Axon Targeting Meets Protein Trafficking: Comm Takes Robo to the Cleaners

Axon guidance at the *Drosophila* midline relies on dynamic regulation of the guidance receptor Robo by its negative regulator Comm. Recent findings demonstrate that Comm collaborates with the ubiquitin-protein ligase DNedd4 to inhibit Robo signaling by promoting the sorting of Robo into the endocytic pathway.

The journey of an axon to its target requires precise execution of navigational decisions. Central nervous system (CNS) axons that reach the midline must decide whether to project ipsilaterally (on the same side of the midline as their cell bodies) or contralaterally (across the midline). Over the past decade, work in the *Drosophila* CNS has uncovered an elegant system that governs axon guidance at the midline involving receptors of the evolutionarily conserved Roundabout (Robo) family and the Robo ligand Slit. This system is essential for controlling axon behavior at the midline (Kidd et al., 1999). In the absence of Robo or Slit, all CNS axons enter the midline and fail to exit it.

Both crossing and noncrossing axons rely on Robo/Slit signaling to govern their behavior. Slit is expressed at the CNS midline by glial cells, while CNS axons express Robo. Slit repels axons that express Robo, and this repulsion prevents ipsilaterally projecting axons from entering the midline. Robo/Slit signaling also keeps contralaterally projecting axons, which normally cross the midline only once, from reentering the midline once they have crossed it (see Figure). But if contralaterally projecting axons express Robo, how are they able to overcome Slit repulsion and cross the midline, and why do they lose this ability to overcome Slit repulsion after they cross?

Genetic evidence has indicated that the transmembrane protein Commissureless (Comm) is a powerful negative regulator of Robo proteins (Tear et al., 1996; Kidd et al., 1998). Comm overexpression strongly reduces Robo family protein expression, consistent with the idea that Comm acts by controlling Robo protein accumulation. This result is intriguing, as Robo protein normally fails to accumulate on the surfaces of axons within the midline. Nonetheless, the molecular mechanism by which Comm regulates axon guidance has been unknown. It was initially hypothesized that Comm produced by midline glia was transferred to crossing axons where it inhibited Robo protein expression. This suggested two possible models for how Comm controls midline crossing. According to one model, Comm would facilitate crossing of axons that express low, but not high levels of Robo. Ipsilaterally projecting axons would then express high levels of Robo, while contralaterally projecting axons would express low levels of Robo when crossing the midline, but high levels after crossing. Alternatively, axons could express equivalent levels of Robo, but differ in their sensitivity to Comm, with crossing

Model for the Regulation of Axon Midline Crossing by Comm

1. Contralaterally projecting neuron; 2, ipsilaterally projecting neuron; 3, commissure; 4, longitudinal axon tracks; 5, Slit-secreting midline glial cells; 6, late endosomes; 7, Golgi; 8, secretory vesicle.

(A–C) Axons of contralaterally projecting neurons but not of ipsilaterally projecting neurons cross the midline. Once contralaterally projecting axons cross the midline, they do not recross it.

(A') Molecular mechanism for regulation of Robo/Slit signaling by Comm.

(A) Growth cones of both ipsilaterally and contralaterally projecting neurons initially express Robo protein (green).

(B) Comm is expressed specifically in the contralaterally projecting neurons while they are crossing the midline. Once contralaterally projecting neurons cross the midline, they do not recross it.

Comm expression results in loss of cell surface Robo.

(B') Once Comm is expressed, it binds Robo and ubiquitin-protein ligase DNedd4. DNedd4 ubiquitinates Comm and/or an unidentified protein X (which associates with Comm). Ubiquitination facilitates the sorting of Comm and Robo from the Golgi directly into the late endosomes. Loss of surface Robo causes a loss of Slit signaling, allowing the contralaterally projecting growth cones to enter the commissures and cross the midline. Note that since ipsilaterally projecting neurons do not express Comm and thus remain sensitive to Slit (see A'), they do not cross the midline.

