Gazzaley, A., Cooney, J.W., McEvoy, K., Knight, R.T., and D'Esposito, M. (2005). J. Cogn. Neurosci. *17*, 507–517.

Grill-Spector, K., Henson, R., and Martin, A. (2006). Trends Cogn. Sci. *10*, 14–23.

Manns, J.R., and Squire, L.R. (2001). Hippocampus *11*, 776–782.

Mehta, M.R. (2001). Neuroscientist *7*, 490–495.

Moores, E., Laiti, L., and Chelazzi, L. (2003). Nat. Neurosci. *6*, 182–189.

Mulligan, N.W. (1998). J. Exp. Psychol. Learn. Mem. Cogn. *24*, 27–47.

Paller, K.A., and Wagner, A.D. (2002). Trends Cogn. Sci. *6*, 93–102.

Ryan, J.D., and Cohen, N.J. (2004). J. Exp. Psychol. Hum. Percept. Perform. *30*, 988–1015.

Summerfield, J.J., Lepsien, J., Gitelman, D.R., Mesulam, M.M., and Nobre, A.C. (2006). Neuron *49*, this issue, 905–916.

Turk-Browne, N.B., Yi, D.-J., and Chun, M.M. (2006). Neuron *49*, this issue, 917–927.

Yi, D.-J., and Chun, M.M. (2005). J. Neurosci. *25*, 3593–3600.

DOI 10.1016/j.neuron.2006.03.008

Navigating the Anterior-Posterior Axis with Wnts

Recent studies have begun to shed light on the molecular guidance cues controlling anterior-posterior axon guidance. Two recent studies in the current issue of *Developmental Cell* show that Wnts play critical roles in patterning processes and directing neuronal migration in *C. elegans*. Together with previous findings in vertebrates and flies, these new results establish conserved function of Wnts in A-P guidance.

Despite their enormous numbers and complexity, axonal networks are extremely carefully organized. Much of the scaffold of the network is established during earlier developmental stages, when growth cones navigate in embryonic structures. Remarkably, early growth cone navigation largely follows the logic of embryonic patterning along the same major body axes: anterior-posterior (A-P) and dorsal-ventral.

Axon guidance cues along the dorsal-ventral axis are relatively well understood. For example, Netrins and Slits are conserved cues that play roles in directing growth cones along the dorsal-ventral axis as they grow toward or away from the midline (Dickson, 2002). Little has been known about the identity of the cues axons recognize along the A-P axis. This gap in understanding has begun to be filled with the realization that Wnt family proteins act as directional guidance cues in the A-P axis of the vertebrate spinal cord (Lyuksyutova et al., 2003; Imondi and Thomas, 2003; Liu et al., 2005; Dickson, 2005). Ascending sensory axons are attracted to higher concentration of Wnts anteriorly via Frizzled 3, a seven transmembrane domain receptor, and conversely, descending corticospinal tract axons are repelled from higher levels of Wnts via Ryk (Derailed), a Wnt receptor first found to mediate Wnt repulsion in *Drosophila*. A guidance role for Wnts is conserved in the *Drosophila* ventral nerve cord, where Wnt5 determines the pathway choice during midline crossing, allowing a subset of commissural axons to cross the midline only through the anterior commissure in each segment because they avoid Wnt5 in the posterior commissure via the Derailed receptor (Yoshikawa et al., 2003). Whether Wnts have a global A-P guidance role in *Drosophila* in addition to this intrasegmental A-P role is unknown.

In the current issue of *Developmental Cell*, two elegant papers provide compelling evidence that multiple Wnts act as directional cues to control the A-P migration of growth cones and neuronal cell bodies as well as the initial polarity of neuronal processes in *C. elegans* (Hilliard and Bargmann, 2006; Pan et al., 2006). A third independent study also led to the finding that Wnt signaling controls neuronal polarity in the A-P axis in *C. elegans* (Prasad and Clark, 2006). These new exciting findings not only establish a conserved role of Wnt family signaling proteins in axon patterning along the long axis in animals but also provide intriguing new insights into the diverse mechanisms neurons adopt to utilize the directional information provided by Wnts. Given the number of Wnt proteins and receptors and their multitude of mechanisms, this family of guidance cues may play a major role in circuit assembly along the A-P axis.

