* L1-stage animals is already elevated above that of *anc-1* or *dgn-1* L1s due to increased mispositioning during late embryogenesis as described above. Thus, loss of both *anc-1*-dependent and *dgn-1*-dependent embryonic maintenance pathways may simply increase the rate of PVQ cell body displacement such that many of the additional defects that would occur during larval development in the single mutants occur instead during late embryogenesis.

Epistasis between *dig-1* and *anc-1* suggests a role for *anc-1* in cell-matrix interactions

Intriguingly, although *dig-1* function is not required for maintenance during larval development, loss of *dig-1* function appears to abrogate the requirement for *anc-1* function during this period, on the basis of two observations. First, *anc-1* single mutants show an increase in PVQ soma anteriorization between L1- and adult-stage animals, whereas *anc-1; dig-1* double mutants show significantly less increase between these stages (Figure 5B). Similarly, *anc-1; dgn-1* double mutants show a substantial L1-to-adult increase in PVQ soma positioning defects, whereas *anc-1; dig-1; dgn-1* triple mutants do not. In contrast, the L1-to-adult increase in PVQ defects is not significantly different between *dgn-1* sin-

Figure 5 Multiple genetic pathways mediate lumbar neuron maintenance. (A) Progressive PVQ displacement in double and triple mutants of *dgn-1*, *dig-1*, and *anc-1*. Percentage of PVQ cell bodies displaced in the threefold embryo, newly hatched L1 larva, and young adult animals for each mutant is shown ($N \geq 200$ neurons for each stage). The standard error of the proportion for each column is no more than $\pm 3.5\%$. (B) Increases in PVQ displacement between threefold embryos and L1 larvae (E-to-L1 increase) and between L1 larvae and adults (L1-to-Ad increase) are graphed on the basis of the values in A. *dig-1* (*n1321*) mutants show a late embryonic (E-to-L1) increase but not a larval-stage (L1-to-Ad) increase. In contrast, *dgn-1* (*cg121*) and *dig-1* (*n1321*); *dgn-1* (*cg121*) double mutants show both late embryonic- and larval-stage increases. *anc-1* (*e1753*) also shows both late embryonic- and larval-stage increases in PVQ displacement. Both *anc-1* (*e1753*); *dig-1* (*n1321*) and *anc-1* (*e1753*); *dgn-1* (*cg121*) double mutants show enhanced late embryonic defects above the single mutants, but the presence of *dig-1* (*n1321*) largely eliminates the additional larval-stage defects due to *anc-1* in the *anc-1* (*e1753*); *dig-1* (*n1321*) double mutants and in the triple mutant. $**P < 0.005$; NS, not significant: $P > 0.2$ (Z test).

gle mutants and *dig-1; dgn-1* double mutants, indicating that the loss of *dig-1* function does not abrogate the requirement for *dgn-1* for larval-stage PVQ soma positional maintenance (Figure 5B). Second, the level of PVQ mispositioning in *anc-1; dig-1* adults is significantly lower than the level in *anc-1* adults (Figure 5A). Similarly, the level of PVQ defects in *anc-1; dig-1; dgn-1* adults is significantly lower than the level

of defects in *anc-1*; *dgn-1* adults, but is not significantly different from the level in *dig-1*; *dgn-1* adults (Figure 5A). Thus, the absence of *anc-1* function during larval development appears to result in additional PVQ cell body mispositioning, above the level accrued during late embryogenesis, only if wild-type *dig-1* function is present. This epistatic relationship between *dig-1* and *anc-1* during larval development implies that ANC-1 acts to modulate some aspect of cell–ECM interaction in neurons to maintain lumbar cell body position.

The nuclear envelope proteins UNC-83 and UNC-84 function in larval maintenance of PVQ soma position

The outer nuclear envelope protein ANC-1 acts in the syncytial hypodermis to maintain nuclear position through the interaction of its C-terminal KASH domain with the SUN domain of UNC-84, an inner nuclear envelope protein (Starr and Han 2002). UNC-84 also interacts with another outer nuclear envelope KASH domain protein UNC-83 during nuclear migration in the developing hypodermis (McGee *et al.* 2006). We examined *unc-84(e1410)* and *unc-83(e1408)* mutants for defects in PVQ soma position. Both mutants have a low level of PVQ soma mispositioning in newly hatched L1s, but a high level of defects in young adult animals (Figure 6). These observations indicate that other nuclear envelope proteins involved in nuclear anchorage and/or nuclear migration participate in neural positional maintenance.

Sensitivity of lumbar neuron position to muscle contraction-induced mechanical stress depends on developmental stage and genetic lesion

We examined the effect of paralysis on the phenotypes of *dgn-1*, *dig-1*, and *anc-1* mutants, since muscle contraction has been previously implicated in the etiology of neural maintenance defects (Benard and Hobert 2009). Loss of *unc-54* myosin function disrupts muscle contractions involved in locomotion and in the defecation motor program (Thomas 1990), the two most obvious types of repetitive mechanical stress to which lumbar neurons are likely subjected. In *dgn-1* mutants neither late embryonic-stage nor larval-stage PVQ soma displacement defects are sensitive to paralysis due to the *unc-54(e1092)* genetic lesion, since the final level of defects is unchanged in *unc-54*; *dgn-1* double mutants (Figure 7A). The *unc-54(e1092)* mutation alone has a negligible level of PVQ displacement (adult level: 1.5%, $N = 200$) comparable to wild-type animals (adult level: 0.9%, $N = 214$). Similarly, no effect on PVC displacement was observed in *unc-54*; *dgn-1* double mutants (not shown). A significant decline in penetrance of PVQ cell body displacement defects in newly hatched L1 larvae and adults is seen in *unc-54*; *dig-1*, indicating partial sensitivity of the PVQ cell body in *dig-1* embryos to muscle contraction-induced displacement (Figure 7B). A significant decline in penetrance of PVQ cell body displacement defects is also seen in *anc-1 unc-54* adults but not in L1 larvae (Figure 7B). However, both *unc-54*; *dig-1* and *anc-1 unc-54* double

Figure 6 Lumbar neuron displacement defects in *unc-83* and *unc-84* mutants. Progressive defects in displacement of PVQ cell bodies between L1 and adult stages in *unc-83(e1408)* and *unc-84(e1410)* mutants. Data for *anc-1(e1753)* shown in Figure 4 is included for comparison. Percentage of displaced neuron cell bodies and standard error of the proportion (error bars) are shown for each mutant at each stage ($N \geq 100$ neurons). ** $P < 0.005$ (Z test).

mutants still show ~25% PVQ soma displacement in newly hatched L1 larvae (i.e., 60–80% of control levels), indicating that accumulation of late embryonic-stage displacement defects is largely unimpaired by muscle paralysis. The etiology of most late embryonic PVQ cell body displacement in *dig-1* and *anc-1* mutants may thus be the same as that in *dgn-1* mutants and reflects a stress not caused by muscle contraction to which PVQ is subjected during late embryogenesis.

