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Review

Netrin, Slit and Wnt receptors allow axons to choose the axis of migration

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ABSTRACT

One of the challenges to understanding nervous system development has been to establish how a fairly limited number of axon guidance cues can set up the patterning of very complex nervous systems. Studies on organisms with relatively simple nervous systems such as *Drosophila melanogaster* and *C. elegans* have provided many insights into axon guidance mechanisms. The axons of many neurons migrate along both the dorsal–ventral (DV) and the anterior–posterior (AP) axes at different phases of development, and in addition they may also cross the midline. Axon migration in the dorsal–ventral (DV) direction is mainly controlled by Netrins with their receptors; UNC-40/DCC and UNC-5, and the Slits with their receptors; Robo/SAX-3. Axon guidance in the anterior–posterior (AP) axis is mainly controlled by Wnts with their receptors; the Frizzleds/Fz. An individual axon may be subjected to opposing attractive and repulsive forces coming from opposite sides in the same axis but there may also be opposing cues in the other axis of migration. All the information from the cues has to be integrated within the growth cone at the leading edge of the migrating axon to elicit a response. Recent studies have provided insight into how this is achieved.

Evidence suggests that the axis of axon migration is determined by the manner in which Netrin, Slit and Wnt receptors are polarized (localized) within the neuron prior to axon outgrowth. The same molecules are involved in both axon outgrowth and axon guidance, for at least some neurons in *C. elegans*, whether the cue is the attractive cue UNC-6/Netrin working though UNC-40/DCC or the repulsive cue SLT-1/Slit working though the receptor SAX-3/Robo (Adler et al., 2006, Chang et al., 2006, Quinn et al., 2006, 2008). The molecules involved in cell signaling in this case are polarized within the cell body of the neuron before process outgrowth and direct the axon outgrowth. Expression of the Netrin receptor UNC-40/DCC or the Slit receptor SAX-3/Robo in axons that normally migrate in the AP direction causes neuronal polarity reversal in a Netrin and Slit independent manner (Levy-Strumpf and Culotti 2007, Watari-Goshima et al., 2007). Localization of the receptors in this case is caused by the kinesin-related VAB-8L which appears to govern the site of axon outgrowth in these neurons by causing receptor localization. Therefore, asymmetric localization of axon guidance receptors is followed by axon outgrowth *in vivo* using the receptor's normal cue, either attractive, repulsive or unknown cues.

Introduction

There are four well-known classes of molecules that serve as instructive guidance cues for the growth cones of pioneer axons. Each of these cues can either attract or repel axons depending on the receptors present on the surface of the migrating growth cones (Chilton 2006). The cues include the Netrins/UNC-6 (Kennedy et al., 2006, Round and Stein 2007), Slits (Dickson and Gilestro, 2006), Semaphorins (Kruger et al., 2005) and Ephrins (Flanagan 2006; Quinn and Wadsworth 2006; Mohamed and Chin-Sang, 2006). The major cues involved in axon guidance in *C. elegans* and *Drosophila* are summarized in Table 1. The locations where the cues to be discussed are expressed in *C. elegans* are shown in Fig. 1. The discovery that classic morphogens can also function as axon guidance molecules has contributed to our understanding of how a rather limited repertoire of secreted molecules can establish patterning in the nervous system. Recent reports have identified the Wnt family (Endo and Rubin, 2007), Sonic hedgehog (Charron et al., 2003; Erskine and Herrera, 2007), TGF-β (Colavita et al., 1998) and FGFs (Lindwall, 2007) as axon guidance molecules. These cues and their receptors are found across species barriers from worms through flies to vertebrates.

Abbreviations: ALM, anterior lateral microtubule neuron; AP, anterior–posterior; AVM, anterior ventral microtubule neuron; CAN, canal associated neuron; CFZ, *C. elegans* frizzled; CWN, *C. elegans* Wnt; DCC, deleted in colorectal cancer; Drl, derailed; DV, dorsal–ventral; EGL, egg laying defective; Fra, Frazzled; Fz, Frizzled; HSN, hermaphrodite specific neuron; Let, lethal; Lin, lineage; MIG, migration defective; MOM, more of MS; PLM, posterior lateral microtubule neuron; PVM, posterior ventral microtubule neuron; Ryk, receptor tyrosine kinase; SLT, Slit; RIAM, Rap-1 interacting molecule; Robo, roundabout; SAX, sensory axon guidance; UNC, uncoordinated; VAB, variable abnormal; VASP, vasodilator stimulated phosphoprotein; Wnt, wingless/int.

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Table 1
Axon guidance molecules of the Netrin, Slit and Wnt pathways in *C. elegans* and *Drosophila*

Ligand	Receptor	Mechanism	References
UNC-6/Netrin A and B	UNC-40/Frazzled/DCC	Neurons and cells expressing UNC-40/DCC/Frazzled are attracted towards Netrin/UNC-6.	Hedgecock et al. (1990) Ishii et al. (1992) Leung-Hagesteijn et al. (1992)
	UNC-5/Unc5	Neurons and cells expressing UNC-5 (with or without UNC-40) are repulsed by UNC-6/Netrin.	Chan et al. (1996) Harris et al. (1996) Mitchell et al. (1996) Kolodziej et al. (1996) Keleman et al. (2001)
Slit/SLT-1	Robo 1, 2, 3/SAX-3+EVA-1	Neurons and cells expressing Robo receptor are repulsed by Slit. Robo receptor prevented from reaching the growth cone surface by Commissureless.	Seeger et al. (1993) Zallen et al. (1998) Hao et al. (2001) Keleman et al. (2002) Keleman et al. (2005) Fujisawa et al. (2007)
Wnt5/EGL-20/LIN-44/CWN-1, 2, MOM-2	Frizzled/MIG-1/LIN-17, CFZ-2, MOM-5	In *Drosophila*, Wnts attract cells expressing Frizzled. Wnts in *C. elegans* direct many cell and axon migrations in the anterior–posterior direction.	Fradkin et al. (1995) Whangbo and Kenyon (1999) Maloof et al. (1999)
	Ryk/Derailed/LIN-18	Neurons are repulsed by Wnt at the midline preventing recrossing.	Yoshikawa et al. (2003) Inoue et al. (2004) Endo and Rubin (2007) Silkankova and Korswagen (2007) Zinovyeva et al. (2008)

Axon guidance cues are shown in the first column, their receptors in the second, and a summary of their function is given in the third column. The growth cones have receptors for several cues allowing the growth cone to take alternate directions.

Pioneer axon guidance can be divided into an axon outgrowth phase and an axon guidance phase where the growth cone at the tip of the migrating axon responds to guidance cues to determine its trajectory towards its final target. The trajectory the axons take may involve several changes in direction. Cues impinging on the growth cone frequently change as the growth cone migrates to new locations, so the cues and the ability to respond to them need to be dynamic. The literature indicates that axons are often subjected to opposing gradients, an attractive source and a repellant source and these need to be integrated in the growth cones. This comprehensive review of older and current literature focuses on how alterations in direction and even axis of migration are affected by Netrins, Slits and Wnts and their receptors, mainly in *C. elegans* and *Drosophila*. Analysis of axon migration in these relatively simple systems greatly expands our ability to understand axon migration in the much more complicated nervous systems of higher organisms.

Netrins and Slits in dorsal–ventral (DV) guidance

UNC-6/Netrin was first described in *C. elegans* mutants that have axon guidance defects in neurons that extend from the ventral to the dorsal nerve cords resulting in animals with an uncoordinated (Unc) phenotype (Hedgecock et al., 1990). UNC-6/Netrin is a laminin-like extracellular cue (Ishi et al., 1992) that is expressed dynamically in a variety of guide-post cells, mainly on the ventral side of the animal (Wadsworth et al., 1996 and shown in Fig. 1). Two transcripts encoding Netrin 1 and 2 were cloned from embryonic chick brain extracts (Serafini et al., 1994). They promote attraction of the commissural neurons and repel the trochlear motor neurons (Serafini et al., 1996, Colamarino and Tessier-Lavigne, 1995). Netrins are found in many other organisms including *Drosophila*, zebrafish and mammals (Harris et al., 1996; Mitchell et al.; 1996, Strahle et al.; 1997, Round and Stein, 2007). UNC-6/Netrin binds to two single-pass transmembrane receptors; UNC-40/DCC/Frazzled (Chan et al., 1996) and UNC-5 (Leung-Hagesteijn et al., 1992, reviewed in Round and Stein, 2007).

