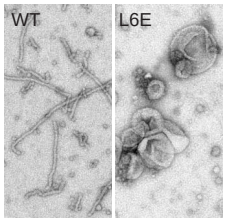
Bending the membrane

The budding of vesicles from membranes requires marked changes in the curvature of the membrane. How these changes are achieved and contribute to vesicle budding is an area of intense interest. One model invokes the enzymatic modification of membrane lipids, which alters membrane curvature by converting inverted-cone-shaped lipids to cone-shaped lipids. Others have proposed that proteins able to induce membrane tubulation by increasing curvature do so, at least in part, by virtue of forming oligomers on the membrane. Recent work from McMahon and colleagues now presents an attractive model for how epsin may promote the formation of clathrin-coated vesicles by directly bending the plasma membrane (*Nature* **419**, 361–366 (2002)).

Epsin 1 has been implicated in clathrin-mediated endocytosis. It interacts both with phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2) through its ENTH domain, as well as several components of the clathrin coat, and has been suggested to recruit and promote polymerization of clathrin. When added to liposomes containing PtdIns(4,5)P_2, epsin 1 can drive the formation of tubules. To better understand how this is achieved, the authors solved the structure of the epsin 1 ENTH domain in the presence of the PtdIns(4,5)P_2 headgroup, inositol-1,4,5-trisphosphate (Ins(1,4,5)P_3). This structure revealed a helix at the amino terminus of the ENTH domain (termed helix 0) that participates in the binding of Ins(1,4,5)P_3 but remained unstructured in a previously determined structure of the ENTH domain alone. Mutation of



helix 0 residues severely affects the ability of the ENTH domain to tubulate liposomes: a larger hydrophobic surface enhances the ability of epsin to tubulate lipsomes, whereas a smaller hydrophobic surface reduces this ability (see figure). In an assay employing lipid monolayers containing PtdIns(4,5)P_2, epsin is sufficient to cause clathrin-coated membrane invagination, and this activity is also critically dependent on the hydrophobic surface of helix 0.

How might helix 0 contribute to the induction of membrane curvature? As an amphipathic helix, helix 0 has the potential to interact with the membrane through its hydrophobic surface. The authors therefore suggest that helix 0 is inserted into the outer leaflet of the lipid bilayer, where it pushes the head-groups apart, thereby bending the membrane. They further speculate that the recruitment of clathrin and subsequent formation of a clathrin cage may stabilize this initial increase in membrane curvature. Adding to the existing ideas, this exciting new model will no doubt spur further research into the understanding of the forces underlying vesicle formation.

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are prevented from prematurely engaging axons. Robo could inhibit the sensing of local cues until the axon is far enough away from Slit to reduce its Robo signalling. This would relieve Robo-mediated inhibition of short-range cues and allow the appropriate longitudinal tract to be joined. Although no biochemical link has been found between Robo and Fas II, the work from Rhee *et al.*[2] uncovers a biochemical inhibition between Robo and another well-known short-range cue, N-cadherin.

N-cadherin is a homophilic cell adhesion molecule that regulates oriented axon outgrowth both *in vivo* and *in vitro*[8,9]. The work of Rhee *et al.* begins with the observation that activation of the Robo signalling cascade caused a specific decrease in N-cadherin-mediated adhesion[2]. The authors begin to fill in the molecular gaps by first examining the role of N-cadherin-associated proteins in the Robo response. In the adhesive state, N-cadherin is bound to β-catenin, which is itself bound to α-catenin, which in turn binds F-actin[10]. This bridge from N-cadherin to the actin cytoskeleton seems to be necessary for N-cadherin-mediated adhesion, as loss of the interaction between N-cadherin and β-catenin causes loss of adhesion[11,12]. Rhee *et al.* found that

Work from Rhee *et al.* in this issue of *Nature Cell Biology* describes convincing evidence for a biochemical inhibition of the cell adhesion molecule N-Cadherin by the guidance receptor Robo.

after activation of Robo, N-cadherin no longer immunoprecipitated β-catenin[2]. This loss of interaction is coincident with an observed increase in tyrosine-phosphorylated β-catenin. The authors go on to show that the tyrosine kinase Abelson (Abl) is constitutively associated with Robo and is the most probable candidate for phosphorylating β-catenin. Activation of Robo results in the formation of a complex consisting of Robo, Abl and N-cadherin, which may facilitate the tyrosine phosphorylation of β-catenin[2]. Although this is an *in vitro* study, there is existing evidence for some of these interactions *in vivo*. For example, in *Drosophila*, Abl interacts genetically with β-catenin and *D*E-Cadherin in various morphogenetic contexts[12,13]. Work in *Drosophila* has also suggested a genetic interaction for Abl and Robo, but one in which Abl inhibits the Robo pathway[14]. The work from Rhee *et al.* predicts that there will also be a positive role for Abl in Robo signalling, consistent with more recent studies[15].

What part might cross-pathway inhibition of N-cadherin by Robo play in axon guidance? One intriguing possibility is that it cooperates with the Robo–Slit pathway in the positioning of longitudinal pathways. The original 'Robo-code' model suggested that axons are first targeted by the Robo–Slit pathway and then local adhesion cues, such as Fas II, guide the axon to a final position[5,6,7]. This model, however, does not address the issue of gridlock. How is it that axons targeted to the lateral tract by their Robo signalling are not paralysed by the positive local adhesion cues in the medial and intermediate pathways that they must traverse? One possibility is that activation of Robo causes cross-pathway inhibition of all