It is possible that the noncontractile stress driving larval-stage displacements in *dgn-1* is simply a continuation of the stress driving late embryonic displacements, and that the function of DGN-1 is to render PVQ unresponsive to this stress throughout development. However, the etiology of larval-stage displacements in *dgn-1* must be distinct from that in *anc-1*, since accumulation of additional larval-stage defects in *anc-1* is largely (~80%) blocked by paralysis (Figure 7B). These conclusions are supported by quantification of PVQ soma displacement in adult mutants grown (from embryonic stages) on plates containing 50 μ M levamisole, an agonist of cholinergic neurotransmission that produces paralysis through hypercontraction of muscle (Figure 7A). Since levamisole does not penetrate the egg shell, only larval-stage defects would be sensitive. These results suggest the existence of a set of parallel soma maintenance pathways, dependent on different maintenance factors, which allow the cell bodies of individual neurons such as PVQ to cope with different types of displacement stresses. Both the differential requirement on maintenance gene function and the distinct etiologies of larval and adult-stage neuron displacements thus support the idea that late embryonic-stage maintenance of PVQ cell body position is mechanistically distinct from larval-stage maintenance.

Figure 7 Effect of muscle paralysis on lumbar neuron maintenance. (A) PVQ displacement defects in adult *dgn-1(cg121)*, *dig-1(n1321)*, or *anc-1(e1753)* mutants grown on standard plates (control) or on plates containing the paralytic cholinergic agonist 50 μ M levamisole (50 uM Lev), and defects in adult *unc-54(e1092)*; *dgn-1*, *unc-54(e1092)*; *dig-1*, or *anc-1 unc-54(e1092)* double mutants grown on standard plates (*unc-54*). (B) PVQ displacement defects in newly hatched (<2 hr) L1 larvae and in adult *dig-1(n1321)* or *anc-1(e1753)* animals or L1 and adult *unc-54(e1092)*; *dig-1* or *anc-1 unc-54(e1092)* double mutants. Percentage of displaced PVQ cell bodies and standard error of the proportion (error bars) are shown for each sample ($N \geq 100$ neurons). ** $P < 0.005$; * $P < 0.05$; NS, not significant: $P > 0.14$ (Z test).

Discussion

Our studies suggest the existence of at least two temporally and mechanistically distinct regimes for maintenance of PVQ cell body position (Figure 8). During late embryonic development, a DIG-1/DGN-1-dependent pathway and a separate ANC-1-dependent pathway act to maintain PVQ soma position in the face of an unidentified stress that is largely independent of muscle contraction. During this late embryonic maintenance regime, the effect of loss of either (or both) *dig-1* or *dgn-1* function appears to be simply additive with the effect of *anc-1* loss of function, indicating parallel independent pathways. The importance of a DIG-1/DGN-1-dependent pathway suggests that cell-matrix interactions are important in embryonic positional maintenance. DGN-1 could interact directly with DIG-1 or indirectly through another matrix component

such as laminin. During larval development, a second maintenance regime is operative, which does not require DIG-1 function but requires both DGN-1 and ANC-1. The dependence of larval-stage maintenance on DGN-1 function is not necessitated solely by passive displacement due to muscle contraction. In contrast, the larval ANC-1 pathway appears specifically to protect against stress caused by muscle contraction. Although DIG-1 is not itself required during larval stages, it appears to impose a dependence on ANC-1 for ongoing PVQ cell body positional maintenance during larval development. These genetic studies suggest that a complex interplay of cell adhesion and nucleus-cytoskeleton interactions governs the cell body positional maintenance of neurons in response to a range of displacement stresses.

Further studies will be required to determine how general a role DGN-1 and ANC-1 play in neuroarchitectural maintenance. DGN-1 is expressed transiently during embryonic and early larval stages in many neuroblasts and neurons (Johnson *et al.* 2006). ANC-1 expression is detectable by the L1 stage in all postembryonic somatic cells (Starr and Han 2002), although presumably it is also expressed in late embryos. These factors may thus play roles in neural maintenance outside of the lumbar ganglion. The secreted factor DIG-1, which is expressed from embryonic comma stage through the adult stage in either intestine or mesodermal tissue, has been previously reported to function in positional maintenance of a number of neuronal cell bodies and axons, possibly through a role in stabilizing the basement membrane (Benard *et al.* 2006).

The cell specificity within the lumbar ganglion for dependence of soma positional maintenance on the factors we have characterized is intriguing and may reflect unique aspects of the immediate environment of each neuron and the repertoire of stresses to which it is subjected. PVQ may be particularly sensitive in this regard, because it is characteristically the most anterior cell body in the ganglion and thus more easily displaced forward away from the more sterically constrained neurons located posterior of it in the ganglion. Previous studies of neural maintenance of cell bodies and axons have similarly revealed a high level of cell specificity of sensitivity to loss of individual maintenance factors (Benard and Hobert 2009), suggesting that the battery of maintenance mechanisms each neuron deploys to protect against the specific “threat matrix” to which it is subjected may constitute a critical aspect of its differentiation.

Complex etiology of neural maintenance defects

Previous characterization has demonstrated the importance of contraction-induced passive displacement of neural cell bodies and axons in the etiology of mispositioning defects in maintenance mutants (Benard and Hobert 2009). The nature of the muscle contraction-independent displacement stress experienced by PVQ soma is thus intriguing. One possibility is that the contraction-independent stress on PVQ cell body position represents aberrant activation of active PVQ motility in late-stage embryos in *dgn-1*, *dig-1*, and *anc-1* mutants,

Figure 8 Model of lumbar maintenance pathways. In late embryos (left), a DIG-1/DGN-1-dependent pathway and a parallel ANC-1-dependent pathway act to block (gray lines) displacement of the PVQ cell body due to an unidentified stress independent of muscle contraction. These factors may also act to maintain PVQ position against passive displacement due to muscle contraction (dashed gray lines). During larval stages (right), ANC-1 protects against passive displacement caused by muscle contraction, whereas DGN-1 function in positional maintenance is not necessitated solely by muscle contraction stress. DIG-1 is not required for maintenance during larval stages but imposes a requirement for ANC-1 function, perhaps by enhancing passive displacement due to muscle contraction (gray arrow).

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perhaps caused by release from a brake on inappropriate PVQ migration. Future studies exploring this hypothesis should help to elucidate the range of displacement stresses against which neurons must be buffered throughout development to ensure neuroarchitectural integrity.

