A challenge to conventional thinking has been met and answered by two new articles in this issue. Hua et al. (page 1035) and Paladino et al. (page 1023) have imaged live three-dimensional cells to reaffirm that a class of proteins takes a direct route to the apical membrane.

The suggestion that GPI-linked apical proteins traffic directly from the Golgi to the apical plasma membrane was made—and widely accepted—years ago based on classical biochemical experiments. But a recent article proposed instead that, in MDCK cells, this class of proteins were delivered first to the basolateral membrane, and then endocytosed and sent across the cell to the apical membrane. Striking images were presented to support this view, but the work also involved a somewhat controversial new assay.

The new papers suggest that the new assay may have been problematic. Paladino found that it caused a partial depolarization of the cells. Both new papers also used live cell imaging to track the transport of newly synthesized GPI-anchored proteins. This required some substantial technical breakthroughs. Most live cell imaging is performed on flat, nearly two-dimensional cell preparations. But fully polarized MDCK cells are at least 10-μm tall, meaning multiple focal planes have to be imaged all at once.

To minimize the main problem of photobleaching, Paladino et al. used the quicker spinning disc confocal technology, but this approach did not permit quantitation. Hua and colleagues stuck with laser scanning microscopy, optimizing every parameter, and carefully quantified the dynamics of transport.

Although resolution was limited in both strategies, fluorescent signals can be followed for the longer periods of time required for trafficking through the taller cells. Both groups saw GPI-GFP move directly from the Golgi, through the trans-Golgi network (TGN), and to the apical membrane. Very little protein ever reached the basolateral membrane.

These new imaging techniques, although powerful in resolving this controversy, may really shine when used to test the requirement for putative trafficking components. JCB

In This Issue

GPI-linked proteins (green) move directly from the TGN to the apical membrane (left to right).

A MAP’s stabilizing mechanism

The Stu2p microtubule-associated protein (MAP) clamps around tubulin heterodimers, as revealed by electron micrograph images from Al-Bassam et al. (page 1009). Stu2p and other members of the XMAP215 family may thus capture and deliver tubulin to the growing ends of microtubules.

Although Stu2p stabilizes microtubules in vivo, it has a destabilizing effect in vitro. This as-yet unexplained difference has made it difficult to determine how the XMAP215 family works at a mechanistic level. The new studies suggest that their stabilizing ability stems from their interaction with free tubulin α/β heterodimers.

Tubulin heterodimers, the authors found, associate with dimers of Stu2p, as shown by affinity chromatography. This association is necessary for Stu2p’s stabilizing ability, as a mutant that bound to filament ends but not to tubulin dimers caused microtubule shortening. Electron micrographs revealed that the long, open dimers of free Stu2p closed up and clamped around tubulin upon binding.

Earlier experiments suggested that Stu2p is transported along microtubules to the growing ends. If Stu2p brings along its captured tubulin, it would increase the local concentration of available new subunits. Microtubule stabilization by the XMAP215 family might therefore result from increased polymerization rates.

The authors are now trying to trim down the Stu2p–tubulin complex into a version that can be crystallized. They hope that higher resolution images will identify interactions that trigger the release of tubulin and free Stu2p for another round of capture. If the interaction is stronger in vitro than in vivo, Stu2p might sequester rather than deliver tubulin, thereby explaining its in vitro destabilizing effects. JCB

Elongated Stu2p (top), upon binding tubulin, forms a compact complex (bottom).