UNC-40/DCC/Frazzled in *C. elegans* has been implicated in the guidance of many neurons and cells in the ventral direction, usually in a Netrin/UNC-6-dependent fashion (Chan et al., 1996). However, there are also mild defects in cells and motor neurons that migrate in the dorsal direction. Vertebrate DCC (deleted in colorectal cancer) is a Netrin receptor and is expressed by commissural neurons that are chemoattracted by Netrin *in vitro* (Kennedy et al., 1994; Keino-Masu et al., 1996). The *Drosophila* homologue of UNC-40/DCC is called Fra for Frazzled (Kolodziej et al., 1996), and a zebrafish homologue of DCC is expressed in the developing brain (Hjorth et al., 2001). A second protein homologous to UNC-40/DCC/Fra called Neogenin also binds to

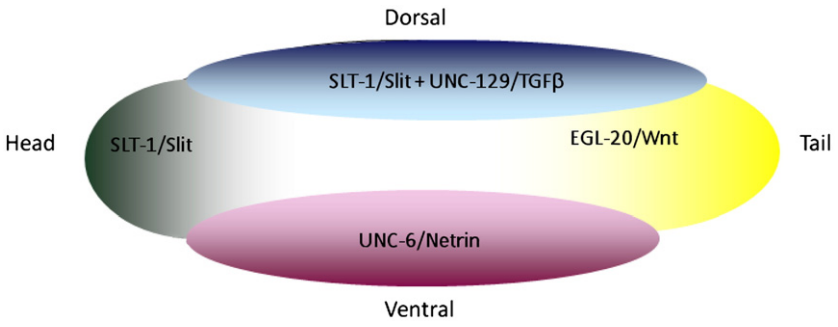

Fig. 1. Location of the major DV and AP guidance cues in *C. elegans* (modified from Fig. 2 of Blelloch et al., 1999). UNC-6/Netrin is expressed by neurons and other cells in the ventral region of *C. elegans* where it attracts cells and neurons expressing UNC-40/DCC and repels those expressing UNC-5. Slit expression by dorsal muscle repels neurons expressing SAX-3/Robo. UNC-129/TGFβ expression in dorsal muscle attracts motor neurons through an unknown mechanism. EGL-20/Wnt and other Wnts repel neurons and cells that express Frizzled receptors. Slit expression in the anterior epidermis during embryogenesis repels cells and neurons that express SAX-3/Robo.

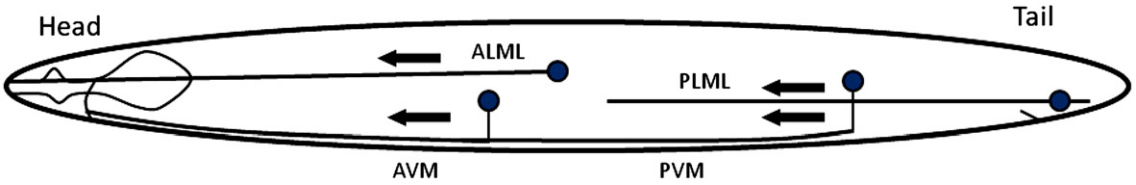

Fig. 2. Simplified schematic of the normal migrations of the axons of four of the six mechanosensory neurons in *C. elegans* (White et al., 1976). The axons of the ALM and PLMs migrate in the anterior direction from their cell bodies. ALMs and PLMs are found on the right and left halves of the body and only the ones on the left side are shown. The AVM and PVM axons migrate ventrally from their cell bodies and then in an anterior direction. AVM is on the right side and PVM on the left side of the body.

Netrin (Keino-Masu et al., 1996). Recently it was shown that the orphan receptor Downs syndrome cell adhesion molecule (DSCAM) is expressed on spinal commissural axons in rat and that it can work independently of, or co-operate with, DCC in mediating attractive turning responses to Netrin-1 (Ly et al., 2008).

Ventral-ward guidance of several neurons in *C. elegans* involves the opposing influences of UNC-6/Netrin, which attracts the axons expressing the UNC-40/DCC/Fra receptor to ventral regions (Chan et al., 1996) and Slit-1 expression in dorsal muscle, which repels axons through its receptor SAX-3/Robo (Hao et al., 2001 and Fig. 1). The SAX-3 molecule also has additional SLT-1-independent functions. Recently, a second SLT-1 receptor was found in *C. elegans*, called EVA-1. It functions as a co-receptor with SAX-3/Robo for SLT-1/Slit allowing the AVM axon (Fig. 2) to be repulsed by dorsal sources of SLT-1, resulting in migration in the ventral-ward direction towards the source of UNC-6. The authors found that UNC-6/Netrin working through UNC-40 and SLT-1 working through SAX-3 and EVA-1 are in two parallel genetic pathways. They hypothesized that SAX-3 could bind to UNC-40 if either SLT-1 or EVA-1 was absent and thus inhibit the guidance of the AVM axon by silencing UNC-40. They suggested that this would allow the axons to remain sensitive to high concentrations of UNC-6/Netrin close to the ventral cord in *C. elegans* (Fujisawa et al., 2007). This proposed inhibition of the UNC-6/Netrin pathway by SAX-3 is consistent with some of the phenomena that have been described in midline crossing where there is also Robo silencing of Frazzled/DCC/UNC-40 to allow axons to cross midline sources of Netrin (Stein and Tessier-Lavigne, 2001).

UNC-5 is a second single-pass transmembrane receptor found in *C. elegans* (Leung-Hagesteijn et al., 1992). *unc-5* mutants have axon guidance and cell migration defects in structures that migrate dorsally away from ventral sources of UNC-6/Netrin. Evidence is consistent with the idea that a ventral source of UNC-6 attracts cells expressing UNC-40, while repelling cells expressing UNC-5, even when UNC-40 is present (Merz and Culotti, 2000). Mammals have several Unc-5s (A–D) (Colamarino et al., 1995; Ackerman et al., 1997; Leonardo et al., 1997; Kennedy et al., 2006; Round and Stein 2007). Several lines of evidence support the idea that expression of UNC-5 on the growth cone is necessary and sufficient for dorsal-ward guidance. Ectopic expression of UNC-5 in touch neurons (Fig. 2) causes their axons to migrate in an abnormal dorsal direction in *C. elegans* (Hamelin et al., 1993). Expression of UNC-5 is also co-incident with dorsal-ward migration of the distal tip cells (Su et al., 2000). The *Drosophila* Unc5 has both long and short-range activities on the neurons (Keleman et al., 2001). UNC-5 may overcome the interaction between UNC-6/Netrin and UNC-40/DCC, since expression of both UNC-40/DCC and UNC-5 in the growth cone causes the cytoplasmic domains to interact and attraction is converted to repulsion (Hong et al., 1999).

Dorsal-ward guidance of at least some cells and axons in *C. elegans* relies on the opposition of attractive and repulsive cues such that there is repulsion of UNC-5 expressing axons and cells by ventral sources of UNC-6/Netrin and attraction towards dorsal muscle sources of UNC-129/TGFβ, possibly functioning either through an effect on UNC-5 itself or an as yet undescribed receptor (Colavita et al., 1998). It is likely that there are additional cues involved in dorsal-ward guidance as absence of both UNC-6 repulsion by ventral sources and UNC-129 attraction towards dorsal muscle does not completely abolish dorsal guidance for some of the motor neurons in *C. elegans*.

Slit and Netrin in midline crossing

Migration of axons across the midline requires the resolution of attractive and repulsive forces both emanating from the midline region. Slit and Netrin are both expressed at the midline in mammals, zebrafish, *Drosophila* and *C. elegans* (Serafini et al., 1994; Harris et al., 1996; Mitchell et al., 1996; Wadsworth et al., 1996; Battye et al., 1999; Kidd et al., 1999; Yuan et al., 1999; Hao et al., 2001; Yeo et al., 2001). Slit is the midline repellent for axons expressing one of the three Robo receptors in *Drosophila* (Seeger et al., 1993; Kidd et al., 1999) and expression of Slit at the midline ensures that ipsilateral axons do not cross the midline inappropriately (Kidd et al., 1999). Netrin expression at the midline attracts the commissures expressing the Frazzled/UNC-40/DCC receptor but they cannot pass through to the contralateral side, unless the attraction is suppressed (Serafini et al., 1996; Kidd et al., 1999). Therefore the opposing signals of Netrin attraction and Slit repulsion by the midline need to be resolved to allow crossing of axons to the contralateral side.