We believe that the finding that some displacement stresses to which neurons in *C. elegans* are subjected cannot be ascribed to muscle contraction is key to establishing the relevance of the *C. elegans* model to neuroarchitectural maintenance in humans and other vertebrates. Although muscle contraction may generate displacement stress in the human spinal column and peripheral nervous system, the brain is not significantly subject to such stresses directly, although accelerations of the head arising from either muscle contraction (e.g., running) or from contraction-independent mechanisms (e.g., falling, being struck, or shaken) may result in displacement stress on the brain due to inertial effects. Cumulative disorganization of brain neuroarchitecture by mechanisms independent of muscle contraction may constitute a possible etiology for slow-onset diseases of brain function. Since such diseases often result in human behavioral and cognitive changes, it will be interesting to explore the behavioral consequences of disruption of soma positional maintenance in the *C. elegans* nervous system.

Cell-matrix interactions are a key component of maintenance pathways

DGN-1/dystroglycan joins a growing list of factors required for the ability of neuronal soma and/or processes to maintain position over time in the face of growth, body movements, and neural remodeling (Benard and Hobert 2009). A common feature of these previously identified maintenance factors is that they potentially mediate cell-cell or cell-matrix interactions. Current models of the role of these maintenance factors in stabilizing neuronal soma and neurite position emphasize their possible function in contributing to cell-matrix attachment (Benard and Hobert 2009).

DGN-1 function may be required only for a critical period during embryogenesis to establish a maintenance pathway that continues to protect PVQ and other lumbar neurons from displacement throughout development, on the basis of the findings that early embryonic but not later embryonic

neural expression of DGN-1 rescues PVQ mispositioning in *dgn-1* null mutants (this report) and that *dgn-1* is expressed in neurons (including the lumbar ganglion neurons) only transiently during embryonic development, with the exception of the PVP neurons in the preanal ganglion (Johnson *et al.* 2006; Johnson and Kramer 2012). An intriguing possibility is that DGN-1 interaction with the matrix modulates some aspect of neural differentiation that establishes a critical maintenance program early in postmitotic neurons. A role for dystroglycan-matrix interactions in aspects of cell differentiation is not unprecedented. Dystroglycan is required for robust response of mammary epithelial cells to prolactin or growth hormone (Leonoudakis *et al.* 2010) and modulates insulin-like growth factor 1 signaling during oligodendrocyte differentiation (Galvin *et al.* 2010).

We recently reported that *dgn-1(cg121)* animals display an axon guidance defect of follower lumbar commissure axons descending from neurons of the lumbar ganglion, in which follower axons frequently fail to extend into the preanal ganglion via the lumbar commissure and instead extend along an oblique trajectory directly toward the preanal ganglion (Johnson and Kramer 2012). This lumbar axon guidance defect appears to be an independent phenotype of *dgn-1(cg121)* for several reasons. First, lumbar ganglion cell body position is largely normal by the threefold stage of embryogenesis, a point at which extension of lumbar ganglion axons through the lumbar commissure is complete (Durbin 1987). Second, PVQ shows the highest penetrance of Pva phenotype among the lumbar neurons we have examined but shows no defects in lumbar commissure guidance of its axon, which pioneers the lumbar commissure. Third, follower lumbar ganglion neurons like PVC can show axon misguidance without cell body displacement and vice versa. Finally, *anc-1* and *dig-1* mutants do not show defects in lumbar commissure guidance of either the PVQ pioneer or of follower PVC axons. We conclude that lumbar neuron cell body displacement and lumbar axon misguidance reflect the loss of distinct functions of dystroglycan in the same DGN-1-dependent neuron population. We note, however, that like cell body positional maintenance, proper follower lumbar axon guidance requires only a membrane-tethered N-terminal domain of DGN-1 (Johnson and Kramer 2012), suggesting

that a specific extracellular binding partner is important in both functions.

Involvement of nuclear anchoring in positional maintenance in the nervous system

We identified two KASH domain proteins, the nesprin/Syne homolog *ANC-1* and *UNC-83*, as well as their binding partner, the SUN domain protein *UNC-84*, as involved in neural maintenance. The binding of the C-terminal KASH domains of *ANC-1* or *UNC-83* to the SUN domain of *UNC-84* allows these factors to form LINC (linkers of nucleus and cytoskeleton) complexes that span the nuclear envelope and thus potentially transmit mechanical forces from the plasma membrane or cytoplasm into the nucleus (Crisp *et al.* 2006; Starr and Fridolfsson 2010). LINC complexes mediate nucleus-centrosome coupling important in nucleokinesis during neuron and photoreceptor cell migration in the developing mammalian central nervous system (Zhang *et al.* 2009; Yu *et al.* 2011). Our identification of a role for KASH and SUN proteins in neuronal maintenance in *C. elegans* suggests that LINC complexes may control neuronal migration/displacement throughout the metazoa.

How does *ANC-1* function to maintain neuron cell body position in *C. elegans*? A simple model invokes a role for *ANC-1* in anchoring the nucleus of the neuron, probably through direct interaction with the actin cytoskeleton. Disruption of this connection in *anc-1* mutants would allow passive nuclear repositioning, rendering the neuron more susceptible to displacement by external mechanical forces. The epistatic effect of *dig-1* on *anc-1* implies that the cellular function of *ANC-1* also impinges on cell-matrix interactions. Intriguingly, recent studies have indicated a possible role for vertebrate nesprin/Syne proteins in cell-matrix adhesion. Nesprin-2 can localize to filopodia (Dawe *et al.* 2009), and tension exerted on the nucleus via nesprin-1 connections can influence the number of focal adhesions and the level of substrate traction (Chancellor *et al.* 2010). Nesprin-3 may mediate connection of the nucleus with hemidesmosomes through its association with the $\alpha 6 \beta 4$ integrin-binding protein plectin (Wilhelmsen *et al.* 2005). Future studies of neural soma maintenance pathways in *C. elegans* should help to elucidate the interplay between cell-matrix adhesion and nuclear anchorage in controlling nervous system architecture.

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Supporting Information

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Neural Maintenance Roles for the Matrix Receptor Dystroglycan and the Nuclear Anchorage Complex in *Caenorhabditis elegans*

Robert P. Johnson and James M. Kramer

File S1

Supporting Methods

Strain Construction

Strains were constructed using standard genetic manipulations. During construction of double and triple mutant strains, the *dgn-1(cg121)* allele was tracked by its gonad morphogenesis defect (Johnson et al., 2006), the *dig-1(n1321)* allele was tracked by its twisted pharynx phenotype (Axang et al., 2007) or displaced gonad phenotype (Thomas et al., 1990), and the *anc-1(e1753)* allele was tracked by its defect in hypodermal nuclear anchorage (Hedgecock and Thomson, 1982). The *unc-54(e1092)*, *unc-84(e1410)* and *unc-83(e1408)* alleles were tracked by their Unc phenotype. The *dys-1(cx18)* and *dyb-1(cx36)* alleles were tracked by their head bending phenotype (Kim et al., 2004), which we observe is also present in *stn-2(ok2417)*. The *sgca-1(ok1529)*, *sgn-1(ok2432)*, *lge-1(tm1051)* and *T07D3.4(ok664)* alleles lack visible phenotypes and were tracked by PCR using primers that distinguish wild-type and mutant alleles (Table S1).

