In This Issue

Visualizing vesicles

Barbero et al. have visualized the movement of individual vesicles from late endosomes to the trans Golgi by using GFP-marked Rab9 (page 511). Their results suggest that vesicles rather than tubules are used to deliver mannose 6-phosphate receptors (MPRs) in their return to the Golgi, after these proteins have transported enzymes from the Golgi to prelysosomes.

The detailed localization of the GFP-Rab9 is also telling. Rab GTPases are thought to contribute delivery address information for many trafficking vesicles, but Barbero et al. note that Rab9 is present on the endosome-derived vesicles from budding through docking. This is consistent with the group’s earlier experiments suggesting that Rab9 has a function during budding. Perhaps as part of this budding process Rab9 clusters in particular regions, as the authors note that Rab9 and Rab7 (a GTPase involved in late endosome fusion) are present in largely nonoverlapping domains within a given late endosome.

Once Barbero et al. have overcome some protein engineering problems, they hope to visualize MPRs and the Rab9-binding cargo collector TIP47. They predict that MPRs will be present in the transport vesicles, and that TIP47 will be released from the vesicles after budding, perhaps to be replaced by an unknown docking factor.

Monitoring MLCK

Myosin light chain kinase (MLCK) is a busy protein—it phosphorylates myosin’s regulatory light chain (and thus activates myosin) during nonmuscle cell contraction, cytokinesis, stress fiber formation, and motility. In general, MLCK is known to be present at the cellular sites implicated in these events, but the results of Chew et al. (page 543) are some of the first hints at the dynamics of that localization and the in situ activity of the kinase.

Chew et al. track MLCK by adding a module with a calcium–calmodulin-binding domain flanked by BFP and GFP. In the absence of calcium, the BFP and GFP are close enough to each other to allow fluorescence resonance energy transfer (FRET) between them. When calcium–calmodulin binds, however, this disrupts FRET. Chew et al. suspect that when there is sufficient calmodulin to bind to the added cassette, there will also be calmodulin binding to a domain in MLCK, an event that activates the kinase. Thus, the absence of FRET is interpreted as a sign of an activated kinase.

The authors measure both the absolute abundance of MLCK (by exciting GFP directly), and the kinase activity (by measuring FRET from BFP to GFP). In motile cells, they find that active MLCK is prominent in the leading lamella, where myosin is present only in small clumps. Activation of myosin may help pull it into larger aggregates, and drag the nucleus toward the front of the cell. At the back of the cell, however, there is less active MLCK, suggesting that other myosin activators, such as Rho kinase, may help in pulling up the back of the cell.

Meanwhile, MLCK can be seen transiently associating with and activating on stress fibers, and localizing at the equator of dividing cells before it gets activated. Chew et al. suspect that a similar technique for monitoring both abundance and activity may be useful for other calmodulin-regulated kinases.