Understanding of the mechanism of Slit activity came in part from work in *Drosophila*. Mutations in Robo (roundabout) cause a phenotype where some of the neurons that are normally ipsilateral, instead project across the midline (Seeger et al., 1993). Mutations in commissureless (comm) in *Drosophila* cause the opposite appearance, as nearly all CNS axon commissures are absent, such that growth cones of the neurons that are normally contralateral, instead now extend only on their own side (Tear et al., 1996). Comm prevents delivery of Robo to the growth cone in axons that are destined to cross the midline by sorting newly synthesized Robo to the late endocytic pathway, thus preventing it from reaching the surface of the growth cone and targeting it for degradation (Keleman et al., 2002; Keleman et al., 2005). Several experiments have suggested that it is the cytoplasmic portion of the Robo molecule that makes the guidance decisions. Robo and Frazzled/DCC/UNC-40 receptors specify attraction or repulsion such that a chimeric molecule with a Robo cytoplasmic domain is repulsed by Slit and one with a Frazzled cytoplasmic domain is attracted to Slit, whether the extracellular domain is from DCC/UNC-40 or Robo (Bashaw and Goodman, 1999). In *Xenopus* spinal explants, it was found that activation of the Slit receptor, Robo, silenced the attractive effect of Netrin-1 through direct binding of the cytoplasmic domain of Robo to the cytoplasmic domain of the Netrin receptor DCC, resulting in silencing of the attractive effect of the midline on neurons expressing both DCC and Robo (Stein and Tessier-Lavigne, 2001). Robo co-immunoprecipitates with UNC-40/DCC in a Slit-dependent manner. This binding is asymmetric, since activation of Robo causes binding to DCC but activation of DCC does not cause binding to Robo. Once midline crossing has occurred, the axons are guided along either rostral or caudal migrations on the contralateral side in tracts parallel to the midline, at least partly by expression of Robo which is repelled by the Slit sources at the midline (Kidd et al., 1999; Rajagopalan et al., 2000). In *C. elegans*, the absence of SAX-3/Robo causes repeated midline crossing by neurons that normally do not cross (Zallen et al., 1998). This crossing is presumably suppressed

by the silencing of Netrin/UNC-6 attraction due to interaction between SAX-3 and EVA-1 with UNC-40/DCC/Fra (Fujisawa et al., 2007).

Netrin and Slit are also involved in midline crossing in vertebrates. Netrin 1 in mice is needed for corpus collosum and hippocampal commissure development in mice (Serafini et al., 1996). Mice lacking Slit1 and Slit2—are deficient in all three major forebrain connections, the corticofugal, callosal, and thalamocortical tracts (Bagri et al., 2002). In mammals, Rig-1/Robo-3 receptor prevents Slit sensitivity and lack of Rig-1 prevents midline crossing (Sabatier et al., 2004). Rig-1 is thought to repress the repulsive activity of any low level of Robo-1 present in pre-crossing neurons. After crossing, Rig-1 is down-regulated and Robo-1 and 2 are upregulated allowing their repulsion by the midline. The mechanism of action of Rig-1/Robo-3 has recently been addressed (Chen et al., 2008). It was found that there are two alternatively spliced variants of Robo-3: Robo-3.1 and 3.2. Robo-3.1 is present on pre-crossing and crossing axons allowing silencing of Slit silencing. Slit 3.2 is expressed after crossing, favouring Slit repulsion.

Netrin and Slit in anterior–posterior or longitudinal guidance

Although Slit and Netrin are known primarily for their roles in DV guidance, including midline crossing, they are also involved in longitudinal or anterior–posterior (AP) guidance. In Slit deficient *Drosophila*, the tracts that normally run parallel to the midline collapse on the midline due to attraction by Net emanating from the midline (Garbe and Bashaw 2007). However, Netrin attraction from the midline also plays a role in longitudinal guidance as the tracts in Net A and B mutants run farther from the midline (Mitchell et al.. 1996, Bashaw and Goodman, 1999; Kidd et al., 1999; Bhat et al., 2007; Garbe and Bashaw, 2007). The Slit phenotype is more penetrant than the Netrin phenotypes and it was found that the two pathways function both independently and interdependently (Garbe and Bashaw, 2007). In higher organisms, longitudinal guidance also relies on Slit–Robo (Devine and Key, 2008).

Wnts are also cues for midline crossing and anterior–posterior guidance

Axon guidance in the AP direction was somewhat more recalcitrant to analysis than DV guidance, probably because of redundancy in signaling molecules. Wnt is a well-studied morphogen that is involved in asymmetric cell divisions in many organisms and at least three signaling pathways have been described, Wnt/ βcatenin, Wnt/calcium and Wnt/planar cell polarity (Endo and Rubin 2007). Wnt signals thorough Fz (Frizzled) and disheveled to allow stabilization of β-catenin, which enters the nucleus and influences transcription of specific genes (Eisenmann, 2005, Logan and Nusse, 2004). Midline crossing in *Drosophila* relies on Wnt signaling in addition to the Netrin and Slit signaling described above (Callahan et al., 1995, Yoshikawa et al., 2003). In each segment of *Drosophila*, there is an anterior and a posterior commissure and axons cross the midline in only one of the commissures (Callahan et al., 1995). The choice of commissure is determined by the Derailed/Ryk atypical receptor tyrosine kinase which is expressed by axons in the anterior commissure. The Wnt5 cue is expressed mainly by axons in or close to the posterior commissure (Yoshikawa et al., 2003). Axons expressing Drl/Ryk are repelled by Wnt5, ensuring that these axons migrate through the anterior and not the posterior commissure. In the absence of Drl, axons are insensitive to Wnt5. The initial path finding of pioneer neurons across the midline is normal in the absence of Wnt5 but later division of the commissures into the anterior and posterior commissure bundles does not occur properly and the follower axons in the anterior commissure do not cross appropriately (Fradkin et al., 2004). It was suggested that the main function of Wnt in this process is in control of defasciculation of neurons to allow them to leave the posterior commissure and enter the anterior commissure. Wnt5 is transcriptionally repressed in the anterior commissure by Drl and this repression allows midline crossing of the anterior commissure. Wnt5 in *Drosophila* also affects midline recrossing by causing repulsion of post-crossing neurons expressing Drl/Ryk (Yoshikawa et al., 2003).

In mammals, Wnt disruption caused abnormalities of the mouse brain (McMahon et al., 1990). Also in the mouse, ascending commissural neurons that have crossed the midline are attracted to high anterior concentrations of Wnt4 by Fz3 (Lyuksyutova et al., 2003). Descending neurons of the corticospinal tract are repulsed by Wnts binding to the receptor Ryk (Imondi and Thomas 2003, Liu et al., 2005, and Dickson and Gilestro 2006). Projections of the cortical axons across the anterior commissure are abnormal in mice lacking Ryk which is the receptor for Wnt5 (Keeble et al., 2006). Fz and Ryk function as co-receptors that stimulate neurite outgrowth in mammalian cells (Lu et al., 2004). 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).

