

Chapter 2

Guidance Molecules Required for Growth Cone Migration of Cells and Axons

Abstract In metazoans, neuronal cell bodies and their axons migrate long distances along both the dorsal–ventral and the anterior–posterior axis during different phases of development; additionally, they may also cross the midline in bilaterally symmetric organisms. The leading edge of a migrating cell or an axon is a highly specialized structure, the growth cone (GC). The GC steers through an increasingly complex environment by perceiving attractive and repulsive cues via their cognate receptors expressed on the GC surface. Interestingly, studies in the past two decades revealed that the axon guidance molecules are highly conserved across eukaryotes including UNC-6/Netrins, acting as a bifunctional cue, attracting and repelling GCs via UNC-40/DCC and UNC-5/UNC-5 receptors, respectively, SLT-1/Slit repelling GCs via its receptor SAX-3/Robo, and Wnts acting through their Frizzled (Fz) and Ryk receptors. Besides these universally conserved guidance molecules, there are molecules specific to nematodes steering the GCs during nervous system development.

Keywords Guidance cues • cell migration • Axon outgrowth • UNC-6 • Netrin • SLIT • Robo • Wnts • UNC-53

Studies in mammals, flies, and nematodes led to identification of molecules required for guidance and extension of growth cone (GC) during nervous system development. The following section discusses the major cues and their cognate receptors required for circumferential or dorsal–ventral (DV), longitudinal or anterior–posterior (AP) GC guidance and midline guidance in bilaterally symmetric organisms.

2.1 Guidance Cues and Their Receptors in Dorsal–Ventral Guidance

2.1.1 *UNC-6/Netrin and UNC-40/DCC/FRA*

A genetic screen for mutations affecting circumferential migrations in *Caenorhabditis elegans* led to identification of three genes *unc-6*, *unc-5*, and *unc-40*, where *unc-5* and *unc-40* gene products affect ventral and dorsal migrations of the GCs of pioneer axons and mesodermal cell along the epidermis in an *unc-6*-dependent manner causing the uncoordinated (Unc) phenotype (Hedgecock et al. 1987, 1990). UNC-6 is ventrally localized laminin-related extracellular matrix protein, expressed in twelve types of neuroglia and pioneer neurons (Hedgecock et al. 1990; Chan et al. 1996), affecting both dorsal and ventral migrations (Wadsworth et al. 1996). UNC-6 serves as a bifunctional guidance cue in both vertebrates and invertebrates, acting both as a chemorepellent and as a chemoattractant (Hedgecock et al. 1990; Tessier-Lavigne and Goodman 1996). The vertebrate, UNC-6 homologues, Netrin-1 (*Ntn-1*) and Netrin-2 (*Ntn-2*), expressed at the ventral midline of chick embryonic spinal cord (Serafini et al. 1994), guide commissural axons toward the ventral midline and telencephalon, acting as attractive cue (Métin et al. 1997) and repulsive cue in guiding trochlear motor axons dorsally away from the ventral floor plate and GABAergic interneurons in the cerebellar cortex (Colamarino and Tessier-Lavigne 1995; Guijarro et al. 2006). Netrin-1 acts as guidance cue in several regions of the central nervous system (CNS), including spinal commissural, hippocampal, retinal, thalamic, and dopaminergic axon guidance (Kennedy et al. 1994; Colamarino and Tessier-Lavigne 1995; Barallobre et al. 2000; Braisted et al. 2000; Shewan et al. 2002; Lin et al. 2005). Similarly, the *Drosophila* homologues, Netrin-A and Netrin-B, expressed at the ventral midline, function as an attractive cue during commissure formation and repulsive cue, repelling the motor axon outgrowth to their targets (Harris et al. 1996; Mitchell et al. 1996). Mutations in Netrin caused partially missing or thinner commissures, a phenotype that is rescued by expression of Netrin from the ventral midline.

UNC-6/Netrin achieves its chemoattractant ventral guidance function through its receptor UNC-40 of *C. elegans*, Neogenin/DCC (deleted in colorectal cancer) of vertebrates, and FRA (Frazzled) of *Drosophila*, expressed on motile cells and pioneer neurons (Hedgecock et al. 1990; Fearon et al. 1990; Keino-Masu et al. 1996; Kolodziej et al. 1996; Chan et al. 1996). DCC was originally identified as a tumor suppressor gene, deleted at high frequency in colorectal cancers (Fearon et al. 1990), expressed on the axons of the dorsal spinal commissural neurons as they extend across the floor plate and to the ventral funiculus. Biochemical assays using antibodies against DCC supported the function of DCC as a receptor for Netrin-1, where Netrin-1-dependent commissural axon outgrowth was stopped in in vitro assays (Keino-Masu et al. 1996). Similarly, *frazzled* (*fra*), *Drosophila* member of DCC gene family, was also found to be expressed on the axons in the embryonic CNS and on motor axons in the periphery, null mutations in *fra*

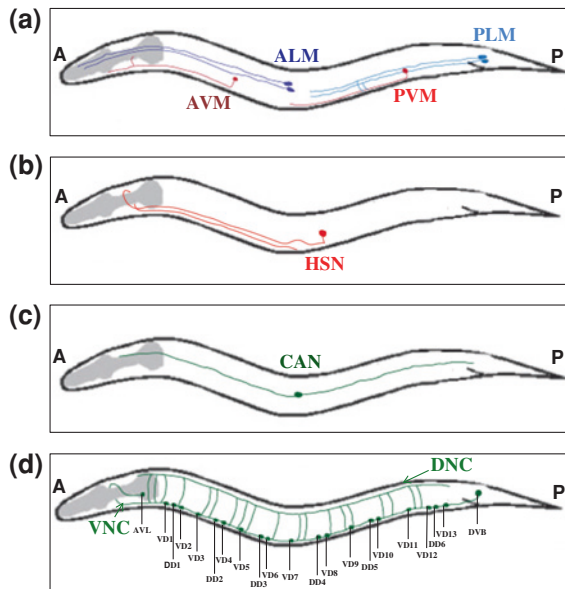


Fig. 2.1 Illustrative of neuronal cell bodies and their axons during *C. elegans* development: **a** The six mechanosensory neurons including two anterior lateral microtubules (ALMs), located in the midbody region, and two posterior lateral microtubules (PLMs), located in the tail of the worm. ALM extends anterior processes ending in the first pharynx bulb, and PLMs are bipolar with a shorter posterior process extended into the tail and an anterior process terminating near the ALM cell body. Anterior ventral microtubule and posterior ventral microtubule (AVM and PVM) extend a ventral process to the ventral nerve cord (VNC), followed by anterior outgrowth toward the head of the worm. **b** Hermaphrodite-specific neurons (HSNs), a pair of bilaterally symmetric neuron, with the cell body located in the midbody region of the worm, extends a ventral process to the VNC, which later extends anteriorly to the nerve ring. **c** Canal-associated neurons (CANs), with their cell bodies located in the midbody region of the worm extending processes in both anterior and posterior directions. **d** The six DD and thirteen VD neurons send a commissural axonal process during embryogenesis (DDs) or larval development (VDs), and commissures extend from the VNC toward the dorsal nerve cord (DNC)

disrupted axon guidance, and the phenotypes resembled those observed in embryos lacking the *Drosophila* Netrin genes (Harris et al. 1996; Mitchell et al. 1996). Additionally, expression of FRA in the neurons rescued the defects in motor axon guidance, strongly supporting it to be a receptor for Netrin (Kolodziej et al. 1996).

In *C. elegans*, *unc-40* gene function is required for GC migrations of several cells including AVM and PVM (anterior and posterior ventral microtubule neuron) mechanosensory or touch receptor neurons that sense gentle body touch (Fig. 2.1a) (Chalfie and Sulston 1981; Chalfie et al. 1985). In wild-type animals, AVM cell body is located laterally in the posterior left side of the worm body, whereas PVM cell body is located laterally in anterior right side of the worm body, and both the neurons send out a ventral process to the ventral nerve cord (VNC), followed by anterior longitudinal extension along the VNC. Phenotypic

analysis of *mec-4::GFP* transgenic worms with *unc-40* mutation revealed that function of UNC-40 is required for the ventral process outgrowth (Chalfie et al. 1994; Chan et al. 1996). *unc-40* mutants also showed defects in the ventral process outgrowth of hermaphrodite-specific neuron (HSN), a pair of bilaterally symmetric neurons extending a ventral process toward the VNC, followed by an anterior process toward the nerve ring (Fig. 2.1b) and in ventral GC migrations of the phasmid (PHA and PHB) and PDE neurons (Hedgecock et al. 1990).

Several lines of evidence suggest requirement of UNC-40 in both ventral and dorsal GC migrations. SDQR a sister neuron of AVM, born in the first larval stage, migrates dorsally to the lateral margin of the body wall muscles, and its axon grows dorsally and anteriorly toward the nerve ring. Phenotypic analysis of *unc-119::GFP* transgenic worms (Chalfie et al. 1994; Maduro and Pilgrim 1995) with *unc-40* mutation revealed defects in the dorsal axon guidance but less severe than the *unc-6* and *unc-5* mutations (Kim et al. 1999).

