HIGHLIGHTS

AXON GUIDANCE

Don't turn around

In the developing *Drosophila* nervous system, axons have to decide whether to project across, away from or parallel to the midline. Most choose to cross, and the crossing event is controlled such that each axon traverses the midline once only, before turning 90° to join one of the longitudinal nerve fascicles. The growth cone must therefore change its affinity for the midline as it crosses. Which molecules control this process? Growth cones are initially attracted to the midline by the Netrins, but as they traverse it, they become responsive to repulsion mediated by the Slit protein by upregulating expression of the Slit receptor Roundabout (Robo).

Unfortunately, this pleasingly straightforward model does not explain a key discrepancy between the *robo* and *slit* mutant phenotypes. In *slit* mutants, the axon scaffold collapses onto the midline, whereas in *robo* mutants, they cross and re-cross several times. If Slit and Robo were the only factors involved, both mutations should prevent axons from exiting the midline. So, there must be additional factors that prevent axons from lingering at the midline, but cannot overcome its attraction completely.

As reported in *Neuron*, Simpson *et al.* looked for *robo*-like sequences in the *Drosophila* database, whereas Rajagopalan *et al.* did a gain-of-function genetic screen for factors that abolish axonal crossing at the midline. This led to the identification of two new *robo* family members, *robo2* and *robo3*. Loss-of-function mutations in either gene cause midline crossing defects at low frequency, but, more strikingly, *robo/robo2* double mutants have a phenotype that closely resembles that of the *slit* mutation. Therefore, although *robo2* plays only a minor role in repelling axons into the longitudinal pathways, it provides enough repulsive response to stop axons from lingering at the midline.

But this is not all. Two papers in *Cell* from the same groups also showed that a Robo combinatorial code determines lateral positioning of axons. Axons in the most medial fascicle express Robo protein only, those in the intermediate fascicle express Robo and Robo3 and those in the lateral fascicle express all three Robos. The authors proposed that a gradient of Slit exists, with the highest levels closest to the midline, and that the combination of Robos expressed by an axon determines its sensitivity to Slit. The researchers did both gain- and loss-of-function experiments to find out whether manipulating the Robo code affected lateral axonal positioning. As predicted by the model, loss of *robo2* or *robo3* causes a medial bias in axon positioning, whereas ectopic expression of either protein on medial axons repels them further from the midline.

Therefore, this wealth of new information provides evidence that both Robo and Robo2 are necessary for Slit-mediated repulsion of axons at the midline, and that the interaction of the three Robos with a putative Slit gradient determines the distance that axons are repelled from the midline. This neatly shows how a relatively small number of factors can be used to coordinate an apparently highly complex sequence of events in neural development.

Heather Wood

References

ORIGINAL RESEARCH PAPERS Simpson, J. H. *et al*. Short-range and long-range guidance by Slit and its Robo receptors: Robo and Robo2 play distinct roles in midline guidance. *Neuron* **28**, 753–766 (2000) | Rajagopalan, S. *et al*. Crossing the midline: roles and regulation of Robo receptors. *Neuron* **28**, 767–777 (2000) | Simpson, J. H. *et al*. Short-range and long-range guidance by Slit and its Robo receptors: a combinatorial code of Robo receptors controls lateral position. *Cell* **103**, 1019–1032 (2000) | Rajagopalan, S. *et al*. Selecting a longitudinal pathway: Robo receptors specify the lateral position of axons in the *Drosophila* CNS. *Cell* **103**, 1033–1045 (2000)