Wnts control cell fate, cell polarity, including axon outgrowth polarity, and cell migration in *C. elegans* (Maloof et al., 1999, Whangbo et al., 1999, Pan et al., 2006, Silkankova and Korswagen, 2007, Zinovyeva et al., 2008). *C. elegans* has proven to be a good organism for the identification of AP axon guidance molecules as well as DV molecules because it has a number of neurons that take a direct anterior or posterior route as part of their journey from their birthplace to their final specific destination along the AP axis, before they polarize and extend axons and dendrites (Fig. 1 of Zinovyeva et al., 2008). Furthermore, the cells are relatively easy to see using appropriate tags (recent review in Silkankova and Korswagen 2007). In addition, *C. elegans* has fewer Wnts and receptors than mammals since it has five Wnts (EGL-20, LIN-44, CWN-1, CWN-2, MOM-2) and four Frizzleds (MOM-5, CFZ-2, MIG-1, LIN-17), along with the Ryk homologue LIN-18 while mouse has 19 Wnts and 12 Frizzled receptors (Pan et al., 2006). However, Wnts frequently function as repulsive signals for cells expressing Frizzled receptors in *C. elegans*, rather than as attractants as in mammals. Maloof et al. (1999) found that the migrations of the Q neuroblast and its descendants were controlled by a Wnt called EGL-20. The Q neuroblast divides to give two sister cells, QL and QR. QR makes a short anterior migration and its descendants migrate anteriorly into the head of the animal. QL migrates a short distance posteriorly and then expresses a Hox transcription factor called MAB-5 whose expression is controlled by EGL-20. Its descendants then make posterior migrations into the tail towards the source of MIG-20/Wnt. The Hox gene control in QL also requires another Wnt signaling pathway gene, BAR-1/β-catenin/Armadillo. A protein called PRY-1 is required to keep MAB-5/Hox off in the QR neuroblasts. Whangbo and Kenyon (1999) found that the migratory behaviour of the QLs and QRs was due to left–right asymmetrical differences in the cellular responsiveness to EGL-20/Wnt signaling. At higher relative concentrations of Wnt/EGL-20, the QL neuron expressed the Wnt pathway proteins, LIN-17/FZ, BAR-1/β catenin and MIG-5/Dishevelled, resulting in posterior migration of the cell while at lower relative EGL-20/Wnt concentrations, QR did not express the proteins in this pathway due to lower sensitivity to Wnt/EGL-20 and migrated in the anterior direction.

The Wnts play somewhat redundant roles in the specification of cell fate and the guidance of cell and axon migrations (Pan et al., 2006, Zinovyeva et al., 2008). Three Wnts (EGL-20, CWN-1 and LIN-44) are expressed by a group of epidermal and muscle cells located in the posterior of the animal near the anus. These together with a fourth WNT, MOM-2, function somewhat redundantly to mediate the repulsive effect on the cells of hermaphrodite specific neurons (HSNs) to promote their anteriorly directed migrations from the tail region to mid-body (Pan et al., 2006). The most important of the Wnts for HSN migration was EGL-20, though the removal of any one of the other three Wnts; CWN-1, CWN-2 or MOM-2, also caused some of the HSNs to under-migrate relative to a wild-type strain. The absence of a

second Wnt in addition to EGL-20, including any one of; CWN-1, LIN-44 or MOM-2, enhanced the HSN under-migration defect caused by the loss of EGL-20. The most important Frizzled receptor for HSN migration was MIG-1. The absence of a second Frizzled, either MOM-5 or CFZ-2, enhanced the defects caused by the loss of MIG-1. Interestingly, LIN-17/Fz antagonized MIG-1, so that it suppressed the defects somewhat. Zinovyeva et al. (2008) also looked at the genes needed for the migrations of many cells and neurons in *C. elegans* and found that the Wnts; CWN-1, CWN-2 and EGL-20 were required for the migrations of many different cells, some migrating anteriorly like the HSNs and QR cell descendents and some moving in the posterior direction like the CAN cells and the descendents of the QL cell. They function redundantly and can sometimes antagonize each other's function. All four Frizzled receptors function in the Q cell migrations. This might be expected, as they migrate in opposite directions to each other as described above. A subset of Frizzled receptors is required for the migrations of other cells. Pan et al. (2006) also looked at axon outgrowth of the AVMs and PVMs (Fig. 2) in different mutant backgrounds and found that they saw no significant axon guidance defect in any single mutant background for either the Wnts or Frizzleds. However, they found misguidance of both the AVM and PVM axons when both CWN-1 and EGL-20 Wnts were absent and when both MIG-1 and MOM-5 Frizzled receptors were absent. In mutant backgrounds, the axonal processes were abnormal, they were shorter, bifurcated in the ventral cord or had axons that migrate in the posterior instead of the anterior direction. Therefore, several Wnts may co-operate to control both cell or axon migrations and either two Wnts or two Frizzleds need to be removed to detect a significant effect on axon guidance.

Polarity of axon outgrowth

Axons are at least sometimes under the control of hierarchical layers of cues and this has been shown for the AVM neuron in *C. elegans*. Slit and Robo are involved in DV and AP guidance as well as midline crossing in *C. elegans* (Hao et al., 2001). As mentioned previously, the axons of the AVM neurons migrate ventrally under the opposing influence of both Netrin and Slit that work through two parallel genetic pathways (Hao et al., 2001; Fujisawa et al., 2007). When both SLT-1 and UNC-6/Netrin are absent, the AVM adopts an anterior trajectory as a result of a guidance mechanism that is uncovered in the absence of DV guidance. Presumably, this indicates that DV guidance is the main function of both Netrin and Slit with AP guidance only becoming apparent in the absence of DV guidance, at least for the AVM axons. The AP guidance in this case probably operates through a different mechanism such as the Wnt system.

Recent experiments on the attractive guidance of neurons by Netrin through UNC-40/DCC have led to the resolution of several interesting questions. One question is whether the same molecules are responsible for axon outgrowth and axon guidance of an individual neuron. A second question is whether the same molecules in an individual neuron are used to respond to opposing repulsive and attractive cues impinging on the neuron. These questions have been addressed in *C. elegans* where orientation of axon outgrowth of some neurons in the ventral direction relies on the same localization molecules for both axon outgrowth and axon guidance, whether the cue is attractive or repulsive. A HSN neuron sends an axon ventrally and then it migrates anteriorly and grows into the head. UNC-6/Netrin in *C. elegans* functions in the HSN neurons for their ventral-ward migration to break symmetry and thus defines the site of axon formation (Adler et al., 2006). When either UNC-6/Netrin or UNC-40/DCC is absent, the HSN grows anteriorly, not ventrally, thus both are required for axon outgrowth, specifically to promote leading edge formation. In the HSNs both UNC-40 and the pleckstrin homology containing adaptor protein MIG-10/RIAM/Lamellipodin are located ventrally on the leading edge of the cell body before axon outgrowth in a Netrin/UNC-6-dependent fashion. MIG-10 is homologous to Lamellipodin, a protein that stimulates F actin formation during

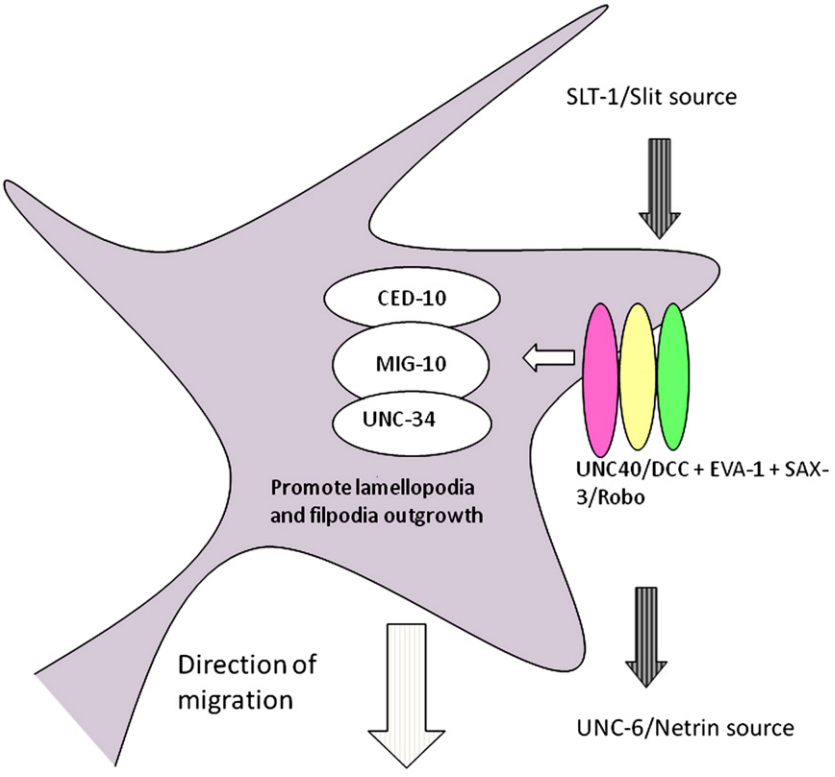

Fig. 3. Ventral guidance of the growth cone in the DV axis. In the case of DV guidance, it is known that both UNC-6/Netrin and SLT-1/Slit cause localization of UNC-40/DCC and SAX-3/Robo and these in turn polarize CED-10, MIG-10 and UNC-34/Ena/VASP leading to the cytoskeletal reorganization in the growth cones of pioneer neurons.