2.1.2 SLT-1/Slit and SAX-3/Robo

Another ventral guidance pathway conserved across species is the SLT-1/Slit (Shiga-like toxin) and its cognate receptor SAX-3/Robo (Roundabout). Initially, *slit* gene was identified in *Drosophila* embryo as a larval cuticle-patterning gene (Nüsslein-Volhard et al. 1984), and later, it was shown that null mutations in *slit* gene resulted in disruption of *Drosophila* embryonic CNS (Rothberg et al. 1988). Antibody staining and in situ hybridization revealed *slit* expression at midline glial cells, and reduction in *slit* expression resulted in disruption of developing midline cells and commissural axon pathways (Rothberg et al. 1990). Roundabout (*robo*), receptor for Slit, was identified along with *slit* and *comm* in a large-scale mutant screen for the genes that control midline axon guidance in *Drosophila* CNS (Seeger et al. 1993). Axons expressing high Robo levels from the outset never crossed the midline, and axons that crossed the midline once, expressed Robo after crossing, suggesting that Robo acts as a repulsive receptor (Kidd et al. 1998a). Phenotypic analysis of transheterozygotes and transgenic for *slit* and *robo* confirmed their role in a common genetic pathway, where Slit acts as a ligand to Robo in midline axon guidance in *Drosophila* embryos (Kidd et al. 1998b; Kidd et al. 1999). Genetic interaction between Slit and Robo was supported by biochemical studies confirming their physical interaction (Brose et al. 1999). Later, a single *slit* gene was identified in *C. elegans* (*slt-1*) and three *slit* genes in humans (*slit-1–slit-3*) (Itoh et al. 1998; Brose et al. 1999).

In *C. elegans*, SLT-1 protein is expressed in larval dorsal muscles and signals through its receptor SAX-3/Robo to promote ventral guidance of GCs (Hao et al. 2001). SAX-3/Robo is required for ventral process guidance of HSN motor neurons (Fig. 2.1b), evidenced by phenotypic analysis of *sax-3* mutants, where HSN fails to extend a ventral process (Zallen et al. 1998). Besides, genetic analysis supported the requirement of SAX-3/Robo for ventral process guidance of PVM and AVM touch neurons (Fig. 2.1a), in SLT-1/Slit-dependent manner (Hao et al. 2001).

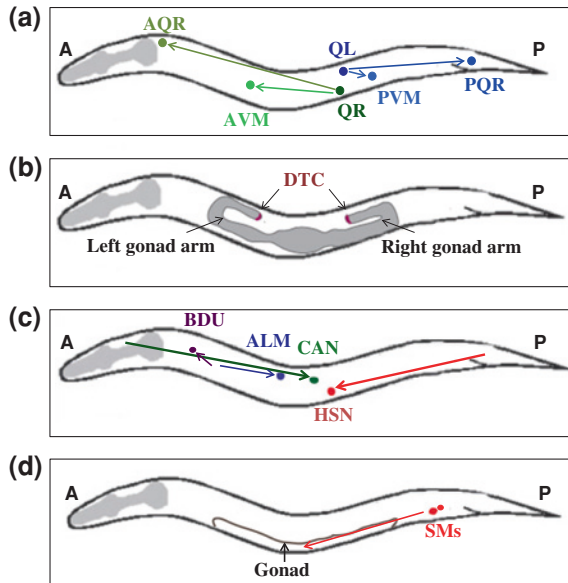


Fig. 2.2 Diagrammatic representation of neuronal and non-neuronal cell migration during *C. elegans* development: **a** Q neuroblasts include QL (left) and QR (right): QR migrates anteriorly and divides into AQR and AVM, and QL migrates posteriorly and divides into PVM and PQR. **b** Distal tip cells (DTC), a pair of cells at the tip of each gonad arm, migrate during larval stage away from gonad premordium on the ventral side and turn dorsally and finally migrate toward the center on dorsal side forming a U-shaped gonad. **c** Anterior lateral microtubule (ALM) and its sister cell BDU migrate during embryogenesis. ALM undergoes posterior migration to the midbody region of the animal, completing its migration by L1 stage, and BDU migrates anteriorly. **d** The two sex myoblasts (SMs) are born in the posterior body region in L1 stage and migrate anteriorly to the midbody region to flank the gonads in the L2 stage

In *C. elegans*, SAX-3/Robo is also required to maintain the sex myoblasts (SMs) along the ventral muscle quadrants (Fig. 2.2d) (Branda and Stern 2000). SMs are bilaterally symmetric pair of cells born during early larval development in the ventral midposterior body region, and after birth, they migrate anteriorly on ventral muscle quadrant to flank the precise center of the gonads, where they divide and differentiate into the muscles required for egg laying (Sulston and Horvitz 1977). Three pathways regulate the anterior migration of SMs including gonad-dependent attraction (GDA), gonad-independent mechanism (GIM), and gonad-dependent repulsion (GDR) (Thomas et al. 1990; Chen et al. 1997; Branda and Stern 2000). Phenotypic analysis of mutants confirmed the function of *egl-17* and *egl-15* in GDA (Stern and Horvitz 1991; DeVore et al. 1995); *unc-53*, *unc-71*, and *unc-73* in GIM (Chen et al. 1997); and *unc-14*, *unc-33*, *unc-44*, and *unc-51* in GDR (Branda and Stern 2000). Moreover, it was found that SAX-3/Robo acts redundantly with GDA to position the SMs in the ventral position, supported by phenotypic analysis of *sax-3*; *egl-17* mutants and *sax-3* mutation with gonad ablation experiments, with more dorsally displaced SMs, were observed (Branda and Stern 2000).

2.1.3 SLT-1/EVA-1

A second SLT-1 receptor has been recently identified in *C. elegans*, called enhancer of ventral axon (EVA-1) guidance defects of *unc-40* mutants, required for ventral process guidance of pioneer AVM neuron (Fig. 2.1a). EVA-1 functions as a co-receptor with SAX-3/Robo. Earlier work supported that SAX-3/Robo in response to SLT-1/Slit repels axons of AVM and PVM pioneering neurons toward VNC (Hao et al. 2001). Recently, genetic evidence pointed toward the presence of two parallel pathways in AVM ventral process guidance including UNC-6/Netrin signaling via UNC-40 and SLT-1 signaling through EVA-1. Based on the genetic evidence where more ventral process guidance defects were detected in *eva-1*, *slt-1* and then in *sax-3* mutants, it was hypothesized that in wild-type worms, SAX-3 binds to EVA-1, whereas in the absence of either SLT-1 or EVA-1, SAX-3 binds to UNC-40, inhibiting the ventral process guidance (Fujisawa et al. 2007). This proposed inhibition of the UNC-6/Netrin pathway by SAX-3 is consistent with the fly midline crossing phenotype, where Robo silencing of Frazzled/UNC-40 allows GCs to cross midline sources of netrin (Stein and Tessier-Lavigne 2001).

2.1.4 UNC-6/Netrin and UNC-5/UNC-5

unc-5 was identified as one of the three genes affecting circumferential migration of pioneer axons and mesodermal cells on *C. elegans* body wall, specifically affecting dorsal migration (Hedgecock et al. 1990). Sequence analysis revealed UNC-5 as a transmembrane protein, expressed on cell surface of pioneer axons and motile cells, repelled by UNC-6 gradient (Hedgecock et al. 1990; Leung-Hagsteijn et al. 1992; Wadsworth et al. 1996). Three vertebrate homologues of *unc-5*, namely *UNC5h1*, *UNC5h2*, and *UNC5h3* have been identified in mouse and human (Leonardo et al. 1997), acting as repulsive receptor for Netrin-1, regulating migration of cerebellar neurons and corticospinal tract (CST) axon (Ackerman and Knowles 1998; Finger et al. 2002). A mouse homologue of UNC-5, rostral cerebellar malformation (*rcm*) gene, was identified, and *rcm* mutants exhibited cerebellar and midbrain defects, apparently as a result of abnormal neuronal migration (Ackerman et al. 1997). Similarly, *Drosophila* UNC-5 was shown to be required for motor axon guidance, and ectopic expression of UNC-5 in CNS caused midline repulsion of axons (Keleman and Dickson 2001). Interestingly, some of the migrations regulated by UNC-5 required DCC function, evidenced by experiments conducted in *Xenopus* spinal axons, where UNC-5-mediated repulsion was achieved by the formation of receptor complex between DCC and UNC-5, in response to Netrin-1. The interaction between the cytoplasmic domains converted the DCC-mediated attraction to UNC-5-/DCC-mediated repulsion (Hong et al. 1999).

In *C. elegans*, phenotypic analysis of *unc-5* single mutants and *unc-5 unc-6* double mutants revealed that *unc-5* gene function requires *unc-6* in various dorsal migrations including motor neuron axons (Fig. 2.1d), extending dorsal

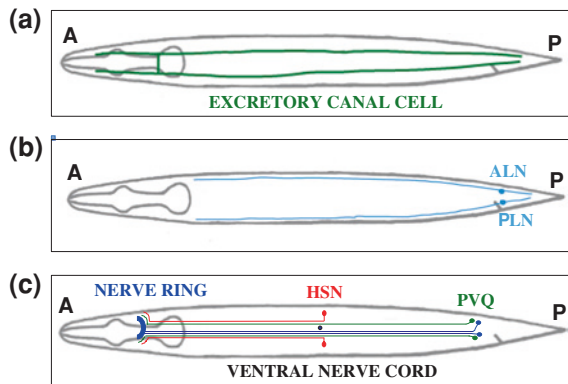


Fig. 2.3 Delineative of neuronal and non-neuronal cell migration and axon outgrowth during *C. elegans* development: **a** The excretory canal is a H-shaped cell, extending out two posterior and two anterior processes with its cell body located in the second pharynx bulb. **b** ALN and PLN neurons, with their cell bodies located in the tail, extend a long anterior process toward the head and a short posterior process to the tail of the worm. **c** The *C. elegans* ventral nerve cord (VNC) is bilaterally asymmetric with the four neurons in the right bundle and forty neurons in the left bundle. The axons in the right bundle cross over the ventral midline to the contralateral side (left bundle), whereas the axons in the left bundle are always ipsilateral and never cross the midline. All the cell bodies send axons to the nerve ring located between the first and second pharynx bulb

commissures from cell bodies located in the VNC (Fig. 2.1d), SDQR, excretory canal cell (EC) (Fig. 2.3a), and distal tip cells (DTCs) (Fig. 2.2b) (Hedgecock et al. 1990; Kim et al. 1999). The role of UNC-5 in dorsal guidance was further supported by ectopic expression of UNC-5 in touch neurons including AVM and PVM (Fig. 2.1a), where the ventral processes were dorsally rerouted (Hamelin et al. 1993), suggesting that UNC-5 is sufficient and necessary for dorsal GC guidance and migration. Analogous to vertebrates where function of UNC-40 is required in guidance mediated by UNC-5, genetic analysis of DTC migration in hypomorphic *unc-5* allele in an *unc-40* wild-type and null background led the authors to propose that UNC-5 and UNC-40 receptors mediate chemorepulsion either independently or together, and this could be due to the formation of various oligomeric receptor complexes (Merz et al. 2001).