Plasmid Construction

Heterologous expression plasmid pBJ230

The *unc-54* promoter region (nt 25-985) of plasmid pPD30.38 (Andrew Fire lab 1995 vector kit) was deleted by mutagenic PCR using *Phu* polymerase (NEB) and the following primer pair introducing *NotI* (single underline) and *Ascl* (double underline) sites:

5'-CCTGCGGCCGCAAGCTTGGCGTAATCATGG-3'
5'-CCTGGCGCGCCGGATTGGCCAAAGGACCCAA-3'

The amplified product was 5' phosphorylated using T4 polynucleotide kinase (NEB) and recircularized by ligation with T4 DNA ligase (NEB). The resulting plasmid pBJ230 contains sequential *NotI* and *Ascl* sites replacing the original *unc-54* control region of pPD30.38, allowing insertion of *NotI* and *Ascl* flanked heterologous promoter regions, but retains the downstream multiple cloning site of pPD30.38 which contains *NheI* and *KpnI* sites into which protein coding cassettes may be inserted. pBJ230 also retains the *unc-54* 3' untranslated region downstream of the multiple cloning site from pPD30.38.

Promotorless *dgn-1(+)* expression plasmid pBJ236

The *dgn-1(+)* coding region was amplified from the *dgn-1(+)* genomic clone pJK600 (Johnson et al., 2006) using *Phu* polymerase and the following primer pair:

NheIdgn1F: 5'-GCTAGCATGCGTCTCATTTTCTGGT-3'
KpnIdgn1R: 5'-GGTACCTTAAGGAGGAATGAATGGAG-3'

Primer NheIdgn1F introduce a 5' *NheI* site (single underline) immediately before the ATG start codon in *dgn-1* exon 2. Primer KpnIdgn1R introduces a 3' *KpnI* site (double underline) immediately following the TAA stop codon in *dgn-1* exon 6. The PCR product was A-tailed using Taq DNA polymerase (NEB) and ligated into pGEM-T (Promega) to make pBJ231. A *NheI/KpnI* fragment of pBJ231 containing the *dgn-1(+)* coding region was inserted into *NheI/KpnI* digested pBJ230 to make the promotorless *dgn-1(+)* expression construct pBJ236.

Promoterless Venus expression plasmid pBJ237

The GFP variant Venus coding region was amplified from plasmid pJK776 using *Phu* polymerase and the following primer pair:

5'-GCTAGCATGAGTAAAGGAGAAGAACT-3'
5'-GGTACCTATTGTATAGTTCATCCA-3'

to introduce a 5' *NheI* site (single underline) immediately before the ATG start and a 3' *KpnI* site (double underline) immediately following the TAG stop codon. The PCR product was A-tailed using Taq polymerase and ligated into pGEM-T to make pBJ232. A *NheI/KpnI* fragment of pBJ232 containing the Venus coding region was inserted into *NheI/KpnI* digested pBJ230 to make the promotorless Venus expression construct pBJ237.

Heterologous promoter-driven *dgn-1(+)* and Venus expression constructs

Promoter regions were amplified from N2 genomic DNA, from plasmid pCB101 in the case of *rgef-1p*, or from plasmid pBJ324 in the case of *ajm-1p*, using the primers indicated in Table S2. Amplification primers introduced 5' *NotI* and 3' *Ascl* sites (single and double underline, respectively). PCR products were A-tailed using Taq DNA polymerase, and ligated into pGEM-T. *NotI/Ascl* fragments representing the promoter regions from the pGEM-T subclones were inserted by ligation into *NotI/Ascl*-cut pBJ236 or *NotI/Ascl*-cut pBJ237 to generate the heterologous expression constructs summarized in Table S2.

Mutagenesis of *dgn-1* coding region

A segment of *dgn-1(+)* representing the coding region (exons 2-6, with intervening introns) and limited flanking non-coding portions of intron 1 and exon 6 was amplified from pJK600 using *Phu* polymerase and the following primer pair:

5'-ACGGTTAATAAGGGTCATCC-3'
5'-CAAAAATGACAGAGAACAACTAAC-3'

The PCR product was A-tailed using Taq DNA polymerase and ligated into pGEM-T to make pBJ195.. Deletion and transmembrane domain substitution mutants of *dgn-1* were generated in pBJ195 by mutagenic PCR using Phu polymerase and the primer pairs indicated in Table S3. Amplified products were 5' phosphorylated using T4 polynucleotide kinase and recircularized by ligation with T4 DNA ligase. The 5' ends of mutagenic primers were designed to anneal at specific codon boundaries so that amplified products would lack the intervening region (including introns) of the *dgn-1* sequence. The codons for the first 51 amino acid (aa) residues of DGN-1, representing the signal sequence and the beginning of the Ig-like subdomain, are retained in all mutants to ensure co-translational translocation of the expressed proteins. The *dgn-1(pat-3TM)* transmembrane substitution mutant was generated using the following primer pair:

5'-TACCCGCAATGACTCCGAGCACAATTGCGAGCACCAACAGCTGCGAGTCAGCTTCTTGAA-3'
5'-TCGTAATCCTCGGAATCTCTCTGTTGCTCTGGAAGAAAGTGGAAAGAAGAAGTCAA-3'

The underlined regions correspond to codons of *pat-3* (Gettner et al., 1995) comprising the N-terminal or C-terminal halves of the PAT-3 transmembrane domain (PAT-3 aa 738-760); the remaining primer sequences correspond to *dgn-1(+)* sequences flanking the native transmembrane domain codons (DGN-1 aa 474-496). Following mutagenic PCR and recircularization, the resulting sequence substitutes the *pat-3* transmembrane domain codons for the native *dgn-1* transmembrane domain codons.