fibroblast lamellipodial protrusion, so presumably the asymmetry of UNC-40 and MIG-10 results in cytoskeletal reorganization typical of migrating growth cones. Thus UNC-6/Netrin orients the HSN ventrally before growth cone formation. Quinn et al., 2008 have shown that CED-10/Rac1 binds to and functions upstream of MIG-10/Lamellipodin to mediate its asymmetric distribution on the ventral edge of the HSN cell body to orient HSN axon outgrowth (Fig. 3). They showed that MIG-10 acts with the Rac binding molecule PAK-1 (p21 activated kinases) and in parallel with another PAK known as MAX-2 to orient HSN axon outgrowth. The PAK molecules involved in this process are known to play roles in reorganization of the cytoskeleton. Therefore, both UNC-6/Netrin and UNC-40/DCC are required for axon outgrowth as well as for axon guidance. UNC-40 was previously shown to be involved in left–right asymmetry decisions in *C. elegans* and this is presumably also the result of polarized axon outgrowth but in the left–right axis (Honiberg et al., 2000).

At least some of the proteins that induce asymmetry before axon outgrowth are the same, whether the cue is having an attractive or a repulsive effect. For instance, both UNC-6/Netrin and SLT-1/Slit induce neuronal asymmetry and define the site of axon outgrowth using MIG-10/RIAM/Lamellopodin, and UNC 34/Enabled/VASP which co-operate to promote both axon outgrowth and axon guidance, even though UNC-6/Netrin attracts and Slit-1/Slt repels the neurons (Chang et al., 2006; Quinn et al., 2006). In the absence of either UNC-6/Netrin or Slit-1/Slt, the AVMs or PVMs (Fig. 2) rarely migrate ventrally at the proper position, into the ventral cord (Quinn et al., 2006). If MIG-10/RIAM is overexpressed in the absence of either UNC-6/Netrin or Slit-1/Slt in the AVMs or PVMs it leads to a multipolar phenotype which can be converted to a monopolar phenotype by either of the cues. Therefore, MIG-10 mediates the asymmetry of the AVMs and PVMs in response to either UNC-6/Netrin or SLT-1/Slit, despite the fact that one repels and the other attracts the axons. MIG-10 interacts genetically and biochemically with UNC-34/Ena/VASP (Fig. 3). In addition, MIG-10 and actin co-localize in HEK 293 cells to promote filopodia formation. Therefore the effect of UNC-6/Netrin and SLT-1/Slit on axon outgrowth is likely to be due to the reorganization of the actin cytoskeleton within the growth cones of the axons of pioneer neurons. Chang et al., (2006) found that UNC-34 is required for finger-like structures on the growth cone called filopodia and MIG-10 increases the number of filopodia in the HSNs. Interestingly, filopodia were not essential for ventral guidance of the HSNs. This suggests that as well as guidance activity, UNC-6/Netrin and Slt-1/Slit can promote asymmetry to allow axon outgrowth at the proper position using at least some of the same proteins, despite the fact that one attracts and the other cue repels the growth cone. Therefore, it appears as if the site of axon formation and axon guidance may be determined by the same signaling molecules, regardless of whether the cue is attractive or repulsive, at least for ventral migrations in the DV axis of the neurons examined.

Wnts in *C. elegans* provide instructional information to cells and neurons that migrate long distances during development as mentioned previously. However, they also serve permissive roles in axon migration once the cells reach their final positions and send out processes that are presumptive dendrites and axons. Wnts organize neuronal polarity of the ALM and PLM mechanosensory neurons in *C. elegans*, (Fig. 2) rather than act as attractive or repellant instructional cues (Prasad and Clark 2006, Hilliard and Bargmann, 2006). LIN-44/Wnt appears to determine the polarity of the PLM mechanosensory neurons by establishing or maintaining an asymmetric distribution of LIN-17/Frizzled in the cell (Hilliard and Bargmann, 2006). In LIN-44/Wnt and LIN-17/Frizzled mutants, the polarity was reversed along the body axis such that the long PLM process, PLM growth cone, and synapses were posterior to its cell body instead of anterior. In embryos, the growth cone was found frequently at the tip of the posterior rather than the anterior process. The long posterior process of the PLMs was enriched in synaptobrevin, which is a presynaptic protein normally localized in the anterior process, supporting the view that the site of axon exit from the cell body was reversed. Therefore, the anterior–posterior process outgrowth was disrupted and the polarity of the neuron was reversed. LIN-17/Frizzled is usually located predominantly in the posterior process of the PLM neurons, which is the presumptive dendrite. In *lin-44* mutants, LIN-17mRFP1 was uniformly distributed between the anterior and posterior process indicating that the asymmetry of the LIN-17 localization was lost. Therefore, LIN-44/Wnt may regulate the subcellular localization of its receptor, LIN-17. The same type of reversal was apparent for the ALMs when the EGL-20/Wnt or the CWN-1/Wnt were absent, suggesting that different Wnt signals control neuronal polarity at different AP positions in the animal. However, the Wnt receptor in the ALMs was not identified (Hilliard and Bargmann, 2006). Prasad and Clarke (2006) also found that disruption of Wnts led to complete inversion of the ALM and PLM process polarity.

VAB-8 localizes overexpressed SAX-3/Robo and UNC-40

One question that has rarely been addressed is whether the same guidance cue that is involved in axon guidance also causes localization of the receptor in the growth cone. Are the same proteins required for receptor localization and function? Two recent papers by Levy-Strumpf and Culotti (2007) and Watari-Goshima et al. (2007) cast some new light on this question and also on the choice of DV or AP guidance by Netrin and Slit receptors, As discussed, these receptors are primarily involved in DV guidance in *C. elegans*, but their ectopic expression (or overespression for SAX-3/Robo) in the touch neurons, which mostly migrate along the AP axis, can sometimes cause a reversal of polarity of the axons, but still along the AP axis. The result may actually be due to an altered site of initial axon outgrowth as described above in the previous paragraph, such that the neuronal processes sometimes run in the opposite direction to their usual trajectory. Therefore, in these cases, the overexpression of the Netrin and Slit receptors can function in a somewhat unusual axis of migration in a manner independent of their usual guidance cues. In addition, since the direction of migration is altered, the receptors appear to overcome the Wnt signaling in the neurons as well as Netrin and Slit signaling in the DV direction. The ALM mechanosensory touch neurons used in these studies are bilaterally symmetrical and have a long anterior process, presumably an axon as described previously (Fig. 2). VAB-8 is a kinesin-related protein expressed in many neurons that normally make posterior migrations. The researchers over-expressed the VAB-8L isoform in the ALMs and found that a high percentage of the axons were misguided in a posterior rather than an anterior direction, exactly 180° relative to their usual route. This suggests that VAB-8L has the ability to steer axons in the posterior direction in a cell-autonomous manner, probably by altering the site of axon outgrowth in the ALM cell body. Both groups determined that the misdirection of the ALMs was suppressed in various genetic backgrounds indicating that all of the genes encoding these proteins are downstream of VAB-8L. The proteins included the Rac guanine nucleotide exchange factor UNC-73/Trio/Kalerin (Steven et al., 1998), its target MIG-2 GTPase (Zipkin et al., 1997), the ligand SLT-1/Slit and its receptor SAX-3/Robo as well as the two UNC-6/Netrin receptors UNC-5, UNC-40 (Levy-Strumpf and Culotti, 2007 and Watari-Goshima et al., 2007).