Wnt Signaling in A-P Guidance in C. elegans

The search for A-P guidance mechanisms in *C. elegans* was first conducted by investigating neuronal cell migration along the A-P axis. Cynthia Kenyon's laboratory identified *wnt/egl-20* as a gene required for normal A-P migration of the QL and QR neuroblasts, which give rise to sensory neurons on the left and right sides of the worm. *frizzled/lin-17* and *frizzled/mig-1* were also shown to be part of this regulatory system (Harris et al., 1996). Subsequent analyses established that QL and QR cells respond differently to EGL-20 in a dose-dependent manner, such that QL is more sensitive to EGL-20 than QR. High levels of EGL-20 promote posterior migration by activating the canonical Wnt gene expression pathway in QL, inducing expression of the Hox gene *mab-5* and a change in cell identity and A-P position (Maloof et al., 1999). Low levels of EGL-20 promote anterior migration of QR through a different and unknown pathway (Whangbo and Kenyon, 1999). Because the QR descendents do not require a localized source of EGL-20 to migrate anteriorly, EGL-20 was thought to be a permissive cue rather than a directional guidance cue for A-P cell migration (Whangbo and Kenyon, 1999).

Taking advantage of the wealth of mutant *wnt* and *frizzled* strains available in *C. elegans*, Gian Garriga's group from the University of California, Berkeley systematically analyzed the function of Wnts and Frizzled receptors in neuronal migration and axon guidance along the A-P axis in *C. elegans*. Remarkably, they found that all five Wnts and four Frizzleds in *C. elegans* function in neuronal migration and a subset of Wnts control anterior axon guidance, and at least Wnt/EGL-20 can function as a repellent that is sensed by Frizzled proteins. The hermaphrodite-specific neurons (HSNs), a pair of motor neurons that control egg laying, are born at the posterior end of the worm body and migrate anteriorly. Wnt/EGL-20 and Frizzled/MIG-1 are required for this anterior migration: mutations in either gene cause HSNs to terminate their migration posterior to their normal positions. Although single mutants of four other *wnt* genes, *cwn-1*, *cwn-2*, *lin-44*, and *mom-2*, showed few or no

posteriorly displaced HSNs, double mutants significantly enhanced *egl-20* mutant phenotypes. Through an impressive series of genetic analyses, involving a sensitized background that eliminated another signaling cell, the Garriga lab convincingly showed that EGL-20 expressed in the tail of embryos and larvae serves as a repellent for the HSN cell bodies. Similarly, single mutations of the *frizzled* genes, *mom-5* and *cfz-2*, and the Derailed/Ryk gene *lin-18*, had no effect on HSN on their own but enhanced the HSN migration defects of *mig-1/frizzled* mutants. Interestingly, another Frizzled gene, *lin-17*, appeared to antagonize *mig-1*, suggesting that Frizzled signaling has unexpected and unexplored complications.

Building on this observation, the Garriga lab found that redundant Wnts and Frizzleds direct axon pathfinding along the A-P axis. The AVM and PVM mechanosensory neurons each extend a single process that initially projects ventrally and enters the ventral nerve cord then turn anteriorly and grow along the A-P axis. None of the Wnt, Frizzled, or *lin-18/Ryk* single mutants showed significant defects in AVM and PVM axon guidance, but double Wnt and Frizzled mutants displayed several A-P guidance defects, including premature stopping, abnormal branching at the ventral nerve cord, and posterior turning at the ventral nerve cord. The repulsion of AVM and PVM growth cones by these Wnts is mediated by two Frizzled receptors, MIG-1 and MOM-5. Importantly, overexpression of EGL-20 showed that a localized source of EGL-20 is important for AVM/PVM process guidance. Ectopic anterior expression of EGL-20 from a *lim4* promoter caused AVM and PVM axon stopping and turning away from the EGL-20-expressing cells. Therefore, multiple Wnts may act as directional cues for axon and cell migrations along the A-P axis of the *C. elegans*.

Looking into the A-P patterning of two other mechanosensory neurons, PLM and ALM, Cori Bargmann's group at the Rockefeller University found a distinct and novel function of Wnt signaling in regulating neuronal polarity. The PLM neuron has a longer anterior process and shorter posterior process that extend directly from the cell body. The ALM neuron has one single anterior process that extends from the cell body. Wnt signaling is again important for the A-P disposition of these processes. However, rather than acting as either attractive or repulsive directional cues, Wnts appear to control neuronal polarity along the A-P axis and thus affect the A-P orientation of the processes. In both *lin-44* and *lin-17* mutants, the PLM anterior process was severely shortened, whereas the posterior process became much lengthened. Analyses of the synaptic vesicle protein SNB-1 indicated that the entire PLM neuron was reversed along the body axis, sending its synapses into the wrong process. In addition, *lin-44* and *lin-17* double mutants showed similar defects to single mutants, suggesting that *lin-44* and *lin-17* are in the same genetic pathway. The gene *lin-17* appears to function autonomously in the PLM, an important control for a *frizzled* gene that has many effects on cell fate patterning. What is interesting is that, in contrast to EGL-20 in HSN migration and AVM/PVM migration, the localization of LIN-44 appears to be relatively unimportant to the A-P orientation of the processes, for which LIN-44 might therefore act as a permissive cue. Alternatively, other Wns may also contribute to polarizing neuronal cell bodies along the A-P axis, because Wnt signaling was suggested to play an instructive role in polarizing nonneuronal cells along the A-P axis in *C. elegans* (Goldstein et al., 2006).