***unc-119p* expression constructs for *dgn-1* mutants**

Construction of *unc-119p::dgn-1* mutant expression plasmids is summarized in Table S3. The *unc-119p* expression constructs for *dgn-1* mutants were generated from the pBJ195-derived mutants by one of two methods:

Method 1. The *dgn-1* mutant coding region was amplified using flanking primers introducing 5' *NheI* (single underline) and 3' *KpnI* sites (double underline) sites:

NheI*dgn1F*: 5'-GCTAGCATGCGTCTCATTTTCTGGT-3'
KpnI*dgn1R*: 5'-GGTACCTTAAGGAGGAATGAATGGAG-3'
KpnI*dgn1delR*: 5'-AAGGTACCTTATTTCTTGATACAAGCAC-3'
KpnI*dgn1delTMCytoR*: 5'-AAGGTACCTTAGTCAGCTTCTGAATTGA-3'

Primers *NheI**dgn1F* and *KpnI**dgn1R* are described above. Primer *KpnI**dgn1delR* introduces a TAA stop codon and a *KpnI* site immediately after the codon for DGN-1 aa K498. Primer *KpnI**dgn1delTMCytoR* introduces a TAA stop codon and a *KpnI* site immediately after the codon for DGN-1 aa D469. Amplified products were A-tailed with Taq DNA polymerase and ligated into pGEM-T. *NheI/KpnI* fragments of the resulting pGEM-T subclones, containing the *dgn-1* mutant coding regions, were then ligated into *NheI/KpnI*-cut pBJ238 to replace the *dgn-1(+)* cassette under the control of *unc-119p*, making the *unc-119p* expression plasmids indicated in Table S3. This method was used for *dgn-1(ΔEC)*, *dgn-1(ΔNterm)*, *dgn-1(ΔCore)*, *dgn-1(ΔCyto)*, *dgn-1(ΔCoreΔCyto)* and *dgn-1(Nterm)*.

Method 2. An *XcmI/BbsI* fragment of the pBJ195-derived mutant, containing the mutated *dgn-1* coding region, was ligated into *XcmI/BbsI*-cut pBJ236 to replace the *dgn-1(+)* cassette between the previously introduced 5' *NheI* and 3' *KpnI* sites. The corresponding *NheI/KpnI* fragment of the resulting pBJ236-derived subclone was then ligated into *NheI/KpnI*-cut pBJ238 to replace the *dgn-1(+)* cassette under the control of *unc-119p*, making the *unc-119p* expression plasmids indicated in Table S3. This method was used for *dgn-1(ΔIgL)*, *dgn-1(ΔrBPL)*, *dgn-1(ΔTrich)*, *dgn-1(ΔCADG)*, *dgn-1(ΔSEA)* and *dgn-1(pat-3TM)*.

Supporting Data

Measurements of neuron displacement presented in graphical format in the figures or referenced in the Results section are summarized in tabular format in Tables S4-8.

Table S4. Stage-specific PVQ displacement defects in mutants.

Table S5. Stage-specific PVC displacement defects in mutants.

Table S6. Stage-specific PHA/B/C displacement defects in mutants.

Table S7. Adult stage PVQ displacement in wild-type and *dgn-1(cg121)* animals bearing heterologous promoter-driven *dgn-1(+)* transgenes. Expression constructs were introduced as extrachromosomal arrays (Ex) by germline injection at 25 mg/ml + 75 mg/ml pRF4 + 10 mg/ml pPD122.45[*myo-3p::GFP-NLS*]. All lines contained *hds26[sra-6p::DsRed2, odr-2p::CFP] III* to visualize the PVQ neurons.

Table S8. Adult stage PVQ and PVC displacement in wild-type and *dgn-1(cg121)* animals bearing *unc-119p*-driven *dgn-1* mutant transgenes as extrachromosomal arrays (Ex). Expression constructs were introduced as extrachromosomal arrays (Ex) by germline

injection at 25 µg/ml + 75 mg/ml pRF4 + 10 µg/ml pPD122.45[*myo-3p::GFP-NLS*]. PVQ and PVC neurons were visualized with the integrated markers *hds26* and *rhls4*, respectively.

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Table S1 Primers used for detection of *lge-1*, T07D3.4, *sgca-1* and *sgn-1* mutants.

Allele	Primer Pair	Wild-type Product (bp)	Mutant Product (bp)
<i>lge-1(tm1061)</i>	5'-GACTCCACTTGCGCTACATT-3'	1033	no product
	5'-ATCCTCCGAACCGCTGATCG-3'		
	5'-GACTCCACTTGCGCTACATT-3'	1701	1330
	5'-GTGTTGCAGTGCCCAACGTC-3'		
<i>T07D3.4(ok664)</i>	5'-GCAGTGTCGGCTGCTAATTT-3'	1546	no product
	5'-TACCATGCCCAGCATTGCCC-3'		
	5'-GCAGTGTCGGCTGCTAATTT-3'	2309	809
	5'-TTTCTGAAACCCGTAGGACG-3'		
<i>sgca-1(ok1529)</i>	5'-CTACACGTGATTGCGAAACG-3'	888	no product
	5'-GAGCCGTTCAATTGGTTTGT-3'		
	5'-TTCCATCGGAATTCCTTCAC-3'	no product	987
	5'-TCGAACGTCGAAATCATTCA-3'		
<i>sgn-1(ok2432)</i>	5'-AATGCGAAGAGAGGTGGAGA-3'	952	no product
	5'-AATGCTTGATGGGTGGTTTC-3'		
	5'-CCCCTTCTCAATATTCGGGT-3'	no product	1483
	5'-AAGCTTCCAGCCTCCTTTGT-3'		