Some surprises emerged when UNC-40/DCC was expressed ectopically and SAX-3/Robo was overexpressed in the ALMs. Since these are both well-known DV guidance receptors, they would be expected to guide the ALMs ventrally towards UNC-6 and away from SLT-1 in the dorsal muscle. This was observed in some cases for UNC-40 and was UNC-6-dependent as expected. However, the ALM axons were misdirected posteriorly in an UNC-6 independent manner when UNC-40 was expressed (Levy-Strumpf and Culotti 2007). SAX-3/Robo receptor overexpression could also reroute the ALM axon posteriorly (Watari-Goshima et al., 2007). Mutations in VAB-8, UNC-73/Trio/

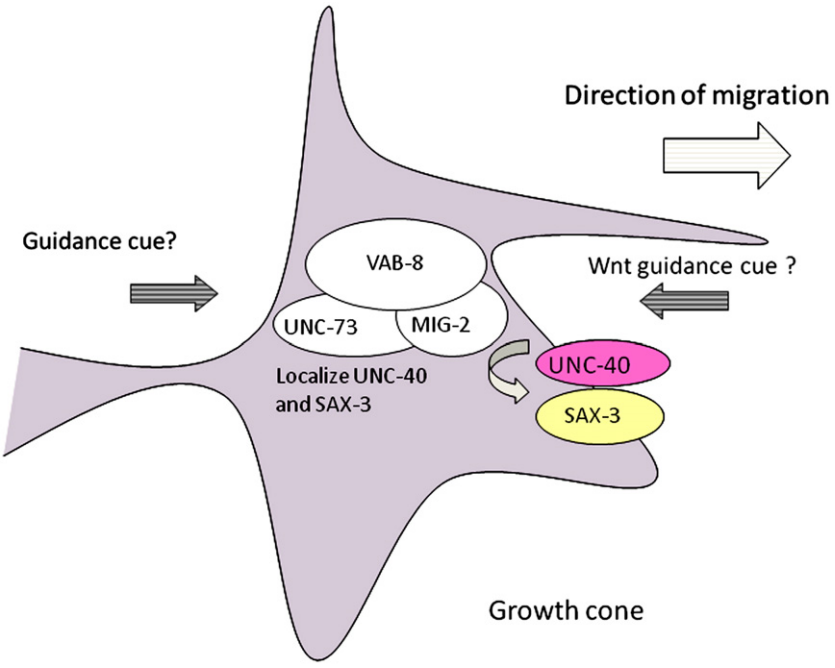

Fig. 4. Overexpression of UNC-40 or SAX-3 causes reversal of AP polarity of the ALM neurons. In the case of the misdirection of axons of the ALMs, VAB-8, UNC-73 and MIG-2 are involved in localization of the receptors in the growth cones but in this case, the normal cues are not involved. Rather, the VAB-8 pathway is responding to an as yet unidentified cue.

Kalirin and MIG-2/Rho failed to suppress the misdirection defects caused by UNC-40 and SAX-3 suggesting that they are upstream of the receptors (Fig. 4). This observation is surprising as it had been thought that UNC-73 and MIG-2 transduce signals from receptors to elicit alterations inside of growth cones in response to external cues (Forsthoefel et al., 2005). The results are consistent with the novel idea that VAB-8 localizes UNC-40 and SAX-3/Robo in the growth cone of the ALM axon through UNC-73 and MIG-2 (Levy-Strumpf and Culotti, 2007, Watari-Goshima et al., 2007). Watari-Goshima et al., (2007) favour the model that VAB-8 may regulate receptor trafficking since UNC-73 interacts with VAB-8 and also with SAX-3, UNC-5 and UNC-40 receptors, suggesting that these protein interactions mediate VAB-8Ls' regulation of the receptors. VAB-8 can also interact with UNC-51, a kinase that regulates membrane trafficking and is involved with the display of UNC-5 in growth cones in *C. elegans* (Ogura et al., 2006). VAB-8 in these experiments promotes receptor localization and thus axon outgrowth and guidance. The cue controlling VAB-8 in this context is unclear but there is a possibility of cross talk between an AP guidance system (possibly Wnt) and the Netrin system. In the case of the ALM studied by Levy-Strumpf and Culotti (2007) and Watari-Goshima et al. (2007), the polarity of the neurons could be normally determined by the Wnts, possibly EGL-20 or CWN-1, and this polarity could be reversed by overexpression of either UNC-40 and/or SAX-3/Robo. Levy-Strumpf and Culotti (2007) consider two scenarios (Supplementary Fig. 2 of their paper). In one scenario, the unknown AP cue acts through VAB-8 to polarize UNC-40, which can be activated by UNC-6/Netrin. Alternatively, UNC-73, MIG-2 and VAB-8 affect UNC-40 localization to allow it to appear on the membrane or possibly in lipid rafts, allowing its activation by a cue that is graded along the AP axis (not UNC-6/Netrin). Overexpression of LIN-17/Frizzled caused disruptions of PLM polarity in wild-type backgrounds (Hilliard and Bargmann, 2006) in the same way that overexpression of SAX-3/Robo, UNC-5 and UNC-40/DCC cause reversals of the ALMs and to a lesser extent, the PLMs in the Watari-Goshima et al. (2007) and the Levy-Strumpf and Culotti (2007) papers. These findings suggest that tight regulation of expression levels of receptors on the growth cones of developing axons might be essential to polarity determination, probably at the axon outgrowth stage. It is possible that the ALMs and PLMs might be responding to anterior sources of SLT-1/Slit that is expressed by anterior cells during embryogenesis, and was shown to affect the posterior migrations of the CAN neurons in the AP direction (Hao et al., 2001). Expression of SLT-1/Slit by all muscle cells could reverse the polarity of the AP migration phases of the AVM neuron (Fig. 2). Possibly, these neurons are under the opposing influences of Wnts emanating from the posterior regions and Slit emanating from the anterior regions in somewhat the same way that ventral migrations of the AVMs and HSNs are influenced by the opposing forces of a dorsal source of SLT-1/Slit and a ventral source of UNC-6/Netrin (Hao et al., 2001). It is not clear if VAB-8 continues to affect the migrating axon, once the initial polarity is determined but it is likely to do so since the molecules involved in DV guidance affect both axon outgrowth and axon migration.

In this review, several examples have been discussed where axon guidance has relied on the same guidance cues in both DV and AP guidance. If this is so, how do axons know which axis to choose or which direction to follow in a particular journey? The answer to this question may lie mainly in how signals are integrated inside the target cells but it is likely that receptors are localized in target cells initially to the sites of future axon outgrowth. It will be interesting to see how much receptor localization will be detected in target cells, once more reagents and methods for detection become available. Several different guidance cues may be involved in some cases, one that determines the selection of the site of axon formation, such as was described by Chang et al. (2006) and Quinn et al. (2006) where either UNC-6/Netrin or Slt-1/Slit polarized the HSN growth cones for DV migration. This cue could cause polarization of the receptors using VAB-8-driven localization through UNC-73 and MIG-2. Once the receptor is in place, the appropriate cue (the same one as the first cue or a different one) could trigger downstream signaling through activation of kinases, and thus exert some changes inside the cell that eventually result in cytoskeletal reorganization typical of migrating growth cones. Much is still to be learned about the complexities of axon guidance and more information can be obtained about how competing signals are integrated within growth cones to produce a response in a migrating neuron. The use of model organisms and the targeted expression of proteins in particular cells can be very

informative about processes that are difficult to tease apart in more complicated systems like vertebrates.

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References

Ackerman, S.L., Kozak, L.P., Przyborski, S.A., Rund, L.A., Boyer, B.B., Knowles, B.B., 1997. The mouse rostral cerebellar malformation gene encodes an UNC-5-like protein. Nature 386, 838–842.

Adler, C.E., Bargmann, C., 2006. UNC-6/Netrin induces neuronal asymmetry and defines the site of axon formation. Nat. Neurosci. 9, 511–518.

Bagri, A., Marín, O., Plump, A.S., Mak, J., Pleasure, S.J., Rubenstein, J.L., 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.

Bashaw, G.J., Goodman, C.S., 1999. Chimeric axon guidance receptors: the cytoplasmic domains of Slit and Netrin receptors specify attraction versus repulsion. Cell 97, 917–926.

Battye, R., Stevens, A., Jacobs, J.R., 1999. Axon repulsion from the midline of the *Drosophila* CNS requires Slit function. Development 126, 2475–2481.

Bhat, K.M., Gaziova, I., Krishnan, S., 2007. Regulation of axon guidance by Slit and Netrin signaling in the *Drosophila* ventral nerve cord. Genetics 176, 2235–2246.

Blelloch, R., Newman, C., Kimble, J., 1999. Control of cell migration during *Caenorhabditis elegans* development. Curr. Opin. Cell Biol. 11, 608–613.