An elegant protein localization experiment from the Bargmann lab revealed a promising clue as to how Wnt signaling regulates neuronal polarity: a LIN-17-mRFP translational fusion protein was enriched in the PLM posterior process, suggesting that the Frizzled protein is distributed asymmetrically along the A-P axis. The asymmetric distribution of LIN-17 is dependent on LIN-44, although LIN-44 overexpression and misexpression had little effect on PLM neurons. For the ALM, located on the anterior side of the worm body, LIN-17 and LIN-44 are not important, but the redundant *wnts*, *cwn-1* and *egl-20*, have an analogous polarity-reversing effect on ALM development. It is currently unknown which Frizzled is involved in ALM, because *lin-17* mutants showed normal A-P polarity. However, overexpression of LIN-17 caused reversed ALM morphology. It is interesting that the A-P polarity phenotype appears reversed rather than randomized, suggesting that Wnt signaling may act as a balancing factor against another polarizing activity along the A-P axis.

Novel Insights into the Mechanisms of Wnt Signaling in A-P Guidance

These new studies help to establish that Wnt signaling is an ancient strategy in patterning axonal connections along the A-P axis, from mammals to nematodes. Meanwhile, several new insights have emerged from these results.

First, Frizzleds are diverse in function. So far, vertebrate studies have only identified attractive function of Frizzleds: Frizzled 3 mediates the attraction of commissural axons to Wnt4 and, probably, Wnt7b, during the anterior turning after midline crossing (Lyuksyutova et al., 2003), and several Frizzleds mediate the attractive response of dorsal retinal ganglion cell axons to low concentrations of Wnt3 in retinotopic mapping (Schmitt et al., 2006); similarly, in *Drosophila*, DFrizzled 2 mediates an attractive response of photoreceptor axons to DWnt4 in ventral lamina (Sato et al., 2006). Conversely, in *Drosophila*, Derailed mediates Wnt5 repulsion during midline pathway selection (Yoshikawa et al., 2003), and vertebrate Ryk mediates repulsion of corticospinal tract axons from Wnt1 and Wnt5a and the repulsion of retinal ganglion cell axons from Wnt3 (Liu et al., 2005; Schmitt et al., 2006). In *C. elegans*, it appears that two Frizzled receptors, MIG-1 and MOM-5, actually mediate repulsion by EGL-20. Therefore, Frizzleds can mediate repulsion as well as attraction. Furthermore, another Frizzled receptor, LIN-17, antagonizes the function of MIG-1. These interactions may add to the repertoire of Frizzled receptor functions in nervous system wiring. It will be informative to compare the sequence differences of these different Frizzled receptors in both *C. elegans* and in vertebrates to discern the molecular mechanisms of this diversity. In a new piece of cell biology reminiscent of planar cell polarity, Frizzleds control neuronal cell polarity along the A-P axis, which has major implication in the studies of neuronal polarity. The diversity of Frizzled

function suggests that interesting results will come from analysis of intracellular signaling cascades downstream of Frizzled in the nervous system.

Second, Frizzled proteins can be asymmetrically localized in neurons. The asymmetric localization of the Frizzled proteins may determine the initial A-P neuronal polarity and potentially the direction of further growth along the A-P axis. Although guidance cues are known to cause directional turning of growth cones, it is still unknown what intracellular asymmetry allows growth cones to turn along a certain direction in response to a guidance cue. In the case of cell polarity, a mechanism involving Frizzled protein localization provides a possible insight. However, this is the first example where a guidance receptor is differentially distributed in the direction of polarity and growth. It will be of great interest to test how other Frizzled proteins, such as MIG-1 and MOM-5, are distributed during the guidance of the AVM and PVM processes along the A-P axis.

Third, the direction of the Wnt gradients can be opposite in different animals. In mammals, Wnt-Frizzled signaling appears to respond to an anterior high, posterior low Wnt gradient (Lyuksyutova et al., 2003). In *C. elegans*, high posterior Wnt expression was observed. In the fly midline, Wnt5 is enriched in the posterior commissural in each embryonic segment, in a posterior high gradient, the same direction as in *C. elegans*. Therefore, although Wnt signaling has a conserved role in controlling A-P directionality, the direction of gradients can be distinct. In retinotectal map formation, vertebrate Wnt3 is expressed in a dorsal high, ventral low gradient and repels most of the retinal ganglion cell axons via Ryk (Schmitt et al., 2006). The *Drosophila* visual system also appears to use Wnt signaling for retinotopic mapping along the dorsal-ventral axis, except that DWnt4 is enriched in the ventral target and attracts photoreceptor axons via DFrizzled 2 (Sato et al., 2006).