2.1.5 UNC-129/TGF- β /BMP and UNC-5/UNC-5

Another gene implicated in migration along the DV axis of the worms is *unc-129*, encoding a TGF- β (transforming growth factor- β)/BMP (bone morphogenetic protein) superfamily molecule, a 407-aa protein (Colavita et al. 1998). In mouse, BMPs are expressed in the dorsal roof plate of developing spinal cord, opposite to Netrin-1, and repel commissural axons toward the ventral floor plate or Netrin-1 source and guide motor neuron away from ventral floor plate, requiring DCC/UNC-40 function (Augsburger et al. 1999; Dillon et al. 2007).

In *C. elegans*, *unc-129* was identified as a suppressor of dorsal migration of touch receptor neuron axons (Fig. 2.1a) induced by ectopic expression of UNC-5 in these cells, analogous to mouse, UNC-129 protein is expressed in dorsal rows of body wall muscles (Colavita and Culotti 1998; Colavita et al. 1998). UNC-129 affects GC migrations that are regulated by UNC-6/Netrin signaling, including migration of DTC (Fig. 2.2b) and motoneurons (Fig. 2.1d) in a TGF- β serine/threonine receptor kinase-independent but UNC-5-dependent manner (Colavita et al. 1998; Hedgecock et al. 1990; Qin and Powell-Coffman 2004). UNC-129 switches the UNC-5 signaling to UNC-5 + UNC-40 signaling so as to increase the sensitivity of GC to decreasing UNC-6/netrin gradient probably by binding to UNC-5 or mobilizing UNC-5 receptor, a mechanism similar to *Drosophila* UNC-5-mediated short- and long-range repulsion (Keleman and Dickson 2001).

2.1.6 UNC-6/Netrin and ENU-3

C. elegans ENU-3 (enhancer of Unc), a predicted transmembrane protein with a signal peptide and a coiled-coil region (Yee et al. 2011), was identified in a genetic enhancer screen in *unc-5* mutants, as an enhancer of dorsal axon outgrowth and guidance defects of motoneurons (Fig. 2.1d). The motoneuron cell bodies are located in the VNC and they send out a dorsal process toward the dorsal nerve cord (DNC), a process regulated by UNC-5 (Leung-Hagesteijn et al. 1992). *enu-3* mutant worms showed enhanced axon outgrowth defects of the DB4, DB5, and DB6 motor neuron axons in *unc-5* and *unc-6* loss-of-function strains (Yee et al. 2011). Mutations in *enu-3* also enhanced the axon migration defects of the motor neuron axons in a hypomorphic strain with defects in UNC-5-mediated migrations of GC, suggesting that ENU-3 and UNC-5 work together in motor axon guidance in an UNC-6-dependent manner (Yee et al. 2011). Besides, *enu-3* mutation enhanced the defects in ventral process guidance of AVM and PVM touch receptor neurons (Fig. 2.1a) in strains lacking *unc-40* and *slt-1* function. These observations suggest that ENU-3 and UNC-40 function in parallel pathways dependent on UNC-6 (Yee et al. 2014), where ENU-3 either functions as a receptor or modulates the function of an unidentified UNC-6 receptor.

Some other molecules implicated in dorsal–ventral guidance include MADD-4 (muscle arm development defective) (Seetharaman et al. 2011) and MADD-2 (Alexander et al. 2010), both acting in UNC-40 attractive pathway.

2.2 Cues and Receptors in Midline Guidance

In organisms with bilaterally symmetric nervous system including insects and vertebrates, a midline structure establishes a partition between the two mirror image halves. Axons that connect these two halves cross the midline forming axon commissures and are called commissural axons. Commissural axons cross the midline

once to leave it on the contralateral side, whereas the ipsilateral axons never cross the midline. Once commissural axons have crossed the midline, they continue to grow longitudinally along the midline never recrossing again.

Studies in Nematodes, flies, rodents, and chick embryos provided insight into the cues and receptors regulating the guidance of commissural and ipsilateral axon GCs, indicating that the midline secretes both attractive and repulsive cues including Netrin/UNC-6 and Slit/SLT-1, in mammals, zebrafish, *Drosophila*, and *C. elegans* (Serafini et al. 1994; Harris et al. 1996; Mitchell et al. 1996; Wadsworth et al. 1996; Batty et al. 1999; Kidd et al. 1999; Yuan et al. 1999; Hao et al. 2001; Yeo et al. 2001). In vertebrates and *Drosophila*, Netrin–DCC/FRA and Slit–Robo function in ventral midline guidance. The midline Netrin/UNC-6 source attracts the commissural axons expressing DCC/FRA, but the axon cannot cross the midline to reach contralateral side, unless the attraction is suppressed (Serafini et al. 1996; Kidd et al. 1999). Subsequent in vivo studies, in *netrin-1*-deficient mice, exhibiting defects in commissure development, confirmed the requirement of Netrin-1 for spinal, corpus callosum, anterior, and hippocampal commissure development (Serafini et al. 1996). A role for Slit/SLT-1 in midline guidance came from studies of *slit-1* and *slit-2* mouse mutants, where callosal axons were unable to project across the midline and were deficient in all three major forebrain tracts, namely the corticofugal, callosal, and thalamocortical tracts (Bagri et al. 2002). In *Drosophila slit* mutant embryos, both ipsilateral and commissural axons enter the midline and never leave it (Rothberg et al. 1990; Batty et al. 1999; Kidd et al. 1999). In vertebrates, Slit achieves its function by signaling through Robo, and the Slit-2–Robo complex repels callosal axons away from the midline (Shu et al. 2003). Similarly, in *Drosophila*, Slit/SLT-1 signals via Roundabout (Robo) to prevent recrossing of axons supported by phenotypic analysis of *robo* mutants, where axons including ipsilateral freely cross and recross the midline multiple times (Seeger et al. 1993; Kidd et al. 1998). Later, two additional receptors for Slit were identified in *Drosophila*, Robo-2 and Robo-3, where Robo-2 and Robo-3 determine the position of longitudinal axons from the midline and Robo/Robo1 and Robo-2 prevented the commissural axons to linger in the midline (Rajgopalan et al. 2000). Based on these studies, it was proposed that Netrin/UNC-6 and FRA/DCC/UNC-40 attract the commissural axons to midline whereas Slit/SLT-1 and Robo/SAX-3 repel them to contralateral side and prevent recrossing.

In mammals, Rig-1/Robo-3 receptor, expressed in commissural axons, acts in midline guidance decisions probably by regulating sensitivity to Slit. Rig-1 functions by repressing the repulsive activity of any low level of Robo-1 present in precrossing axons. These results were validated by high level of Rig-1 expression before midline crossing and its downregulation after crossing. After crossing, Rig-1 is downregulated and Robo-1 and Robo-2 are upregulated in commissural GC, allowing their repulsion by the midline (Sabatier et al. 2004).

In *C. elegans*, the ventral midline is flanked on right and left sides by asymmetric VNC, containing bundle of longitudinal axon tracts (Fig. 2.3c), composed of two asymmetric bundles, a right bundle of 40 neurons and the left bundle of 4 neurons; in wild-type worms, the axons from the left bundle cross and extend on the contralateral side, whereas, the axons of right bundle remain ipsilateral. Phenotypic analysis of PVQ neuron (Fig. 2.3c) in *sax-3* mutants suggested that SAX-3/Robo functions

to maintain the asymmetry of the VNC. In wild-type worms, the cell bodies of the two PVQ neurons, PVQR and PVQL, located in the tail of the worm and their axons travel anteriorly the length of the VNC on the right and left sides, respectively, whereas *sax-3* mutants exhibited repeated midline crossing by PVQ neuron observed in worms with *sra-6::GFP* transgene (Troemel et al. 1995), and the defects resembled the midline crossing defects in *Drosophila robo* mutants (Seeger et al. 1993; Zallen et al. 1998). Similar, crossing defects were also observed for HSN neuron (Fig. 2.3c). HSN neuron sends out a ventral process in response to UNC-6/Netrin acting through its receptor UNC-40/DCC/FRA to the VNC, followed by the extension of the process anteriorly without crossing the midline. In *sax-3* mutants, the longitudinal HSN process, cross and recross the ventral midline many times, suggesting a role for SAX-3 in HSN longitudinal process guidance (Zallen et al. 1998).