Callahan, C.A., Muralidhar, M.G., Lundgren, S.E., Scully, A.L., Thomas, J.B., 1995. Control of neuronal pathway selection by a *Drosophila*-tyrosine kinase family member. Nature 376, 171–174.

Chan, S.S., Zheng, H., Su, M.W., Wild, R., Killeen, M.T., Hedgecock, E.M., Culotti, J.G., 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.

Chang, C., Adler, C.E., Krause, M., Clark, S.G., Gertler, F.B., Tessier-Lavigne, M., Bargmann, C.I., 2006. MIG-10/lamellipodin and AGE-1/PI3K promote axon guidance and outgrowth in response to Slit and Netrin. Curr. Biol. 16, 854–862.

Charron, F., Stein, E., Jeong, J., McMahon, A.P., Tessier-Lavigne, M., 2003. The morphogen sonic hedgehog is an axonal chemoattractant that collaborates with Netrin-1 in midline axon guidance. Cell 113, 11–23.

Chen, Z., Gore, B.B., Long, H., Ma, L., Tessier-Lavigne, M., 2008. Alternative splicing of the Robo3 axon guidance receptor governs the midline switch from attraction to repulsion. Neuron 58, 325–332.

Chilton, J.K., 2006. Molecular mechanisms of axon guidance. Dev. Biol. 292, 13–24.

Colamarino, S.A., Tessier-Lavigne, M., 1995. The axonal chemoattractant Netrin-1 is also a chemorepellent for trochlear motor axons. Cell 81, 621–629.

Colavita, A., Krishna, S., Zheng, H., Padgett, R.W., Culotti, J.G., 1998. Pioneer axon guidance by UNC-129, a *C. elegans*-beta. Science 28, 706–709.

Devine, C.A., Key, B., 2008. Robo–Slit interactions regulate longitudinal axon pathfinding in the embryonic vertebrate brain. Dev. Biol. 313, 371–383.

Dickson, B.J., Gilestro, G.F., 2006. Regulation of commissural axon pathfinding by Slit and its Robo receptors. Annu. Rev. Cell Dev. Biol. 22, 651–675.

Eisenmann, D.M., 2005. Wnt Signaling. WormBook, pp. 1–17.

Endo, Y., Rubin, J.S., 2007. Wnt signaling and neurite outgrowth: insights and questions. Cancer Science 98, 1311–1317.

Erskine, L., Herrera, E., 2007. The retinal ganglion cell axon's journey: insights into molecular mechanisms of axon guidance. Dev. Biol. 308, 1–14.

Flanagan, J.G., 2006. Neural map specification by gradients. Curr. Opin. Neurobiol. 16, 59–66.

Forsthoefel, D.J., Liebl, E.C., Kolodziej, P.A., Seeger, M.A., 2005. The Abelson tyrosine kinase, the Trio GEF and Enabled interact with the Netrin receptor Frazzled in *Drosophila*. Development 132, 1983–1994.

Fradkin, L.G., Noordermeer, J.N., Nusse, R., 1995. The *Drosophila*-3 is a secreted glycoprotein localized on the axon tracts of the embryonic CNS. Dev. Biol. 168, 202–213.

Fradkin, L.G., van Schie, M., Wouda, R.R., de Jong, A., Kamphorst, J.T., Radjkoemar-Bansraj, M., Noordermeer, J.N., 2004. The *Drosophila* Wnt5 protein mediates selective axon fasciculation in the embryonic central nervous system. Dev. Biol. 272, 362–375.

Fujisawa, K., Wrana, J.L., Culotti, J.G., 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.

Garbe, D.S., Bashaw, G.J., 2007. Independent functions of Slit–Robo repulsion and Netrin–Frazzled attraction regulate axon crossing at the midline in *Drosophila*. J. Neurosci. 27, 3584–3592.

Hamelin, M., Zhou, Y., Su, M.W., Scott, I.M., Culotti, J.G., 1993. Expression of the UNC-5 guidance receptor in the touch neurons of *C. elegans* steers their axons dorsally. Nature 364, 327–330.

Hao, J.C., Yu, T.W., Fujisawa, K., Culotti, J.G., Gengyo-Ando, K., Mitani, S., Moulder, G., Barstead, R., Tessier-Lavigne, M., Bargmann, C.I., 2001. *C. elegans* Slit acts in midline, dorsal–ventral, and anterior–posterior guidance via the SAX-3/Robo receptor. Neuron 32, 25–38.

Harris, R., Sabatelli, L.M., Seeger, M.A., 1996. Guidance cues at the *DrosophilaDrosophila*-6 homologs. Neuron 17, 217–228.

Hedgecock, E.M., Culotti, J.G., Hall, D.H., 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.

Hilliard, M.A., Bargmann, C.I., 2006. Wnt signals and frizzled activity orient anterior–posterior axon outgrowth in *C. elegans*. Dev. Cell 10, 379–390.

Hjorth, J.T., Gad, J., Cooper, H., Key, B., 2001. A zebrafish homologue of deleted in colorectal cancer, (zdcc) is expressed in the first neuronal clusters of the developing brain. Mech. Dev. 109, 105–109.

Hong, K., Hinck, L., Nishiyama, M., Poo, M.M., 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.

Honigberg, L., Kenyon, C., 2000. Establishment of left/right asymmetry in neuroblast migration by UNC-40/DCC, UNC-73/Trio and DPY-19 proteins in *C. elegans*. Development 127, 4655–4668.

Imondi, R., Thomas, J.B., 2003. Neuroscience. The ups and downs of Wnt signaling. Science 302, 1903–1904.

Inoue, T., Oz, H.S., Wiland, D., Gharib, S., Deshpande, R., Hill, R.J., Katz, W.S., Sternberg, P.W., 2004. *C. elegans*-18 is a Ryk ortholog and functions in parallel to LIN-17/Frizzled in Wnt signaling. Cell 118, 795–806.

Ishii, N., Wadsworth, W.G., Stern, B.D., Culotti, J.G., Hedgecock, E.M., 1992. UNC-6, a laminin-related protein, guides cell and pioneer axon migrations in *C. elegans*. Neuron 9, 873–881.

Keeble, T.R., Halford, M.M., Seaman, C., Kee, N., Macheda, M., Anderson, R.B., Stacker, S.A., Cooper, H.M., 2006. The Wnt receptor Ryk is required for Wnt5a-mediated axon guidance on the contralateral side of the corpus callosum. J. Neurosci. 26, 5840–5848.

Keino-Masu, K., Masu, M., Hinck, L., Leonardo, E.D., Chan, S.S., Culotti, J.G., Tessier-Lavigne, M., 1996. Deleted in Colorectal Cancer (DCC) encodes a Netrin receptor. Cell 87, 175–185.

Keleman, K., Dickson, B.J., 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, L.A., Technau, G.M., Dickson, B.J., 2002. Comm sorts robo to control axon guidance at the *Drosophila* midline. Cell 110, 415–427.

Keleman, K., Ribeiro, C., Dikson, B.J., 2005. Comm function in commissural axon guidance: cell-autonomous sorting of Robo in vivo. Nat. Neurosci. 8, 156–163.

Kennedy, T.E., Serafini, T., de la Torre, J.R., Tessier-Lavigne, M., 1994. Netrins are diffusible chemotropic factors for commissural axons in the embryonic spinal cord. Cell 78, 425–435.

Kennedy, T.E., Wang, H., Marshall, W., Tessier-Lavigne, M., 2006. Axon guidance by diffusible chemoattractants: a gradient of netrin protein in the developing spinal cord. J. Neurosci. 26, 8866–8874.

Kidd, T., Bland, K.S., Goodman, C.S., 1999. Slit is the midline repellent for the robo receptor in *Drosophila*. Cell 96, 785–794.

Kolodziej, P.A., Timpe, L.C., Mitchell, K.J., Fried, S.R., Goodman, C.S., Jan, L.Y., Jan, Y.N., 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, R.P., Aurandt, J., Guan, K.L., 2005. Semaphorins command cells to move. Nat. Rev. Mol. Cell Biol. 6, 789–800.

Leonardo, E.D., Hinck, L., Masu, M., Keino-Masu, K., Ackerman, S.L., Tessier-Lavigne, M., 1997. Vertebrate homologues of *C. elegans* -5 are candidate Netrin receptors. Nature 386, 833–838.