Table S2 Heterologous promoter-driven *dgn-1(+)* and Venus constructs

Source gene	Amplification primers	Region amplified relative to ATG start	<i>NotI/AscI</i> promoter fragment inserted into	Resulting plasmid	Expression cassette
<i>unc-119</i>	5'-GCGGCCGCGCAATTCTATGAAAATGTC-3' 5'-GGCGCGCCCTGTTGTAGCTGAAAATTTT-3'	-1671 bp to -6 bp	pBJ236	pBJ238	<i>unc-119p::dgn-1(+)</i>
			pBJ237	pBJ239	<i>unc-119p::Venus</i>
<i>unc-33</i>	5'-AAGCGGCCGCGCACTCGTTGAGGTACGGTAGA-3' 5'-AAGGCGCGCCCTTCCAGTGATGTCCGCCAAC-3'	-2746 bp	pBJ236	pBJ279	<i>unc-33p::dgn-1(+)</i>
			pBJ237	pBJ280	<i>unc-33p::Venus</i>
<i>unc-14</i>	5'-AAGCGGCCGCGCATTGACAAAAAAGATG-3' 5'-AAGGCGCGCCCTTGGTGGAAGAATTGAGGG-3'	-1398 bp	pBJ236	pBJ277	<i>unc-14p::dgn-1(+)</i>
			pBJ237	pBJ278	<i>unc-14p::Venus</i>
<i>rgef-1</i>	5'-AAGCGGCCGCGCGTTTCCGATACCCCCTTAT-3' 5'-AAGGCGCGCCCGTCGTCGTCGTCGATGCCG-3'	-3440 bp	pBJ236	pBJ261	<i>rgef-1p::dgn-1(+)</i>
			pBJ237	pBJ262	<i>rgef-1p::Venus</i>
<i>rab-3</i>	5'-AAGCGGCCGCGACGACGACGACCTCGACGG-3' 5'-AAGGCGCGCCCTGAAAATAGGGCTACTGTA-3'	-1343 bp	pBJ236	pBJ297	<i>rab-3p::dgn-1(+)</i>
			pBJ237	pBJ295	<i>rab-3p::Venus</i>
<i>aex-3</i>	5'-AAGCGGCCGCAACTGCCGCTTCAATTCTCC-3' 5'-AAGGCGCGCCCTTTTATTAGGATAGGTACA-3'	-1320 bp	pBJ236	pBJ296	<i>aex-3p::dgn-1(+)</i>
			pBJ237	pBJ294	<i>aex-3p::Venus</i>
<i>sra-6</i>	5'-AAGCGGCCGCGTGGGACGTTGGAAAAAGAA-3' 5'-AAGGCGCGCCCGGCAAAATCTGAAATAATAA-3'	-2987 bp	pBJ236	pBJ256	<i>sra-6p::dgn-1(+)</i>
			pBJ237	pBJ257	<i>sra-6p::Venus</i>
<i>ajm-1</i>	5'-AAGCGGCCGCGGATTTGACCGTTCGATAAGA-3' 5'-AAGGCGCGCCCTCGTCGGTAGTACTCGTCCTG-3'	-6325 bp to -830 bp (C25A11.4e form ATG)	pBJ236	pBJ327	<i>ajm-1p::dgn-1(+)</i>
			pBJ237	pBJ328	<i>ajm-1p::Venus</i>
<i>lin-26</i>	5'-AAGCGGCCGCGACCTCGTGCATATCGCTAAG-3' 5'-AAGGCGCGCCCTGAAAATAATCAATTAATAA-3'	-4000 bp	pBJ236	pBJ345	<i>lin-26FGH_{ip}::dgn-1(+)</i>
			pBJ237	pBJ346	<i>lin-26FGH_{ip}::Venus</i>
<i>dpy-7</i>	5'-AAGCGGCCGCGACATTCCTTTTCGCATGTACTCA-3' 5'-AAGGCGCGCCCTTATCTGGAACAAAATGTAAGAAT-3'	-303 bp	pBJ236	pBJ247	<i>dpy-7p::dgn-1(+)</i>
			pBJ237	pBJ248	<i>dpy-7p::Venus</i>

Table S3 Construction of *unc-119p*-driven *dgn-1* mutant expression plasmids

Mutant	Primers for mutagenesis of pBJ195	Domain of DGN-1 deleted (Deleted DGN-1 aa)	<i>NheI/KpnI</i> primers	<i>unc-119p</i> expression plasmid
<i>dgn-1</i> (ΔEC)	5'-GAGCCATGAAGGAAGTGGGC-3' 5'-GAGACAGTCGAAATTAGCAC-3'	Extracellular domain (M52-D450)	<i>NheI</i> dgn1F + KpnIdgn1R	pBJ341
<i>dgn-1</i> (ΔNterm)	5'-GAGCCATGAAGGAAGTGGGC-3' 5'-ACTGACAACAAACCAATCCT-3'	N-terminal domain (M52-I235)	<i>NheI</i> dgn1F + KpnIdgn1R	pBJ342
<i>dgn-1</i> (ΔIgL)	5'-GAGCCATGAAGGAAGTGGGC-3' 5'-AATGTCGAGGAAGAACTAT-3'	Ig-like subdomain (M52-L86)	(<i>NheI</i> and <i>KpnI</i> sites introduced by subcloning into pBJ236)	pBJ365
<i>dgn-1</i> (ΔrRBPL)	5'-CAGGTACAGGTAAACGGGCT-3' 5'-ACGACTGCCGAGTACTTGCG-3'	rRBP-like subdomain (N87-P216)	(<i>NheI</i> and <i>KpnI</i> sites introduced by subcloning into pBJ236)	pBJ366
<i>dgn-1</i> (ΔTrich)	5'-GGGATCTTCAGCCTCTTTG-3' 5'-GACAACAAACCAATCCTTCT-3'	T-rich region (T217-T236)	(<i>NheI</i> and <i>KpnI</i> sites introduced by subcloning into pBJ236)	pBJ367
<i>dgn-1</i> (ΔCore)	5'-GATTCTTCTCGTGGTACGGA-3' 5'-GAGACAGTCGAAATTAGCAC-3'	Core domain (T236-D450)	<i>NheI</i> dgn1F + KpnIdgn1R	pBJ343
<i>dgn-1</i> (ΔCADG)	5'-GATTCTTCTCGTGGTACGGA-3' 5'-GTCAATGTGAAGCCAGCTCT-3'	CADG subdomain (T236-Q328)	(<i>NheI</i> and <i>KpnI</i> sites introduced by subcloning into pBJ236)	pBJ368
<i>dgn-1</i> (ΔSEA)	5'-ATTGGTTGGAAGAGCTGGCT-3' 5'-GAGACAGTCGAAATTAGCAC-3'	SEA subdomain (H339-D450)	(<i>NheI</i> and <i>KpnI</i> sites introduced by subcloning into pBJ236)	pBJ369
<i>dgn-1</i> (<i>pat-3</i> TM)	see below	TM domain (replaced with <i>pat-3</i> TM) (L474-I496)	(<i>NheI</i> and <i>KpnI</i> sites introduced by subcloning into pBJ236)	pBJ371
<i>dgn-1</i> (ΔCyto)	5'-TTTCTTGATACAAGCACAGT-3' 5'-TAATTCCGAGCGAGAAATTG-3'	Cytoplasmic domain (S499-P584)	<i>NheI</i> dgn1F + KpnIdgn1delR	pBJ344
<i>dgn-1</i> (ΔCoreΔCyto)	generated from <i>dgn-1</i> (ΔCore) mutant by amplification with <i>NheI/KpnI</i> primers	Core and Cyto domains (T236-Q328 and S499-P584)	<i>NheI</i> dgn1F + KpnIdgn1delR	pBJ363
<i>dgn-1</i> (ΔNterm)		Core, TM and Cyto domains (T236-D450 and S470-P584)	<i>NheI</i> dgn1F + KpnIdgn1delTMcytoR	pBJ364
<i>dgn-1</i> (<i>pat-3</i> TM) mutagenesis primers:				
5'-TACCCGCAATGACTCCGAGCACAATTGCGAGCACCAACAGCTGCGAGTCAGCTTCTTGAA-3'				
5'-TCGTAATCCTCGGAATTCTTCTTGTGTGCTCTGGAAGAAAAGTGGAAGAAGAAGTCAA-3'				