2.2.1 *Wnt/Wnt and Ryk/Lin-18*

Midline crossing in *Drosophila* deploys Wnt signaling in addition to the Netrin and Slit signaling pathways (Callahan et al. 1995; Yoshikawa et al. 2003). Wnts are secreted glycoproteins also called Wingless, the segment polarity gene of *Drosophila*, involved in the formation of body axis during embryonic development (Nusse and Varmus 1992). Wnts signal via Frizzled (Fz) receptors by binding to the CRDs (cysteine-rich domain) of Frizzled (Bhanot et al. 1996; Dann et al. 2001; Lin et al. 1997) and regulate key developmental processes including embryonic patterning, cell fate specification, and polarity determination (Ciani and Salinas 2005), mediating their responses through three signaling pathways including Wnt/ β -catenin (canonical pathway), Wnt/planar cell polarity (non-canonical pathway), and Wnt/calcium pathway (Endo and Rubin 2007). Additionally, Wnts function during development of mammalian and fly CNS (Lyuksytova et al. 2003; Liu et al. 2005). There are fourteen body segments in the *Drosophila*, each segment has an anterior and a posterior commissure, and axons cross the ventral midline once and choose one of the commissures (Callahan et al. 1995). The choice of commissure is determined by the Drl (derailed)/Ryk (receptor related to tyrosine kinase), an atypical receptor tyrosine kinase, expressed in the axons of the anterior commissure responding to Wnt5 protein, expressed mainly by axons in or close to the posterior commissure (Yoshikawa et al. 2003). Genetic analysis supported Wnt5 as a repulsive cue signaling via axons expressing Drl/Ryk, ensuring GC migration through the anterior commissure. In the absence of the Drl/Ryk receptor, axons were insensitive to Wnt5, resulting in improper commissure bundle formation, inappropriate crossing of the follower axons in the anterior commissure, and projecting inappropriately across the midline in between the commissures (Fradkin et al. 2004). Rescue experiments, expressing *WNT5* under a panneural promoter, confirmed the requirement of Wnt5 protein in commissure formation (Fradkin et al. 2004). Similarly, in mammals Wnt5a acts a repulsive ligand for Ryk receptor driving callosal axons towards the contralateral hemisphere after crossing the midline (Keeble et al. 2006).

Semaphorin protein, expressed at the midline and/or ventral tissues, have been shown to function as repulsive cue in midline guidance, genetic analysis of mice lacking neuropilin-2, a semaphorin receptor, exhibited midline guidance defects, confirming their role in this process (Zou et al. 1999). In worms, the VAB-1/Eph receptor also functions to prevent aberrant axon crossing at the ventral midline (Zallen et al. 1999).

2.3 Guidance Cues and Receptors Implicated in the Migration Along Anterior–Posterior Axis

Identification of molecules involved in axon guidance and cell migration along the AP or longitudinal axis has remained enigmatic, probably owing to the redundancy in DV and AP guidance and outgrowth signaling pathways. Section below discusses guidance molecules regulating GC migration along the AP axis of vertebrates and invertebrates.

2.3.1 *Wnt and Fz*

A number of studies have shown that Wnt proteins regulate AP guidance by regulating the polarity of migrating cells and neurons (Montcouquiol et al. 2006; Endo and Rubin 2007). Mammalian CNS is composed of sensory pathways that form ascending fibers relaying sensory stimuli to higher brain centers and descending fibers sending motor command and regulatory signals down from the brain. In the spinal cord, somatosensory fibers project anteriorly and motor pathways project posteriorly, making spinal cord an excellent model system for studying axon guidance along the AP axis. Mouse genome encodes for nineteen Wnt proteins and twelve Frizzled receptors (Miller 2002). Several of these genes are expressed in the mouse spinal cord gray matter, cupping the dorsal funiculus, in an anterior-to-posterior decreasing gradient along the cervical and thoracic cord. Biochemical studies found that descending neurons of the CST are repelled by Wnts and anti-Ryk antibodies blocked the posterior growth of CST axons, supporting the role of Wnts and Ryk receptor in posterior guidance of CST axons (Imondi and Thomas 2003; Liu et al. 2005). Similarly, commissural neurons in the mammalian dorsal spinal cord send axons ventrally toward the floor plate, where they cross the midline and turn anteriorly toward the brain. Several Wnt proteins stimulate the anterior extension of commissural axons after midline crossing. *WNT4* mRNA, expressed in a decreasing anterior-to-posterior gradient in the floor plate, attracts postcrossing commissural axons. Commissural axons in mice lacking the Wnt receptor Fz3 (Frizzled3) displayed AP guidance defects after midline crossing implicating Wnt4 and Fz3 in anterior guidance of commissural axons (Lyukshutova et al. 2003).

In *C. elegans*, Wnts are known to regulate cell fate, cell polarity, including axon outgrowth, and cell migration (Maloof et al. 1999; Whangbo and Kenyon 1999; Pan et al. 2006; Silkankova and Korswagen 2007; Zinovyeva et al. 2008). The worm genome encodes five Wnt proteins (EGL-20, LIN-44, CWN-1, CWN-2, and MOM-2) and four Frizzled receptors (MOM-5, CFZ-2, MIG-1, and LIN-17), along with the Ryk homologue LIN-18 (Pan et al. 2006). Wnts and their Fz receptors function in both cell and axon GC migrations in worms.

Q neuroblasts are born as bilaterally symmetric cells on the left (QL) and right (QR) sides in the posterior body region, each dividing asymmetrically and generating three neurons and two apoptotic cells. In the first larval stage (L1), QR and its descendants (AQR, SDQR, and AVM) migrate anteriorly and QL and its descendants (PQR, SDQL, and PVM) migrate posteriorly (Fig. 2.2a). Maloof et al. (1999) identified, EGL-20/Wnt, regulating the migrations of the Q neuroblast and its descendants by controlling the expression of Hox transcription factor MAB-5 (Male-ABnormal) in QL neuroblast through the canonical Wnt signaling pathway, migrating posteriorly into the tail towards the source of EGL-20/Wnt. Studies conducted by Whangbo and Kenyon (1999), implicated LIN-17/Fz as the receptor since due to higher concentrations of EGL-20/Wnt, the QL neuron expressed the Wnt pathway proteins such as LIN-17/Fz, BAR-1/ β -catenin, and MIG-5/Dishevelled, resulting in posterior migration while relatively lower EGL-20/Wnt concentrations and absence of the Wnt pathway proteins resulted in QR migration in the anterior direction. Later, genetic analysis showed that EGL-20 functions in QL migration by signaling through MIG-1/Fz and LIN-17/Fz proteins (Forrester et al. 2004; Harris et al. 1996; Maloof et al. 1999).

Wnts were also implicated in several other cell migrations along the AP axis of the worm including CAN and ALM neurons (Fig. 2.2c), which undergo posterior migration near the middle of the worm body and HSNs and BDUs (Fig. 2.2c), migrating anteriorly during development. Loss-of-function mutations in Wnts, including *egl-20*, *cwn-1*, and *cwn-2*, resulted in migration defects in several neurons including ALM, BDU, CAN, HSN, and QR evidenced by phenotypic analysis of *cwn-1 cwn-2* double mutants; moreover, studies supported CWN-2 signaling via CFZ-2/Fz (Zinovyeva and Forrester 2005).

EGL-20, CWN-1, and LIN-44, expressed by a group of epidermal and muscle cells located in the tail of the worm together with a fourth Wnt, MOM-2, function redundantly to repel HSN neurons and promote their anterior migrations from the tail region to midbody (Fig. 2.2c), where EGL-20 carried out its repulsive function via Frizzled, MIG-1 and MOM-5 (Pan et al. 2006). Besides, Wnt signaling has been associated with GC guidance of the anterior processes of AVM and PVM neurons (Fig. 2.1a), phenotypic analysis of various single and double mutants supported the requirement of CWN-1 and EGL-20 Wnts and MIG-1 and MOM-5 Frizzled in the GC migrations (Pan et al. 2006).

Wnts and Frizzled have been also associated with polarity establishment and process outgrowth in ALM and PLM neurons (Fig. 2.1a) along the AP axis of the worm. PLM, which normally sends an anterior process in wild-type worm, extended a posterior process in *lin-44* and *lin-17* loss-of-function mutants. Two Wnts, including CWN-1 and EGL-20 were associated with ALM polarity reversal (Hilliard and Bargmann 2006).

2.3.2 *EGL-17/FGF and EGL-15/FGFR*

The fibroblast growth factor (FGF) and its receptor FGFR, receptor tyrosine kinase pathway, have been implicated in numerous cell migrations during development, in both vertebrates and invertebrates (Chen and Stern 1998; Metzger and Krasnow 1999; Hogan 1999). In the *Drosophila* trachea and the vertebrate lung, FGF directs branching and morphogenesis, a process that includes both cell migrations and cell shape changes (Chu et al. 2013; Bae et al. 2012). In *Drosophila*, *breathless* (*btl*), homologue of FGF, signals via *branchless* (*bln*), homologue of FGFR, for normal migration of tracheal cells (Glazer and Shilo 1991; Samakovlis et al. 1996). In vertebrates, FGF and FGFR signaling has been implicated in neuronal migration, neurite outgrowth, morphogenesis, and cell fate specification (Hu et al. 2013; Neiiendam et al. 2004; Hossain and Morest 2000). In *C. elegans*, FGF–FGFR signaling pathway has been implicated in migrations of SMs and canal-associated neurons (CANs) along the AP axis. SM cells are born in the posterior of the animal during the first larval stage, and in hermaphrodite, they migrate anteriorly to the central gonad and developing vulva, generating uterine and vulval musculature (Fig. 2.2d). Proper positioning of SM requires an FGF-like ligand encoded by the *egl-17* gene, expressed in somatic gonadal cells, acting as attractive cue, and a receptor belonging to the FGF receptor subfamily, encoded by *egl-15*, expressed in SMs (Burdine et al. 1997, 1998; DeVore et al. 1995), constituting the GDA mechanism of SM migration. Mutations in either *egl-17* or *egl-15* result in SM migration defects. Recently, *egl-17* and *egl-15* signaling pathway has been implicated in CAN neuron (Fig. 2.2c) migration, where EGL-17 acts as a repulsive guidance cue evidenced by repelling CAN neurons from sources of ectopic expression of EGL-17, possibly mediating its effect through adaptor protein, SEM-5/GRB-2 (Fleming et al. 2005).