Leung-Hagesteijn, C., Spence, A.M., Stern, B.D., Zhou, Y., Su, M.-W., Hedgecock, E.M., Culotti, J.G., 1992. UNC-5, a transmembrane protein with immunoglobulin and thrombospondin type 1 domains, guides cell and pioneer axon migrations in *C. elegans*. Cell 71, 289–299.

Levy-Strumpf, N., Culotti, J.G., 2007. VAB-8, UNC-73 and MIG-2 regulate axon polarity and cell migration functions of UNC-40 in *C. elegans*. Nat. Neurosci. 10, 161–168.

Lindwall, C., Fothergill, T., Richards, L.J., 2007. Commissure formation in the mammalian forebrain. Curr. Opin. Neurobiol. 17, 3–14.

Liu, Y., Shi, J., Lu, C.C., Wang, Z.B., Lyuksyutova, A.I., Song, X.J., Zou, Y., 2005. Ryk-mediated Wnt repulsion regulates posterior-directed growth of corticospinal tract. Nat. Neurosci. 8, 1151–1159.

Logan, C.Y., Nusse, R., 2004. The Wnt signaling pathway in development and disease. Annu. Rev. Cell Dev. Biol. 20, 781–810.

Lu, W., Yamamoto, V., Ortega, B., Baltimore, D., 2004. Mammalian Ryk is a Wnt coreceptor required for stimulation of neurite outgrowth. Cell 119, 97–108.

Ly, A., Nikolaev, A., Suresh, G., Zheng, Y., Tessier-Lavigne, M., Stein, E., 2008. DSCAM is a netrin receptor that collaborates with DCC in mediating turning responses to netrin-1. Cell 133, 1241–1254.

Lyuksyutova, A.I., Lu, C.C., Milanesio, N., King, L.A., 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.

Maloof, J.N., Whangbo, J., Harris, J.M., Jongeward, G.D., Kenyon, C., 1999. A Wnt signaling pathway controls hox gene expression and neuroblast migration in *C. elegans*. Development 126, 37–49.

McMahon, A.P., Bradley, A., 1990. The Wnt-1 (int-1) proto-oncogene is required for development of a large region of the mouse brain. Cell 62, 1073–1085.

Merz, D.C., Culotti, J.G., 2000. Genetic analysis of growth cone migrations in *Caenorhabditis elegans*. J. Neurobiol. 44, 281–288.

Mitchell, K.J., Doyle, J.L., Serafini, T., Kennedy, T.E., Tessier-Lavigne, M., Goodman, C.S., Dickson, B.J., 1996. Genetic analysis of Netrin genes in *Drosophila*: Netrins guide CNS commissural axons and peripheral motor axons. Neuron 17, 203–215.

Mohamed, A.M., Chin-Sang, I.D., 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 III, E.B., Kelley, M.W., 2006. Noncanonical Wnt signaling and neural polarity. Annu. Rev. Neurosci. 29, 363–386.

Ogura, K., Goshima, Y., 2006. The autophagy-related kinase UNC-51 and its binding partner UNC-14 regulate the subcellular localization of the Netrin receptor UNC-5 in *Caenorhabditis elegans*. Development 133, 3441–3450.

Pan, C.L., Howell, J.E., Clark, S.G., Hilliard, M., Cordes, S., Bargmann, C.I., Garriga, G., 2006. Multiple Wnts and frizzled receptors regulate anteriorly directed cell and growth cone migrations in *Caenorhabditis elegans*. Dev. Cell 10, 367–377.

Prasad, B.C., Clark, S.G., 2006. Wnt signaling establishes anteroposterior neuronal polarity and requires retromer in *C. elegans*. Development 133, 1757–1766.

Quinn, C.C., Wadsworth, W.G., 2006. Axon guidance: ephrins at WRK on the midline. Curr. Biol. 16, R954–R955.

Quinn, C.C., Pfeil, D.S., Wadsworth, W.G., 2008. CED-10/Rac1 mediates axon guidance by regulating the asymmetric distribution of MIG-10/lamellipodin. Curr. Biol. 18, 808–813.

Quinn, C.C., Pfeil, D.S., Chen, E., Stovall, E.L., Harden, M.V., Gavin, M.K., Forrester, W.C., Ryder, E.F., Soto, M.C., Wadsworth, W.G., 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, B.J., 2000. Selecting a longitudinal pathway: Robo receptors specify the lateral position of axons in the *Drosophila* CNS. Cell 103, 1033–1045.

Round, J., Stein, E., 2007. Netrin signaling leading to directed growth cone steering. Curr. Opin. Neurobiol. 17, 15–21.

Sabatier, C., Plump, A.S., Le Ma, Brose, K., Tamada, A., Murakami, F., Lee, E.V., 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.

Seeger, M., Tear, G., Ferres-Marco, D., Goodman, C.S., 1993. Mutations affecting growth cone guidance in *Drosophila* genes necessary for guidance toward or away from the midline. Neuron 10, 409–426.

Serafini, T., Colamarino, S.A., Leonardo, E.D., Wang, H., Beddington, R., Skarnes, W.C., Tessier-Lavigne, M., 1996. Netrin-1 is required for commissural axon guidance in the developing vertebrate nervous system. Cell 87, 1001–1014.

Serafini, T., Kennedy, T.E., Galko, M.J., Mirzayan, C., Jessell, T.M., Tessier-Lavigne, M., 1994. The Netrins define a family of axon outgrowth-promoting proteins homologous to *C. elegans* -6. Cell 78, 409–424.

Silhankova, M., Korswagen, H.C., 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.

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.

Steven, R., Kubiseski, T.J., Kulkrarni, S., Mancillas, J., Ruiz-Morales, A., Hogue, C.W., Pawson, T., Culotti, J.G., 1998. UNC-73 activates the Rac GTPase and is required for cell and growth cone migrations in *C. elegans*. Cell 92, 785–795.

Strähle, U., Fischer, N., Blader, P., 1997. Expression and regulation of a Netrin homologue in the zebrafish embryo. Mech. Dev. 62, 147–160.

Su, M., Merz, D.C., Killeen, M.T., Zhou, Y., Zheng, H., Kramer, J.M., Hedgecock, E.M., Culotti, J.G., 2000. Regulation of the UNC-5 Netrin receptor initiates the first reorientation of migrating distal tip cells in *Caenorhabditis elegans*. Development 127, 585–594.

Tear, G., Harris, R., Sutaria, S., Kilomanski, K., Goodman, C.S., Seeger, M.A., 1996. commissureless controls growth cone guidance across the CNS midline in *Drosophila* and encodes a novel membrane protein. Neuron 16, 501–514.

Wadsworth, W.G., Bhatt, H., Hedgecock, E.M., 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.

Watari-Goshima, N., Ogura, K.I., Wolf, F.W., Goshima, Y., Garriga, G., 2007. *C. elegans*-8 and UNC-73 regulate the SAX-3 receptor to direct cell and growth-cone migrations. Nat. Neurosci. 10, 169–176.

Whangbo, J., Kenyon, C., 1999. A Wnt signaling system that specifies two patterns of cell migration in *C. elegans*. Mol. Cell 4, 851–858.

White, J.G., Southgate, E., Thomson, J.N., Brenner, S., 1976. The structure of the ventral nerve cord of *Caenorhabditis elegans*. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 275, 327–348.

Yeo, S.Y., Little, M.H., Yamada, T., Miyashita, T., Halloran, M.C., Kuwada, J.Y., Huh, T.L., 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, R.D., Kokel, M., Thomas, J.B., 2003. Wnt-mediated axon guidance via the *Drosophila* Derailed receptor. Nature 422, 583–588.

Yuan, W., Zhou, L., Chen, J.H., Wu, J.Y., Rao, Y., Ornitz, D.M., 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, J.A., Yi, B.A., Bargmann, C.I., 1998. The conserved immunoglobulin superfamily member SAX-3/Robo directs multiple aspects of axon guidance in *C elegans*. Cell 92, 217–227.

Zinovyeva, A.Y., Yamamoto, Y., Sawa, H., Forrester, W.C., 2008. Complex network of Wnt signaling regulates neuronal migrations during *Caenorhabditis elegans*development. Genetics 179, 1357–1371.