Table S4 Stage-specific PVQ displacement defects in mutants

	<u>3-fold embryos</u>		<u>L1 larvae</u>		<u>Adults</u>	
	%PVQ displaced	N [†]	%PVQ displaced	N [†]	%PVQ displaced	N [†]
<i>oyIs14</i>	1.4	216	0	200	0.9	214
<i>oyIs14;dgn-1(cg121)</i>	8.4	202	38.9	208	75.9	232
<i>dig-1(n1321);oyIs14;dgn-1(cg121)</i>	7.8	206	37.3	204	68.1	210
<i>dig-1(n1321);oyIs14</i>	7.1	210	41.7	228	35.8	218
<i>anc-1(e1753);dig-1(n1321);oyIs14</i>	10.4	212	63.1	214	67.4	218
<i>anc-1(e1753);oyIs14</i>	9.9	202	33.3	210	88.1	210
<i>anc-1(e1753);oyIs14;dgn-1(cg121)</i>	13.0	200	60.8	204	86.0	214
<i>anc-1(e1753);dig-1(n1321);oyIs14;dgn-1(cg121)</i>	16.5	200	60.0	200	64.5	200
<i>oyIs14;unc-84(e1410)</i>	nd		12.7	236	67.7	220
<i>unc-83(e1408) oyIs14</i>	nd		3.4	206	38.0	208
<i>unc-54(e1092);oyIs14</i>	nd		nd		1.5	200
<i>unc-54(e1092);oyIs14;dgn-1(cg121)</i>	nd		nd		76.0	100
<i>unc-54(e1092);dig-1(n1321);oyIs14</i>	nd		24.8	234	22.5	200
<i>anc-1(e1753) unc-54(e1092);oyIs14</i>	nd		27.0	226	38.0	200
<i>dys-1(cx18);oyIs14</i>	nd		nd		0.9	110
<i>dyb-1(cx36);oyIs14</i>	nd		nd		4.1	122
<i>oyIs14;stn-2(ok2417)</i>	nd		nd		2.0	100
<i>oyIs14;sgca-1(ok1529)</i>	nd		nd		2.0	150
<i>sgn-1(ok2432);oyIs14</i>	nd		nd		0	110
<i>oyIs14;lge-1(tm1051)</i>	nd		nd		1.7	116
<i>T07D3.4(ok664);oyIs14</i>	nd		nd		1.7	118
<i>oyIs14;dgn-1(cg121), 50 μM Levamisole</i>	nd		nd		66.0	244
<i>dig-1(n1321);oyIs14, 50 μM Levamisole</i>	nd		nd		23.6	106
<i>anc-1(e1753);oyIs14, 50 μM Levamisole</i>	nd		nd		62.3	114

[†] Number of PVQ neurons scored (2 per animal scored)

nd, not determined

Table S5 Stage-specific PVC displacement defects in mutants

	<u>3-fold embryos</u>		<u>L1 larvae</u>		<u>Adults</u>	
	%PVC displaced	N [†]	%PVC displaced	N [†]	%PVC displaced	N [†]
<i>rhIs4</i>	0	100	0	200	0	200
<i>rhIs4;dgn-1(cg121)</i>	0.96	104	12.0	108	41.3	104
<i>dig-1(n1321) rhIs4</i>	0	122	0.8	124	8.5	106
<i>anc-1(e1753);rhIs4</i>	0	106	1.9	104	24.5	102
<i>dys-1(cx18);rhIs4</i>	nd		nd		0	106
<i>dyb-1(cx36);rhIs4</i>	nd		nd		0	110
<i>rhIs4;stn-2(ok2417)</i>	nd		nd		0	118
<i>rhIs4;sgca-1(ok1529)</i>	nd		nd		0.8	128
<i>sgn-1(ok2432);rhIs4</i>	nd		nd		0	128
<i>rhIs4;lge-1(tm1051)</i>	nd		nd		0.9	110
<i>T07D3.4(ok664);rhIs4</i>	nd		nd		0.0	140

[†] Number of PVC neurons scored (2 per animal scored)

nd, not determined

Table S6 Stage-specific PHA/B/C displacement defects in mutants

	<u>3.5/4-fold embryos</u>		<u>L1 larvae</u>		<u>Adults</u>	
	%PHA/B/C displaced	N [†]	%PHA/B/C displaced	N [†]	%PHA/B/C displaced	N [†]
<i>inIs179</i>	0	300	0.3	600	0.2	600
<i>inIs179;dgn-1(cg121)</i>	1.3	300	19.1	330	36.4	354
<i>inIs179;dig-1(n1321)</i>	0	330	2.2	318	2.8	318
<i>anc-1(e1753);inIs179</i>	0.64	312	3.1	318	2.9	306
<i>dys-1(cx18);inIs179</i>	nd		nd		0	402
<i>dyb-1(cx36);inIs179</i>	nd		nd		0	312
<i>inIs179;stn-2(ok2417)</i>	nd		nd		0	348
<i>inIs179;sgca-1(ok1529)</i>	nd		nd		0	372
<i>inIs179;lge-1(tm1051)</i>	nd		nd		0.2	414

[†] Number of PHA/B/C neurons scored (6 per animal scored)

nd, not determined

Table S7 Adult stage PVQ displacement in wild-type and *dgn-1(cg121)* animals bearing heterologous promoter-driven *dgn-1(+)* transgenes

	line	%PVQ displaced	N	Average
<i>dgn-1(cg121)</i>	-	71.3	94	-
wt	-	0.9	108	-
<i>dgn-1(cg121);Ex[unc-119p::dgn-1(+)]</i>	#1	19.2	120	15.8±3.3
	#2	12.5	104	
wt;Ex[unc-119p::dgn-1(+)]	#1	8.8	102	8.6±0.2
	#2	8.3	132	
<i>dgn-1(cg121);Ex[unc-33p::dgn-1(+)]</i>	#1	21.2	118	26.1±4.9
	#2	31.0	100	
wt;Ex[unc-33p::dgn-1(+)]	#1	7.5	106	14.3±6.7
	#2	21.0	100	
<i>dgn-1(cg121);Ex[unc-14p::dgn-1(+)]</i>	#1	63.7	124	62.1±1.6
	#2	60.5	124	
wt;Ex[unc-14p::dgn-1(+)]	#1	20.0	100	23.5±3.5
	#2	27.0	100	
<i>dgn-1(cg121);Ex[rgef-1p::dgn-1(+)]</i>	#1	75.9	112	78.8±2.9
	#2	81.7	120	
wt;Ex[rgef-1p::dgn-1(+)]	#1	7.5	106	9.1±1.5
	#2	10.6	132	
<i>dgn-1(cg121);Ex[rab-3p::dgn-1(+)]</i>	#1	71.4	126	70.4±1.1
	#2	69.3	114	
wt;Ex[rab-3p::dgn-1(+)]	#1	6.6	106	6.1±0.5
	#2	5.6	108	
<i>dgn-1(cg121);Ex[aex-3p::dgn-1(+)]</i>	#1	73.1	108	74.5±1.4
	#2	75.9	108	
wt;Ex[aex-3p::dgn-1(+)]	#1	2.7	112	5.9±3.2
	#2	9.1	110	
<i>dgn-1(cg121);Ex[sra-6p::dgn-1(+)]</i>	#1	59.6	114	61.8±2.2
	#2	64.0	100	
wt;Ex[sra-6p::dgn-1(+)]	#1	2.0	98	4.8±2.8
	#2	7.5	106	
<i>dgn-1(cg121);Ex[ajm-1p::dgn-1(+)]</i>	#1	66.1	112	69.8±3.7
	#2	73.5	102	
wt;Ex[ajm-1p::dgn-1(+)]	#1	0	104	0.5±0.5
	#2	1.0	100	
<i>dgn-1(cg121);Ex[lin-26FGHip::dgn-1(+)]</i>	#1	77.9	104	78.1±0.2
	#2	78.3	106	
wt;Ex[lin-26FGHip::dgn-1(+)]	#1	1.8	110	1.9±0.1
	#2	2.0	100	
<i>dgn-1(cg121);Ex[dpy-7p::dgn-1(+)]</i>	#1	63.8	116	64.4±0.6
	#2	65.0	100	
wt;Ex[dpy-7p::dgn-1(+)]	#1	0	106	2.0±2.0
	#2	4.0	100	