2.3.3 *SLT-1/Slit and SAX-3/Robo*

SLT-1 functions in multiple guidance pathways including midline, DV, and AP guidance decision. Along the longitudinal axis, it functions in guidance of CAN and ALM neurons (Fig. 2.2c). In embryos, SLT-1 is expressed at high levels in the anterior epidermis, providing guidance for posterior CAN migration to midbody region. CAN migration defects were observed in SLT-1 loss-of-function worms with *ceh-23::GFP* transgene (Zallen et al. 1999; Hao et al. 2001; Yu et al. 2002). SLT-1 functions through its receptor SAX-3/Robo in CAN migration evidenced by CAN migration defects in *slt-1* mutant worms with *myo-3::SLT-1* transgene, where SLT-1 is expressed in muscles. Mutations in *slt-1* and *sax-3* also affected the posterior migrations of ALM neurons (Fig. 2.2c) supported by phenotypic analysis of mutant worms. Longitudinal migration of GCs in VNC (Fig. 2.3c), are also affected by mutation in *sax-3*, phenotypic analysis of VNC structure in *sax-3* mutants visualized with *glr-1::GFP* transgene (Maricq et al. 1995), revealed premature axon termination (Zallen et al. 1998), supporting SAX-3 requirement in VNC axon elongation.

2.3.4 *MIG-13*

mig-13 gene encodes a transmembrane protein, composed of LDL (low-density lipoprotein) receptor repeats and CUB (for complement C1r/C1s, Uegf, Bmp1) domain, affecting the anterior migration of QR and its descendants (QR.x) (Fig. 2.2a), acting cell autonomously. MIG-13 is expressed in the anterior and central regions of the worm under the act of Hox gene. Loss of MIG-13 activity shifts the QR.x (QR and its descendent) posteriorly, whereas increasing the level of MIG-13 shifts them anteriorly in a dose-dependent manner (Sym et al. 1999; Wang et al. 2013). Moreover, recently, it has been shown that LIN-39 establishes QR anterior polarity by binding to the *mig-13* promoter and enhancing its expression, whereas expression of *lin-39* is inhibited in QL.x (QL and its descendent), thereby providing a link between *mig-13* expression and anterior migration of QR.x. MAB-5, a Wnt pathway protein, implicated in posterior migration of QL, inhibits QL anterior polarity by associating with the *lin-39* promoter and down-regulating *lin-39* and thereby *mig-13* expression (Wang et al. 2013).

2.3.5 *UNC-53/NAV*s

unc-53 gene, which regulates the AP directional migrations (Hedgecock et al. 1987), encodes an actin-binding scaffolding protein with calponin homology actin-binding domain, AAA domain, coiled-coil regions, and two polyproline-rich region (Stringham et al. 2002). Hypomorphic alleles of *unc-53* display guidance defects in the anterior migration of the SM (Chen et al. 1997) and the process outgrowth of mechanosensory neurons (Fig. 2.1a), ALN and PLN (Fig. 2.2b) neurons, motor neurons (Fig. 2.1d), and the excretory canals (Fig. 2.3a) (Hekimi and Kershaw 1993; Stringham et al. 2002). Three mammalian UNC-53 homologues, NAV-1/UNC53H1, NAV-2/UNC53H2 and NAV-3/UNC53H3 (neuron navigator-1, 2, 3) coined navigators for their role in axon guidance, have been identified (Merrill et al. 2002; Maes et al. 2002), shown to be involved in neurite induction, supporting the functional conservation of UNC-53/NAV in both vertebrates and invertebrates.

2.3.6 *VAB-8*

VAB-8 (variable abnormal morphology), a kinesin-like protein, functioning primarily in posterior GC migration, was identified in a screen for mutants affecting CAN neuron migration based on the “withered tail” phenotype, which is associated with abnormal CAN migration (Manser and Wood 1990). VAB-8 affects GC migration both during embryogenesis and during larval stages, and mutations in *vab-8* disrupt

Table 2.1 Guidance molecules involved in growth cone migration and axon outgrowth

Cue/Receptor complex	Organism	References
<i>Dorsal–ventral (DV) guidance molecules</i>		
UNC-6/UNC-40	Nematode	Chan et al. (1996), Wadsworth et al. (1996)
Netrin/Neogenin/DCC	Vertebrates	Serafini et al. (1994), Keino-Masu et al. (1996)
SLT-1/SAX-3	Nematode	Brose et al. (1999), Hao et al. (2001)
SLT-1/EVA-1	Nematode	Fujisawa et al. (2007)
UNC-6/UNC-5	Nematode	Leung-Hagesteijn et al. (1992), Wadsworth et al. (1996)
UNC-6/UNC-5/Rcm	Vertebrate	Ackerman et al. (1997), Ackerman and Knowles (1998), Finger et al. (2002)
UNC-129/UNC-5	Nematode	Colavita et al. (1998)
TGF- β /TGFR- β	Vertebrates	Augsburger et al. (1999), Dillon et al. (2007)
UNC-6/ENU-3	Nematode	Yee et al. (2011)
<i>Midline guidance molecules</i>		
SLIT-1/Robo	Vertebrates	Rothberg et al. (1990), Seeger et al. (1993), Kidd et al. (1998)
SLT-1/SAX-3	Nematodes	Zallen et al. (1998)
Wnt5/Drl/Ryk	Fly	Callahan et al. (1995), Yoshikawa et al. (2003), Fradkin et al. (2004)
<i>Anterior–posterior (AP)/Longitudinal guidance molecules</i>		
Wnt/Ryk	Vertebrates	Imondi and Thomas (2003), Liu et al. (2005), and Dickson and Gilestro (2006)
Wnt/Fz3	Vertebrates	Lyuksyutova et al. (2003)
Wnts/Fz	Nematode	Maloof et al. (1999), Zinovyeva and Forrester (2005), Pan et al. (2006), Hilliard and Bargmann (2006)
FGF/FGFR	Vertebrates	Hu et al. (2013), Neiiendam et al. (2004), Hossain and Morest (2000)
EGL-17/EGL-15	Nematodes	Burdine et al. (1997, 1998), Fleming et al. (2005)
SLT-1/SAX-3	Nematodes	Zallen et al. (1998), Zallen et al. (1999), Hao et al. (2001), Yu et al. (2002)
UNC-53	Nematodes	Hekimi and Kershaw (1993), Stringham et al. (2002)
VAB-8	Nematode	Wightman et al. (1996)
MIG-13	Nematodes	Sym et al. (1999), Wang et al. (2013)

fourteen of the seventeen posteriorly directed migrations (Wightman et al. 1996), a role further validated by expression of VAB-8 in the mechanosensory neuron (Fig. 2.1a) under the control of *mec-4* promoter, causing rerouting of ALM anterior process toward the tail of the worm. Besides, *vab-8* regulates the posterior migration of ALM during embryogenesis (Wolf et al. 1998). Genetic analysis implicated VAB-8 and UNC-73B function in EC process outgrowth (Fig. 2.2b), working in a parallel pathway to UNC-53 and UNC-73E (Marcus-Gueret et al. 2012).

Table 2.1 provides a list of Guidance molecules involved in GC steering during embryogenesis discussed in the above sections, and a schematic is depicted in Fig. 2.4.

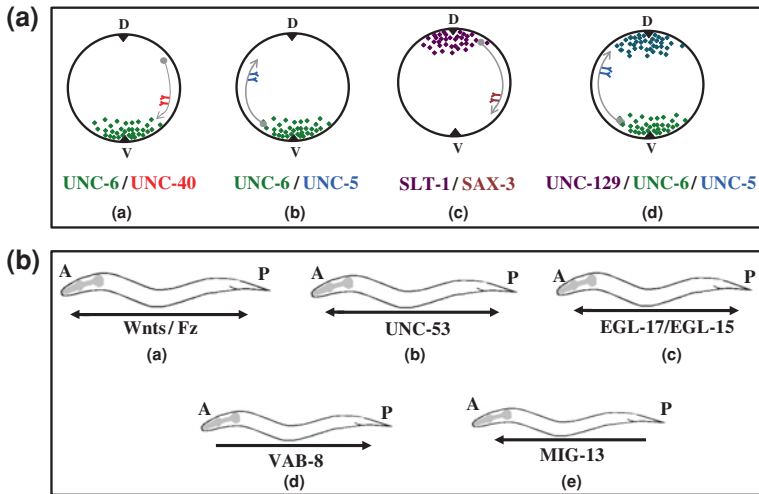


Fig. 2.4 Graphic of guidance molecules in dorsal–ventral and anterior–posterior growth cone migrations and guidance in *C. elegans*: **a** Guidance molecules involved in dorsal–ventral (DV) migration in worms. Cross section of the worm depicting dorsal (D) and ventral (V) sides. *a* UNC-6/UNC-40, where UNC-6 is concentrated ventrally and attracts GCs expressing UNC-40 receptor. *b* UNC-6/UNC-5, ventral source of UNC-6 repels GCs expressing UNC-5 dorsally. *c* SLT-1/SAX-3, SLT-1 is concentrated dorsally and repels growth cones expressing SAX-3 receptor ventrally. *d* UNC-129/UNC-5, attract growth cones expressing UNC-5 receptor repelled by ventrally located UNC-6. **b** Lateral view of the worm depicting anterior (A) and posterior (P) growth cone migrations. Guidance molecules involved in AP guidance include the following: *a* Wnts/Fz, Wnts acting through the Frizzled (Fz) receptors guiding growth cones along the AP axis. *b* EGL-15/EGL-17, EGL-15 a secreted cue binds EGL-17 receptor, regulating GC migrations along the longitudinal axis. *c* UNC-53, a cytoplasmic scaffolding protein steering GCs in both anterior and posterior directions. *d* MIG-13, a transmembrane protein regulating anterior GC migrations. *e* VAB-8, a kinesin-like protein, regulating posterior GC migrations

Some of the molecules involved in axon guidance that is outside the scope of discussion of this book include semaphorins, a family of secreted proteins signaling through plexins (Kruger et al. 2005) and ephrins acting through their Eph receptors (Flanagan 2006; Quinn and Wadsworth 2006; Mohamed and Chin-Sang 2006).