(C) After crossing is completed, Comm expression is extinguished, which restores Robo trafficking to the cell surface. The growth cones regain sensitivity to Slit (A'), and the continuous Robo/Slit signaling will prevent these axons from recrossing the midline.
axons being more sensitive. The work of Keleman et al. in the August 23 issue of Cell, as well as work by Myat et al. (2002) and Georgiou and Tear (2002), supports a different model, in which the transient expression of Comm in contralaterally projecting neurons transiently downregulates Robo signaling in these cells, permitting their axons to cross the midline once and only once.

Keleman et al. and Georgiou and Tear demonstrate that Comm function is required in CNS neurons for axon targeting. These authors also find that Comm is expressed in a cell type-specific fashion in CNS neurons. Comm is expressed in contralaterally but not ipsilaterally projecting neurons. Furthermore, Keleman et al. find that Comm expression is temporally regulated. Comm RNA is detected in contralaterally projecting neurons for a brief period of time, while their axons are crossing the midline. These results suggest that the regulation of Comm expression is critical for determining whether axons cross the midline.

Recent findings also shed light on the molecular mechanism by which Comm regulates Robo protein accumulation. Keleman et al. and Myat et al. find that coexpression of Comm and Robo in tissue culture cells alters Robo’s subcellular localization. In the absence of Comm, Robo protein accumulates at the cell surface; however, when both proteins are present, Robo colocalizes with Comm to intracellular compartments, which are probably late endosomes. As robust endocytosis of Robo protein is detected only in Comm’s absence, this change in Robo localization likely results from altered intracellular trafficking of Robo protein (Keleman et al., 2002). Keleman et al. also demonstrate that Comm needs to physically interact with Robo to affect Robo’s localization. These findings suggest that Comm prevents Robo from reaching the cell surface by binding Robo and targeting it directly to endosomes (see Figure).

How does Comm alter Robo trafficking? Myat et al. supply a piece of the puzzle by demonstrating that Comm binds the ubiquitin-protein ligase DNe4. Ubiquitin-protein ligases facilitate the conjugation of ubiquitin moieties to target proteins, a modification that can serve multiple functions including targeting proteins to the endocytic pathway (Hicke, 2001). Myat et al. demonstrate that perturbing the Comm/DNe4 interaction or DNe4 catalytic activity interferes with Comm’s regulation of Robo localization and with Comm’s gain-of-function activity at the midline. Consistent with this, Keleman et al. identify the region of Comm containing DNe4 binding sites as important for Robo sorting and for Comm’s in vivo overexpression phenotype. One model suggested by these findings is that ubiquitination of Comm (or of an associated protein) by DNe4 allows Comm to sequester Robo protein away from the cell surface by trafficking Robo from the Golgi to the endosomes. Interestingly, in Saccharomyces cerevisiae, polyubiquitination of the transmembrane protein Gap1p by the DNe4 homolog Rsp5p prevents cell surface accumulation of Gap1p by diverting it to the late endosome (Roberg et al., 1997; Helliwell et al., 2001).

These findings open several avenues of investigation. Since regulation of Comm expression appears to dictate whether an axon will cross the midline, it will be interesting to discover what governs the timing and cell type specificity of Comm expression. Future experiments should also identify the cellular machinery responsible for sorting the Robo/Comm complex to the endosome and determine whether there are additional targets of Comm. Also, given the evolutionary conservation of Robo/Slit signaling from worms to humans, might this mechanism for controlling Robo delivery similarly be conserved? More generally, how widespread is the divergence of receptors via selective trafficking? Axon guidance depends on dynamic temporal and spatial control of receptor signaling. These papers provide an elegant example of how the nervous system has taken advantage of an ancient strategy to meet these demands.

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Selected Reading


**Dual Role of Ang2 in Postnatal Angiogenesis and Lymphangiogenesis**

The maturation of the vascular system and the adjustment of blood vessel density in tissues require the opposing processes of vessel growth and regression. A new study in this issue of Developmental Cell shows that Angiopoietin-2 (Ang2), a ligand for the endothelial Tie2 receptor tyrosine kinase, has a dual function in the processes of postnatal angiogenesis and vascular remodeling. Also, Ang2 signals are required for the proper development and function of the lymphatic vessels.

Tissue metabolic needs dictate the blood vascular density of each organ. Tissue hypoxia is a powerful inhibitor of the prolyl hydroxylases that control the ubiquitin-mediated degradation of hypoxia-inducible transcrip-