New Questions

In recently years, the question of axon guidance along the A-P axis has received increasing attention. With the identification of these molecular cues, many new questions can be addressed.

Wnt signaling has been suggested as a conserved mechanism to convey A-P directionality. In addition, Shh was proposed to regulate A-P guidance in the chick (Bourikas et al., 2005; Stoeckli, 2006). It is interesting to ask whether all axons are guided by Wnts and Shh or whether there are additional guidance cues. Along the vertebrate dorsal-ventral axis, Netrin and Shh pull axons ventrally, and BMPs push axons from the dorsal side (Schnorrer and Dickson, 2004). Along the A-P axis, Wnts appear to be one major force to direct A-P growth. Are there any other forces that push and pull axons together with Wnts along this axis? Do Wnts and Shh act together on the same axons, or on different axons? In the case of cell polarity control in *C. elegans*, loss of Wnt signaling appears to reverse the anterior and posterior processes, suggesting that there is another opposing force and that, once the Wnt activity is eliminated, the other force, which is likely weaker, takes over. What is the other competing force? This unidentified force could be another A-P guidance mechanism. Yet another important question is how these axons stop in the right A-P position. Certainly, understanding these guidance systems along the long axis will significantly advance our knowledge of how the nervous system is wired into highly organized networks. In human spinal cord, there are over 40 different longitudinal tracts. Knowing how they are guided along the A-P axis will likely benefit the efforts of therapeutic design for spinal cord injury and regeneration.

Converging studies in mammals, flies, and *C. elegans* point to a common mechanism whereby directionality along the A-P axis may be conveyed by Wnt concentration gradients. Much remains to be done to explore the mechanisms of gradients in axonal guidance. Questions like how steep these gradients are in vivo, how they are detected to allow directional response of the growth cone, and how they are established will continue to stimulate investigation in years to come.

Yimin Zou[1]
[1] Department of Neurobiology, Pharmacology and Physiology
The University of Chicago
Chicago, Illinois 60637

Selected Reading

Bourikas, D., Pekarik, V., Baeriswyl, T., Grunditz, A., Sadhu, R., Nardo, M., and Stoeckli, E.T. (2005). Nat. Neurosci. *8*, 297–304.

Dickson, B.J. (2002). Science *298*, 1959–1964.

Dickson, B.J. (2005). Nat. Neurosci. *8*, 1130–1132.

Goldstein, B., Takeshita, H., Mizumoto, K., and Sawa, H. (2006). Dev. Cell *10*, 391–396.

Harris, J., Honigberg, L., Robinson, N., and Kenyon, C. (1996). Development *122*, 3117–3131.

Hilliard, M.A., and Bargmann, C.I. (2006). Dev. Cell *10*, 379–390.

Imondi, R., and Thomas, J.B. (2003). Science *302*, 1903–1904.

Liu, Y., Shi, J., Lu, C.C., Wang, Z.B., Lyuksyutova, A.I., Song, X., and Zou, Y. (2005). Nat. Neurosci. *8*, 1151–1159.

Lyuksyutova, A.I., Lu, C.C., Milanesio, N., King, L.A., Guo, N., Wang, Y., Nathans, J., Tessier-Lavigne, M., and Zou, Y. (2003). Science *302*, 1984–1988.

Maloof, J.N., Whangbo, J., Harris, J.M., Jongeward, G.D., and Kenyon, C. (1999). Development *126*, 37–49.

Pan, C.-L., Howell, J.E., Clark, S.G., Hilliard, M., Cordes, S., Bargmann, C.I., and Garriga, G. (2006). Dev. Cell *10*, 367–377.

Prasad, B.C., and Clark, S.G. (2006). Development, in press.

Sato, M., Umetsu, D., Murakami, S., Yasugi, T., and Tabata, T. (2006). Nat. Neurosci. *9*, 67–75.

Schmitt, A.M., Shi, J., Wolf, A.M., Lu, C.C., King, L.A., and Zou, Y. (2006). Nature *439*, 31–37.

Schnorrer, F., and Dickson, B.J. (2004). Curr. Biol. *14*, R19–R21.

Stoeckli, E.T. (2006). Curr. Opin. Neurobiol. *16*, 35–39.

Whangbo, J., and Kenyon, C. (1999). Mol. Cell *4*, 851–858.

Yoshikawa, S., McKinnon, R.D., Kokel, M., and Thomas, J.B. (2003). Nature *422*, 583–588.

DOI 10.1016/j.neuron.2006.03.004