References

- Ackerman SL, Knowles BB (1998) Cloning and mapping of the UNC5C gene to human chromosome 4q21–q23. *Genomics* 52(2):205–208
- Ackerman SL, Kozak LP, Przyborski SA, Rund LA, Boyer BB, Knowles BB (1997) The mouse rostral cerebellar malformation gene encodes an UNC-5-like protein. *Nature* 386(6627):838–842
- Adler CE, Fetter RD, Bargmann CI (2006) UNC-6/Netrin induces neuronal asymmetry and defines the site of axon formation. *Nat. Neurosci.* 9:511–518

- Alexander F (2000) Neuroblastoma. *Urol Clin North Am* 3:383–392 vii
- Alexander M, Selman G, Seetharaman A, Chan KK, D'Souza SA, Byrne AB, Roy PJ (2010) MADD-2, a homolog of the Opitz syndrome protein MID1, regulates guidance to the midline through UNC-40 in *Caenorhabditis elegans*. *Dev. Cell.* 18(6):961–972
- Augsburger A, Schuchardt A, Hoskins S, Dodd J, Butler S (1999) BMPs as mediators of roof plate repulsion of commissural neurons. *Neuron* 24:127–141
- Bae YK, Trisnadi N, Kadam S, Stathopoulos A (2012) The role of FGF signaling in guiding coordinate movement of cell groups: guidance cue and cell adhesion regulator? *Cell Adh Migr* 6(5):397–403
- Bagri A, Marin O, Plump AS, Mak J, Pleasure SJ, Rubenstein JL, Tessier-Lavigne M (2002) Slit proteins prevent midline crossing and determine the dorsoventral position of major axonal pathways in the mammalian forebrain. *Neuron* 33:233–248
- Barallobre MJ, Del Rio JA, Alcantara S, Borrell V, Aguado F, Ruiz M, Carmona MA, Martin M, Fabre M, Yuste R, Tessier-Lavigne M, Soriano E (2000) Aberrant development of hippocampal circuits and altered neural activity in Netrin 1-deficient mice. *Development* 127:4797–4810
- Battye R, Stevens A, Jacobs JR (1999) Axon repulsion from the midline of the *Drosophila* CNS requires Slit function. *Development* 126:2475–2481
- Bhanot P, Brink M, Samos CH, Hsieh JC, Wang Y, Macke JP, Andrew D, Nathans J, Nusse R (1996) A new member of the frizzled family from *Drosophila* functions as a Wingless receptor. *Nature* 382:225–230
- Braisted JE, Catalanano SM, Stimac R, Kennedy TE, Tessier-Lavigne M, Shatz CJ, O'Leary DD (2000) Netrin-1 promotes thalamic axon growth and is required for proper development of the thalamocortical projection. *J. Neurosci.* 20:5792–5801
- Branda CS, Stern MJ (2000) Mechanisms controlling sex myoblast migration in *Caenorhabditis elegans* hermaphrodites. *Dev Biol* 226(1):137–151
- Brose K, Bland KS, Wang KH, Arnott D, Henzel W, Goodman CS, Tessier-Lavigne M, Kidd T (1999) Slit proteins bind Robo receptors and have an evolutionarily conserved role in repulsive axon guidance. *Cell* 96(6):795–806
- Burdine RD, Branda CS, Stern MJ (1998) EGL-17(FGF) expression coordinates the attraction of the migrating sex myoblasts with vulval induction in *C. elegans*. *Development* 125:1083–1093
- Burdine RD, Chen EB, Kwok SF, Stern MJ (1997) *egl-17* encodes an invertebrate fibroblast growth factor family member required specifically for sex myoblast migration in *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* 94:2433–2437
- Callahan CA, Muralidhar MG, Lundgren SE, Scully AL, Thomas JB (1995) Control of neuronal pathway selection by a *Drosophila*-tyrosine kinase family member. *Nature* 376:171–174
- Ciani L, Salinas PC (2005) Wnts in the vertebrate nervous system: from patterning to neuronal connectivity. *Nat. Rev. Neurosci.* 6:351–362
- Chalfie M, Sulston JE, White JG, Southgate E, Thomson JN, Brenner S (1985) The neural circuit for touch sensitivity in *Caenorhabditis elegans*. *J Neurosci.* 5(4):956–964
- Chalfie M, Sulston J (1981) Developmental genetics of the mechanosensory neurons of *Caenorhabditis elegans*. *Dev Biol.* 82(2):358–370
- Chalfie M, Tu Y, Euskirchen G, Ward WW, Prasher DC (1994) Green fluorescent protein as a marker for gene expression. *Science* 263:802–805
- Chan SS, Zheng H, Su MW, Wild R, Killeen MT, Hedgecock EM, Culotti JG (1996) UNC-40, a *C. elegans* homolog of DCC (deleted in colorectal cancer), is required in motile cells responding to UNC-6 Netrin cues. *Cell* 87:187–195
- Chen EB, Branda CS, Stern MJ (1997) Genetic enhancers of *sem-5* define components of the gonad-independent guidance mechanism controlling sex myoblast migration in *Caenorhabditis elegans* hermaphrodites. *Dev Biol* 182(1):88–100
- Chen EB, Stern MJ (1998) Understanding cell migration guidance: lessons from sex myoblast migration in *C. elegans*. *Trends Genetics* 14:322–327
- Chu WC, Lee YM, Henry Sun Y (2013) FGF/FGFR signal induces trachea extension in the drosophila visual system. *PLoS ONE* 8(8):e73878

- Colamarino SA, Tessier-Lavigne M (1995) The axonal chemoattractant Netrin-1 is also a chemorepellent for trochlear motor axons. *Cell* 81:621–629
- Colavita A, Culotti JG (1998) Suppressors of ectopic UNC-5 growth cone steering identify eight genes involved in axon guidance in *Caenorhabditis elegans*. *Dev Biol* 194:72–85
- Colavita A, Krishna S, Zheng H, Padgett RW, Culotti JG (1998) Pioneer axon guidance by UNC-129, a *C. elegans* TGF- β . *Science* 28:706–709
- Dann CE, Hsieh JC, Rattner A, Sharma D, Nathans J, Leahy DJ (2001) Insights into Wnt binding and signaling from the structures of two frizzled cysteine-rich domains. *Nature* 412:86–90
- DeVore DL, Horvitz HR, Stern MJ (1995) An FGF receptor signaling pathway is required for the normal cell migrations of the sex myoblasts in *C. elegans* hermaphrodites. *Cell* 83:611–620
- Dickson BJ, Gilestro GF (2006) Regulation of commissural axon pathfinding by Slit and its Robo receptors. *Annu. Rev. Cell Dev. Biol.* 22:651–675
- Dillon AK, Jevince AR, Hinck L, Ackerman SL, Lu X, Tessier-Lavigne M, Kaprielian Z (2007) UNC5C is required for spinal accessory motor neuron development. *Mol Cell Neurosci* 35:482–489
- Endo Y, Rubin JS (2007) Wnt signaling and neurite outgrowth: insights and questions. *Cancer Science* 98:1311–1317
- Fearon ER, Cho KR, Nigro JM, Kern SE, Simons JW, Ruppert JM, Mamliton SR, Preisinger AC, Thomas G, Kinzler KW, Vogelstein B (1990) Identification of a chromosome 18q gene that is altered in colorectal cancers. *Science* 247:49–56
- Finger JH, Bronson RT, Harris B, Johnson K, Przyborski SA, Ackerman SL (2002) The Netrin 1 Receptors *Unc5h3* and *Dcc* are necessary at multiple choice points for the guidance of corticospinal tract axons. *J Neurosci* 22(23):10346–10356
- Flanagan JG (2006) Neural map specification by gradients. *Curr Opin Neurobiol* 16:59–66
- Fleming TC, Wolf FW, Garriga G (2005) Sensitized genetic backgrounds reveal a role for *C. elegans* FGF EGL-17 as a repellent for migrating CAN neurons. *Development* 132:4857–4867
- Forrester WC, Perens E, Zallen JA, Garriga G (1998) Identification of *Caenorhabditis elegans* genes required for neuronal differentiation and migration. *Genetics* 148(1):151–165
- Fradkin LG, van Schie M, Wouda RR, de Jong A, Kamphorst JT, Radjkoemar-Bansraj M, Noordermeer JN (2004) The *Drosophila* Wnt5 protein mediates selective axon fasciculation in the embryonic central nervous system. *Dev Biol* 272:362–375
- Fujisawa K, Wrana JL, Culotti JG (2007) The slit receptor EVA-1 coactivates a SAX-3/robo mediated guidance signal in *C. elegans*. *Science* 317:1934–1938, Erratum in: *Science* 318:570
- Guijarro P, Simó S, Pascual M, Abasolo I, Del Río JA, Soriano E (2006) Netrin1 exerts a chemorepulsive effect on migrating cerebellar interneurons in a Dcc-independent way. *Mol Cell Neurosci* 33(4):389–400
- Glazer L, Shilo BZ (1991) The *Drosophila* FGF-R homolog is expressed in the embryonic tracheal system and appears to be required for directed tracheal cell extension. *Genes Dev* 5:697–705
- Hamelin M, Zhou Y, Su MW, Scott IM, Culotti JG (1993) Expression of the UNC-5 guidance receptor in the touch neurons of *C. elegans* steers their axons dorsally. *Nature* 364(6435):327–330
- Hao JC, Yu TW, Fujisawa K, Culotti JG, Gengyo-Ando K, Mitani S, Moulder G, Barstead R, Tessier-Lavigne M, Bargmann CI (2001) *C. elegans* Slit acts in midline, dorsal–ventral, and anterior–posterior guidance via the SAX-3/Robo receptor. *Neuron* 32:25–38
- Harris J, Honigberg L, Robinson N, Kenyon C (1996) Neuronal cell migration in *C. elegans*: regulation of Hox gene expression and cell position. *Development* 122:3117–3131
- Hedgecock EM, Culotti JG, Hall DH (1990) The *unc-5*, *unc-6*, and *unc-40* genes guide circumferential migrations of pioneer axons and mesodermal cells on the epidermis in *C. elegans*. *Neuron* 4:61–85
- Hedgecock EM, Culotti JG, Hall DH, Stern BD (1987) Genetics of cell and axon migrations in *Caenorhabditis elegans*. *Development* 100:365–382