Table S8 Adult stage PVQ and PVC displacement in wild-type and *dgn-1(cg121)* animals bearing *unc-119p*-driven *dgn-1* mutant transgenes

	line	%PVQ displaced	N	Average	%PVC displaced	N	Average
<i>dgn-1(cg121)</i>	-	71.3	94	-	41.3	104	-
wt	-	0.9	108	-	0	200	-
<i>dgn-1(cg121);Ex[unc-119p::dgn-1(+)]</i>	#1	19.2	120	15.8±3.3	4.2	120	3.3±0.8
	#2	12.5	104		2.5	100	
wt;Ex[<i>unc-119p::dgn-1(+)</i>]	#1	8.8	102	8.6±0.2	1.0	120	0.5±0.5
	#2	8.3	132		0	100	
<i>dgn-1(cg121);Ex[unc-119p::dgn-1(ΔEC)]</i>	#1	64.2	106	66.4±2.2	33.0	106	33.7±0.6
	#2	68.6	102		34.3	102	
wt;Ex[<i>unc-119p::dgn-1(ΔEC)</i>]	#1	5.8	104	7.4±1.6	0	104	0±0
	#2	9.0	122		0	122	
<i>dgn-1(cg121);Ex[unc-119p::dgn-1(ΔNterm)]</i>	#1	71.4	140	71.7±0.3	29.3	140	29.3±0
	#2	72.0	150		29.3	150	
wt;Ex[<i>unc-119p::dgn-1(ΔNterm)</i>]	#1	9.6	104	9.1±0.6	0	104	0.5±0.5
	#2	8.5	106		0	106	
<i>dgn-1(cg121);Ex[unc-119p::dgn-1(ΔIgL)]</i>	#1	70.0	100	69.1±0.9	32.0	100	28.3±3.7
	#2	68.2	110		24.5	110	
wt;Ex[<i>unc-119p::dgn-1(ΔIgL)</i>]	#1	1.0	100	2.0±1.0	0	100	0±0
	#2	3.0	100		0	100	
<i>dgn-1(cg121);Ex[unc-119p::dgn-1(ΔrRBPL)]</i>	#1	70.4	108	71.5±1.2	30.6	108	33.5±2.9
	#2	72.7	110		36.4	110	
wt;Ex[<i>unc-119p::dgn-1(ΔrRBPL)</i>]	#1	3.0	100	1.5±1.5	0	100	0±0
	#2	0	100		0	100	
<i>dgn-1(cg121);Ex[unc-119p::dgn-1(ΔTrich)]</i>	#1	3.8	104	7.4±3.5	1.9	104	2.3±0.4
	#2	10.9	110		2.7	110	
wt;Ex[<i>unc-119p::dgn-1(ΔTrich)</i>]	#1	2.0	100	2.5±0.5	1.0	100	0.5±0.5
	#2	3.0	100		0	100	
<i>dgn-1(cg121);Ex[unc-119p::dgn-1(ΔCore)]</i>	#1	9.8	102	9.8±0.0	0	102	0.9±0.9
	#2	9.8	112		1.8	112	
wt;Ex[<i>unc-119p::dgn-1(ΔCore)</i>]	#1	2.3	130	2.5±0.2	0	130	0±0
	#2	2.7	110		0	110	
<i>dgn-1(cg121);Ex[unc-119p::dgn-1(ΔCADG)]</i>	#1	42.6	108	43.0±0.4	5.6	108	11.4±5.8
	#2	43.4	122		17.2	122	
wt;Ex[<i>unc-119p::dgn-1(ΔCADG)</i>]	#1	9.0	100	7.5±1.5	0	100	0±0
	#2	6.0	100		0	100	
<i>dgn-1(cg121);Ex[unc-119p::dgn-1(ΔSEA)]</i>	#1	16.0	100	10.7±5.3	3.0	100	2.0±1.0
	#2	5.5	110		0.9	110	
wt;Ex[<i>unc-119p::dgn-1(ΔSEA)</i>]	#1	3.0	100	4.5±1.5	0	100	0±0
	#2	6.0	100		0	100	
<i>dgn-1(cg121);Ex[unc-119p::dgn-1(pat-3TM)]</i>	#1	17.0	112	12.6±4.4	2.7	112	1.8±0.9
	#2	8.2	110		0.9	110	
wt;Ex[<i>unc-119p::dgn-1(pat-3TM)</i>]	#1	5.0	100	4.0±1.0	0	100	0±0
	#2	3.0	100		0	100	
<i>dgn-1(cg121);Ex[unc-119p::dgn-1(ΔCyto)]</i>	#1	31.0	100	28.0±3.0	2.0	100	3.5±1.5
	#2	25.0	140		5.0	140	
wt;Ex[<i>unc-119p::dgn-1(ΔCyto)</i>]	#1	20.2	114	16.8±3.4	0.9	114	0±0
	#2	13.4	112		0.9	112	
<i>dgn-1(cg121);Ex[unc-119p::dgn-1(ΔCoreΔCyto)]</i>	#1	17.3	104	15.9±1.4	2.9	104	2.4±0.5
	#2	14.5	110		1.8	110	
wt;Ex[<i>unc-119p::dgn-1(ΔCoreΔCyto)</i>]	#1	7.0	100	5.5±1.5	2.0	100	1.0±1.0
	#2	4.0	100		0	100	

<i>dgn-1(cg121);Ex[unc-119p::<i>dgn-1(Nterm)</i>]</i>	#1	75.5	106	78.0±2.5	43.4	106	49.9±6.5
	#2	80.6	108		56.5	108	
wt;Ex[<i>unc-119p::<i>dgn-1(Nterm)</i>]</i>	#1	38.0	100	41.5±3.5	11.0	100	12.5±1.5
	#2	45.0	100		14.0	100	