Neuronal growth cones have detergent-resistant membranes (DRMs), specialized cell membrane fractions enriched in GPI-anchored proteins and intracellular signaling molecules. But are the DRMs involved in regulating neurite outgrowth? Yes, say Nakai and Kamiguchi (page 1097), who developed a new technique for selectively disrupting DRMs, and used it to demonstrate the functional importance of these structures in neuronal growth cones for the first time.

The authors modified a technique called micro-scale chromophore-assisted laser inactivation (micro-CALI) to specifically disrupt the integrity of DRMs in living cells. A ligand with attached dye was bound to GM1 ganglioside in the DRMs. Upon laser irradiation, the dye produced short-lived free radicals that disrupted nearby membrane structures.

Disrupting DRMs in the peripheral domain stops growth cone migration on L1 or N-cadherin substrates, but not on a laminin substrate. The cell adhesion molecules L1 and N-cadherin apparently require DRMs for normal functioning, whereas B1 integrin, which mediates growth on laminin and is not found in DRMs, does not. Disrupting DRMs in the central domain of the growth cone instead of the peripheral domain has no effect on growth cone migration.

The findings imply that homophilic binding of L1 or N-cadherin in the peripheral domain of the growth cone generates DRM-dependent signals that direct growth cone motility and shape. The new micro-CALI technique should be useful in studying other DRM-dependent signals, and the authors also hope to determine whether the growth cone DRMs are similar or identical to lipid rafts.

Making Dictyostelium stick

Using a clever genetic screen, Fey et al., reporting on page 1109, have identified the first cell–substrate adhesion molecule in the social amoeba Dictyostelium discoideum. The protein shares some structural features with adhesion molecules in higher eukaryotes, suggesting that the relatively simple amoeba will be a useful model system for understanding the contributions of substrate adhesion to cell movement.

The authors generated a panel of Dictyostelium insertional mutants, and then screened the transformants by repeatedly transferring them to new culture dishes. Only mutants that lacked the ability to attach to the dish were transferred. This screen identified nine independent substrate adhesion deficient (sad) mutants, one of which has now been characterized. sadA defines a novel gene encoding a molecule critical for cell–substrate adhesion in vegetative cells. GFP-tagged SadA protein localizes to the plasma membrane. In addition to multiple membrane-spanning regions, the protein’s predicted structure includes three EGF-like domains, suggesting that these domains may be an evolutionarily conserved feature of adhesion molecules.

Mammalian cell–substrate adhesion is more complex than one molecule, but the identification of nine genes essential for adhesion in Dictyostelium is an encouraging start. The authors are now characterizing the other Sad genes in an effort to determine how their gene products interact.

Arp2/3 gets inhibited

The Arp2/3 protein complex is a central regulator of actin assembly. Although a number of Arp2/3 activators have been identified in recent years, Humphries et al., on page 993, are the first to find a direct inhibitor of the complex: the highly conserved actin cytoskeleton component coronin. Using a combination of biochemical and genetic strategies, the authors found that the yeast coronin protein Crn1 physically and functionally interacts with the Arp2/3 complex in vivo via an evolutionarily conserved coiled-coil domain of Crn1. Although Crn1 strongly inhibits Arp2/3-mediated nucleation of actin in vitro, the addition of preformed actin filaments overrides this inhibition.

Based on the results, Humphries et al. propose that Crn1 may spatially restrict the activity of Arp2/3, permitting nucleation of new filaments only from the sides of existing filaments. This would promote the growth of a branched actin filament network like those often seen at the leading edges of motile cells. The mechanism by which coronin exerts this effect is not clear, although biochemical experiments have ruled out a simple steric block of Arp2/3 binding to either activators or actin.
How to start a motor

The longest journey may begin with a single step, but, in the case of myosin motor proteins moving along actin filaments, the start of the walking stride has been nearly impossible to observe. Now Burgess et al., reporting on page 983, reveal the prepower stroke conformation of myosin on actin, clearing a major hurdle to understanding myosin activity.

Previous work on myosin has focused primarily on myosin II, which has the unfortunate habit of dissociating from actin in the presence of ATP, making its prepower stroke conformation all but impossible to observe. As motor domains are highly conserved among different myosins, Burgess et al. looked at myosin V, a highly processive motor that drives mRNA, vesicle, and membrane trafficking. By combining electron microscope images of myosin V with crystallographic data from myosin II heads, the authors developed high resolution models of myosin conformations on and off actin and in the presence and absence of ATP.

The results provide a detailed model of myosin movement. When ATP is added to myosin V molecules not on actin, there is a gross change such that the myosin bends by ~90° at the junction of the motor domain and lever arm. When attached to actin, the leading head has a similar bent structure, but its attachment to the trailing head results in distortion either at the junction of the motor domain and lever arm or throughout the lever arm. When the trailing head detaches, the leading head straightens, and the release of this distortion, combined with the reversal of the bending induced by ATP, drives movement along the actin filament.

The work gives strong support to a longstanding hypothesis that ATP-driven shape changes within myosin heads generate motive force, but shows that cycles of distortion are also important.

Using acid to find direction

For a cell with signaling receptors distributed uniformly on its plasma membrane, deciding which direction to move in response to a stimulus is a serious problem. Earlier work traced this polarity decision to the amplification of phosphoinositide signaling at the cell’s leading edge. But on page 1087, Denker and Barber follow the signal back one step further, to the highly conserved ion exchange protein NHE1. The exchanger appears to be necessary not only for defining the front and rear of the cell, but also for coordinating events at the two ends.

The authors previously found that NHE1 is not only a sodium proton exchanger, but also a plasma membrane anchor for the cytoskeleton. Both functions are needed for PI-3 kinase activation and localization of ion exchange at the leading edge of lamellipodia.

In fibroblasts expressing a mutant form of NHE1 that is defective in ion translocation but retains the ability to anchor the cytoskeleton and localize to lamellipodia, focal adhesions appear to form, but they are not properly remodeled or disassembled. The result is fan-shaped lamellipodia and elongated cell tails, and an inhibition of migration. Cells expressing a form of NHE1 that is a functional ion transporter but defective in cytoskeletal anchoring and localization develop multiple pseudopodia extending in all directions, also slowing migration.

Others have shown that migratory receptors stimulate ion transport by NHE1, which should increase cytoplasmic pH at the leading edge and possibly activate actin-regulating proteins such as ADF/ coflin and gelsolin. As yet, changes in pH have not been seen in migrating cells, perhaps because the available techniques for measuring pH lack the necessary resolution. And other questions remain. It is not clear, for example, what determines the initial localization of NHE1 to the leading edge, or what signal NHE1 generates at the front of a cell to regulate events at the rear.