- Hekimi S, Kershaw D (1993) Axonal Guidance defects in a *Caenorhabditis elegans* mutant reveal cell-extrinsic determinants of neuronal morphology. *The Journal of Neuroscience* 13(10):4254–4271
- Hilliard MA, Bargmann CI (2006) Wnt signals and frizzled activity orient anterior–posterior axon outgrowth in *C. elegans*. *Dev. Cell* 10:379–390
- Hogan BLM (1999) Morphogenesis. *Cell* 96:225–233
- Hong K, Hinck L, Nishiyama M, Poo MM, Tessier-Lavigne M, Stein E (1999) A ligand-gated association between cytoplasmic domains of UNC5 and DCC family receptors converts Netrin-induced growth cone attraction to repulsion. *Cell* 97:927–941
- Hossain WA, Morest DK (2000) Fibroblast growth factors (FGF-1, FGF-2) promote migration and neurite growth of mouse cochlear ganglion cells in vitro: immunohistochemistry and antibody perturbation. *J Neurosci Res* 62(1):40–55
- Hu Y, Poopalasundaram S, Graham A, Bouloux PM (2013) GnRH neuronal migration and olfactory bulb neurite outgrowth are dependent on FGF receptor 1 signaling, specifically via the PI3K p110 α isoform in chick embryo. *Endocrinology* 154(1):388–399
- Imondi R, Thomas JB (2003) Neuroscience. The ups and downs of Wnt signaling. *Science* 302:1903–1904
- Itoh A, Miyabayashi T, Ohno M, Sakano S (1998) Cloning and expressions of three mammalian homologues of *Drosophila* slit suggest possible roles for slit in the formation and maintenance of the nervous system. *Brain Res. Mol. Brain Res.* 62:175–186
- Keeble TR, Halford MM, Seaman C, Kee N, Macheda M, Anderson RB, Stacker SA, Cooper HM (2006) The Wnt receptor Ryk is required for Wnt5a-mediated axon guidance on the contralateral side of the corpus callosum. *J Neuroscience* 26:5840–5848
- Keino-Masu K, Masu M, Hinck L, Leonardo ED, Chan SS, Culotti JG, Tessier-Lavigne M (1996) Deleted in colorectal cancer (DCC) encodes a Netrin receptor. *Cell* 87:175–185
- Keleman K, Dickson BJ (2001) Short- and long- range repulsion by the *Drosophila* Unc5 Netrin receptor. *Neuron* 32:605–617
- Keleman K, Rajagopalan S, Cleppien D, Teis D, Paiha K, Huber LA, Technau, GM, Dickson BJ (2002) Comm sorts robo to control axon guidance at the *Drosophila* midline. *Cell* 110:415–427
- Keleman K, Ribeiro C, Dickson BJ (2005) Comm function in commissural axon guidance: cell-autonomous sorting of Robo in vivo. *Nat. Neurosci.* 8:156–163
- Kennedy TE, Serafini T, de la Torre JR, Tessier-Lavigne M (1994) Netrins are diffusible chemotropic factors for commissural axons in the embryonic spinal cord. *Cell* 78:425–435
- Kidd T, Bland KS, Goodman CS (1999) Slit is the midline repellent for the robo receptor in *Drosophila*. *Cell* 96(6):785–794
- Kidd T, Brose K, Mitchell KJ, Fetter RD, Tessier-Lavigne M, Goodman CS, Tear G (1998a) Roundabout controls axon crossing of the CNS midline and defines a novel subfamily of evolutionarily conserved guidance receptors. *Cell* 92(2):205–215
- Kidd T, Russell C, Goodman CS, Tear G (1998b) Dosage-sensitive and complementary functions of roundabout and commissureless control axon crossing of the CNS midline. *Neuron* 20:25–33
- Kim S, Ren X-C, Fox E, Wadsworth WG (1999) SDQR migrations in *Caenorhabditis elegans* are controlled by multiple guidance cues and changing responses to netrin UNC-6. *Development* 126:3881–3890
- Kolodziej PA, Timpe LC, Mitchell KJ, Fried SR, Goodman CS, Jan LY, Jan YN (1996) Frazzled encodes a *Drosophila* member of the DCC immunoglobulin subfamily and is required for CNS and motor axon guidance. *Cell* 87:197–204
- Kruger RP, Aurandt J, Guan KL (2005) Semaphorins command cells to move. *Nat Rev Mol Cell Biol* 6:789–800
- Leonardo ED, Hinck L, Masu M, Keino-Masu K, Ackerman SL, Tessier-Lavigne M (1997) Vertebrate homologues of *C. elegans* UNC-5 are candidate netrin receptors. *Nature* 386(6627):833–838

- Leung-Hagesteijn C, Spence AM, Stern BD, Zhou Y, Su MW, Hedgecock EM, Culotti JG (1992) UNC-5, a transmembrane protein with immunoglobulin and thrombospondin type 1 domains, guides cell and pioneer axon migrations in *C. elegans*. *Cell* 71(2):289–299
- Lin L, Rao Y, Isacson O (2005) Netrin-1 and slit-2 regulate and direct neurite growth of ventral midbrain dopaminergic neurons. *Mol. Cell Neurosci.* 28:547–555
- Lin K, Wang S, Julius MA, Kitajewski J, Moos M, Luyten FP (1997) The cysteine-rich frizzled domain of Frzb-1 is required and sufficient for modulation of Wnt signaling. *Proc. Natl. Acad. Sci. U. S. A.* 94:11196–11200
- Liu Y, Shi J, Lu CC, Wang ZB, Lyuksyutova AI, Song XJ, Zou Y (2005) Ryk-mediated Wnt repulsion regulates posterior-directed growth of corticospinal tract. *Nat. Neurosci.* 8:1151–1159
- Lyuksyutova AI, Lu CC, Milanese N, King LA, Guo N, Wang Y, Nathans J, Tessier-Lavigne M, Zou Y (2003) Anterior–posterior guidance of commissural axons by Wnt-frizzled signaling. *Science* 302:1984–1988
- Maduro M, Pilgrim D (1995) Identification and cloning of *unc-119*, a gene expressed in the *Caenorhabditis elegans* nervous system. *Genetics* 141:977–988
- Maes T, Barcelo A, Buesa C (2002) Neuron navigator: a human gene family with homology to *unc-53*, a cell guidance gene from *Caenorhabditis elegans*. *Genomics* 80:21–30
- Maloof JN, Whangbo J, Harris JM, Jongeward GD, Kenyon C (1999) A Wnt signaling pathway controls *hox* gene expression and neuroblast migration in *C. elegans*. *Development* 126:37–49
- Manser J, Wood WB (1990) Mutations affecting embryonic cell migrations in *Caenorhabditis elegans*. *Dev. Genet.* 11(1):49–64
- Marcus-Gueret N, Schmidt KL, Stringham EG (2012) Distinct cell guidance pathways controlled by the Rac and Rho GEF domains of UNC-73/TRIO in *Caenorhabditis elegans*. *Genetics* 190:129–142
- Maricq AV, Peckol E, Driscoll M, Bargmann CI (1995) Mechanosensory signaling in *C. elegans* mediated by the GLR-1 glutamate receptor. *Nature* 378:78–81
- Merz DC, Zheng H, Killeen MT, Krizus A, Culotti JG (2001) Multiple signaling mechanisms of the UNC-6/Netrin receptors UNC-5 and UNC-40/DCC in vivo. *Genetics* 158(3):1071–1080
- Métin C, Deléglise D, Serafini T, Kennedy TE, Tessier-Lavigne M (1997) A role for netrin-1 in the guidance of cortical efferents. *Development* 124(24):5063–5074
- Metzger RJ, Krasnow MA (1999) Genetic control of branching morphogenesis. *Science* 284:1635–1639
- Merrill RA, Plum LA, Kaiser ME, Clagett-Dame M (2002) A mammalian homolog of *unc-53* is regulated by all-trans retinoic acid in neuroblastoma cells and embryos. *Proc Natl Acad Sci U.S.A* 99(6):3422–3427
- Miller JR (2002) The Wnts. *Genome Biol* 3:3001
- Mitchell KJ, Doyle JL, Serafini T, Kennedy TE, Tessier-Lavigne M, Goodman CS, Dickson BJ (1996) Genetic analysis of Netrin genes in *Drosophila*: Netrins guide CNS commissural axons and peripheral motor axons. *Neuron* 17:203–215
- Mohamed AM, Chin-Sang ID (2006) Characterization of loss-of-function and gain-of-function Eph receptor tyrosine kinase signaling in *C. elegans* axon targeting and cell migration. *Dev Biol* 290:164–176
- Montcouquiol M, Crenshaw EB III, Kelley MW (2006) Noncanonical Wnt signaling and neural polarity. *Annu Rev Neurosci* 29:363–386
- Neiendam JL, Køhler LB, Christensen C, Li S, Pedersen MV, Ditlevsen DK, Kornum MK, Kiselyov VV, Berezin V, Bock E (2004) An NCAM-derived FGF-receptor agonist, the FGL-peptide, induces neurite outgrowth and neuronal survival in primary rat neurons. *J Neurochem* 91(4):920–935
- Nusse R, Varmus HE (1992) Wnt genes. *Cell* 26(7):1073–1087
- Nüsslein-Volhard C, Wieschaus E, Kluding H (1984) Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster* I. Zygotic loci on the second chromosome Roux's Arch. *Dev Biol* 193:267–282

