A new study in this 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 contraterlatally 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 contraterlatally but not ipsilaterally projecting neurons. Furthermore, Keleman et al. find that Comm expression is temporally regulated. Comm RNA is detected in contraterlatally 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 DNedd4. 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/DNedd4 interaction or DNedd4 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 DNedd4 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 DNedd4 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 DNedd4 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 diversification 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-
Ang1 and platelet-derived growth factor (PDGF)-BB are involved in periendothelial cell (yellow) recruitment (arrow) and vessel stabilization, while Ang2 destabilizes these interactions in blood vessels (BV), allowing endothelial cells to respond to the angiogenic stimuli, such as VEGF. The results of Gale et al. (2002) now show that Ang2 is involved in recruitment of smooth muscle cells that are required for the stability and proper function of the collecting lymphatic vessels (LV). The growth factor-receptor signaling pathways are shown in this figure. Ang1 and Ang2 mediate their signals via the Tie2 receptor, while the various VEGFs bind to specific VEGF receptors and neuropilin (NRP) coreceptors. Via these receptors they regulate the development and function of the blood vessels (VEGF, PlGF, VEGF-B, Ang1, and Ang2) and lymphatic vessels (VEGF-C, VEGF-D, and Ang2).

Data from several experimental systems have led to a model in which Ang2 is presumed to destabilize blood vessels by interfering with the Ang1-Tie2 signals and with endothelial-periendothelial cell interactions (Holash et al., 1999). Ang2 upregulation, along with that of VEGF, is one of the first molecular changes in tumor angiogenesis, but, unlike VEGF, which is expressed in hypoxic tumor cells, Ang2 marks the neovascular endothelium (Holash et al., 1999). Endothelial Ang2 could inhibit Tie2 signals, thus promoting the sprouting of new vessels. On the other hand, a very strong induction of Ang2 is seen in the regressing corpus luteum during a phase of the menstrual cycle when VEGF levels have already declined and this expression is associated with vessel regression (Maisonpierre et al., 1997). In the presence of VEGF, the vessels destabilized by Ang2 would then undergo angiogenesis, but they would regress by endothelial cell apoptosis in the absence of VEGF (Holash et al., 1999). Transgenic Ang2 expression in the embryonic endothelium produces a similar phenotype as the deletion of the Tie2 gene, supporting the view that Ang2 is an antagonistic ligand (Maisonpierre et al., 1997). However, the effects of Ang1 and Ang2 are often similar in various cell culture models, suggesting that Ang2 is an agonist of Tie2 receptor signaling (Teichert-Kuliszewska et al., 2001).

By genetic deletion of Ang2, Gale et al. (2002) now provide further evidence that Ang2 is not redundant with Ang1 but that it has unique functions. They show that the developmentally programmed regression of the hyaloidal vasculature in the eye does not occur in the Ang2−/− mice and that their retinal blood vessels fail to sprout out from the central retinal artery. The development of the retinal vasculature, which occurs in response to hypoxia-driven VEGF expression (Alon et al., 1995), is clearly linked to the regression of the hyaloidal vascular system, as they share a common arterial supply from the optic nerve head. While the reason for the failure of hyaloidal vessel regression in the Ang2 knockout mice is not entirely clear, these data make Ang2 the first growth factor shown to be dispensable for the proper development of the blood vascular system, but necessary for postnatal angiogenesis and vascular remodeling. The blood vascular phenotype of the Ang2 knockout mice would then be consistent with the proposed model of Ang2 acting as a factor that primes endothelial cells for angiogenesis.
Nucleocytoplasmic Transport: More Than the Usual Suspects

A paper in the August 9 issue of Cell describes a novel role for the nucleoporin Nnap60/Nup50 as a soluble cofactor in importin-α-β-mediated nuclear protein import. These findings add a new dimension of complexity to the current understanding of protein transport pathways.

Nucleocytoplasmic transport, a signal- and energy-dependent process, takes place through nuclear pore complexes (NPC) that are embedded within the nuclear envelope. Import of cargo bearing the classic NLS motif can be broadly subdivided into the following steps. The NLS import receptor, a heterodimer of importin-α and importin-β proteins, initiates import by direct binding of the NLS via the importin-α subunit. The molecular details of passage through the NPC are still poorly understood. However, interactions between importin-β and the phenylalanine-glycine (FG) repeats of several distinct NPC components, also referred to as nucleoporins, are of the essence. Within the nuclear interior, importin-β encounters Ran, a small GTPase, in its GDP-bound state, resulting in the formation of an importin-β:RanGTP complex and the concomitant release of importin-α and NLS cargo. Importin-α is then bound by the nuclear export