- Pan CL, Howell JE, Clark SG, Hilliard M, Cordes S, Bargmann CI, Garriga G (2006) Multiple Wnts and frizzled receptors regulate anteriorly directed cell and growth cone migrations in *Caenorhabditis elegans*. *Dev. Cell* 10:367–377
- Qin H, Powell-Coffman JA (2004) The *Caenorhabditis elegans* aryl hydrocarbon receptor, AHR-1, regulates neuronal development. *Dev Biol* 270:64–75
- Quinn CC, Wadsworth WG (2006) Axon guidance: ephrins at WRK on the midline. *Curr. Biol.* 16:R954–R955
- Quinn CC, Pfeil DS, Chen E, Stovall EL, Harden MV, Gavin MK, Forrester WC, Ryder EF, Soto MC, Wadsworth WG (2006) UNC-6/Netrin and SLT-1/slit guidance cues orient axon outgrowth mediated by MIG-10/RIAM/lamellipodin. *Curr Biol* 16:845–853
- Rajagopalan S, Vivancos V, Nicolas E, Dickson BJ (2000) Selecting a longitudinal pathway: Robo receptors specify the lateral position of axons in the *Drosophila* CNS. *Cell* 103:1033–1045
- Rothberg JM, Hartley DA, Walther Z, Artavanis-Tsakonas S (1988) *slit*: an EGF-homologous locus of *D. melanogaster* involved in the development of the embryonic central nervous system. *Cell* 55:1047–1059
- Rothberg JM, Jacobs JR, Goodman CS, Artavanis-Tsakonas S (1990) *Slit*: an extracellular protein necessary for development of midline glia and commissural axon pathways contains both EGF and LRR domains. *Genes Dev* 4:2169–2187
- Sabatier C, Plump AS, Ma L, Brose K, Tamada A, Murakami F, Lee EV, Tessier-Lavigne M (2004) The divergent robo family protein *rig-1/robo3* is a negative regulator of Slit responsiveness required for midline crossing by commissural axons. *Cell* 117:157–169
- Samakovlis C, Hacohen N, Manning G, Sutherland D, Guillemin K, Krasnow MA (1996) Branching morphogenesis of the *Drosophila* tracheal system occurs by a series of morphologically distinct but genetically coupled branching events. *Development* 122:1395–1407
- Seeger M, Tear G, Ferres-Marco D, Goodman CS (1993) Mutations affecting growth cone guidance in *Drosophila*: genes necessary for guidance toward or away from the midline. *Neuron* 10:409–426
- Seetharaman A, Selman G, Puckrin R, Barbier L, Wong E, D'Souza SA, Roy PJ (2011) MADD-4 is a secreted cue required for midline-oriented guidance in *Caenorhabditis elegans*. *Dev Cell.* 21(4):669–680
- Serafini T, Kennedy TE, Galko MJ, Mirzayan C, Jessell TM, Tessier-Lavigne M (1994) The Netrins define a family of axon outgrowth-promoting proteins homologous to *C. elegans* UNC-6. *Cell* 78:409–424
- Serafini T, Colamarino SA, Leonardo ED, Wang H, Beddington R, Skarnes WC, Tessier-Lavigne M (1996) Netrin-1 is required for commissural axon guidance in the developing vertebrate nervous system. *Cell* 87:1001–1014
- Shewan D, Dwivedy A, Anderson R, Holt CE (2002) Age-related changes underlie switch in Netrin-1 responsiveness as growth cones advance along visual pathway. *Nat Neurosci* 5:955–962
- Shu T, Sundaresan V, McCarthy M, Richards LJ (2003) Slit2 guides both precrossing and post-crossing callosal axons at the midline in vivo. *J Neurosci* 23:8176–8184
- Silhankova M, Korswagen HC (2007) Migration of neuronal cells along the anterior–posterior body axis of *C. elegans*: Wnts are in control. *Curr. Opin. Genet. Dev.* 17:320–325
- Stern MJ, Horvitz HR (1991) A normally attractive cell interaction is repulsive in two *C. elegans* mesodermal cell migration mutants. *Development* 113:797–803
- Stein E, Tessier-Lavigne M (2001) Hierarchical organization of guidance receptors: silencing of Netrin attraction by Slit through a Robo/DCC receptor complex *Science* 291:1928–1938
- Stringham E, Pujol N, Vanderchove J, Bogaert T (2002) *unc-53* controls longitudinal migration in *C. elegans* *Development* 129:3367–3379
- Sulston JE, Horvitz HR (1977) Post-embryonic cell lineages of the nematode *Caenorhabditis elegans*. *Dev. Biol.* 56:110–156
- Sulston JE, Schierenberg E, White JG, Thomson JN (1983) The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev Biol* 100:64–119

- Sym M, Robinson N, Kenyon C (1999) MIG-13 positions migrating cells along the anteroposterior body axis of *C. elegans*. *Cell* 98(1):25–36
- Tessier-Lavigne M, Goodman CS (1996) The molecular biology of axon guidance. *Science* 274: 1123–1133
- Thomas JH, Stern MJ, Horvitz HR (1990). Cell interactions coordinate the development of the *C. elegans* egg-laying. *Cell* 62:1041–1052
- Troemel ER, Chou JH, Dwyer ND, Colbert HA, Bargmann CI (1995) Divergent seven transmembrane receptors are candidate chemosensory receptors in *C. elegans*. *Cell* 83:207–218
- Wadsworth WG, Bhatt H, Hedgecock EM (1996) Neuroglia and pioneer neurons express UNC-6 to provide global and local netrin cues for guiding migrations in *C. elegans* *Neuron* 16:35–46
- Wadsworth WG (2006) UNC-6/netrin and SLT-1/slit guidance cues orient axon outgrowth mediated by MIG-10/RIAM/lamellipodin. *Curr. Biol.* 16:845–853
- Wang X, Zhou F, Lv S, Yi P, Zhu Z, Yang Y, Feng G, Li W, Ou G (2013) Transmembrane protein MIG-13 links the Wnt signaling and Hox genes to the cell polarity in neuronal migration *Proc Natl Acad Sci U S A* 110(27):11175–80
- Whangbo J, Kenyon C (1999) A Wnt signaling system that specifies two patterns of cell migration in *C. elegans*. *Mol Cell* 4:851–858
- Wightman B, Clark SG, Taskar AM, Forrester WC, Maricq AV, Bargmann CI, Garriga G (1996) The *C. elegans* gene *vab-8* guides posteriorly directed axon outgrowth and cell migration. *Development* 122:671–682
- Wolf FW, Hung MS, Wightman B, Way J, Garriga G (1998) *vab-8* is a key regulator of posteriorly directed migrations in *C. elegans* and encodes a novel protein with kinesin motor similarity *Neuron* 20(4):655–66
- Yee C, Florica R, Fillingham J, Killeen MT (2014) ENU-3 functions in an UNC-6/Netrin dependent pathway parallel to UNC-40/DCC/Frazzled for outgrowth and guidance of the touch receptor neurons in *C. elegans*. *Dev Dyn.* 2013 Oct 7
- Yee CS, Sybingco SS, Serdetchania V, Kholkina G, Bueno de Mesquita M, Naqvi, Z, Park SH, Lam K, Killeen MT (2011) ENU-3 is a novel motor axon outgrowth and guidance protein in *C. elegans*. *Dev Biol* 352:243–253
- Yeo SY, Little MH, Yamada T, Miyashita T, Halloran MC, Kuwada JY, Huh TL, Okamoto H (2001) Overexpression of a slit homologue impairs convergent extension of the mesoderm and causes cyclopia in embryonic zebrafish. *Dev Biol* 230:1–17
- Yoshikawa S, McKinnon RD, Kokel M, Thomas JB (2003) Wnt-mediated axon guidance via the *Drosophila* derailed receptor. *Nature* 422:583–588
- Yu TW, Hao JC, Lim W, Tessier-Lavigne M, Bargmann CI (2002) Shared receptors in axon guidance: SAX-3/Robo signals via UNC-34/Enabled and a Netrin-independent UNC-40/DCC function. *Nat Neurosci.* 5(11):1147–1154
- Yuan W, Zhou L, Chen JH, Wu JY, Rao Y, Ornitz DM (1999) The mouse SLIT family: secreted ligands for ROBO expressed in patterns that suggest a role in morphogenesis and axon guidance. *Dev. Biol.* 212:290–306
- Zallen JA, Yi BA, Bargmann CI (1998) The conserved immunoglobulin superfamily member SAX-3/Robo directs multiple aspects of axon guidance in *C. elegans* *Cell* 92(2):217–227
- Zallen JA, Kirch SA, Bargmann CI (1999) Genes required for axon pathfinding and extension in the *C. elegans* nerve ring. *Development* 126(16):3679–3692
- Zinov'yeva AY, Yamamoto Y, Sawa H, Forrester WC (2008) Complex network of Wnt signaling regulates neuronal migrations during *Caenorhabditis elegans* development. *Genetics* 179:1357–1371
- Zou Y, Stoeckli E, Chen H, Tessier-Lavigne M (1999) Squeezing axons out of the gray matter: a role for slit and semaphorin proteins from midline and ventral spinal cord *Cell* 102(3):363–375

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