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.

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.
**Escaping a checkpoint**

On page 999, Holway et al. reveal that worm embryos would rather risk genomic mutation than disturb the timing of cell division. DNA damage–induced checkpoints, the group finds, are silenced during early embryogenesis.

The need for the silencing arises because of the importance of timing in embryogenesis. At the worm’s two-cell stage, for instance, a two-minute lag between the division times of the two cells is needed for germ line formation. Embryogenesis fails if this lag is extended, as would be expected upon DNA damage.

Holway and colleagues found, however, that genomic damage from UV or MMS treatments did not stall division in worm embryos. In somatic cells, DNA lesions block the progress of replication forks, whose stalling initiates the damage checkpoint. But replication forks advanced directly through the lesions in the embryos. This bypass required a translesion polymerase called Polh-1, which is able to synthesize DNA opposite damaged bases. The high expression levels of Polh-1 in embryos might at least partly explain their unusual ability to silence the checkpoint.

Fly and frog embryos do not actively silence the DNA damage checkpoint; mammalian systems have not yet been tested. Based on these findings, however, one might speculate that tumor cells that are resistant to chemotherapy might have acquired a heightened capacity for translesion synthesis.

**Serum keeps order in the wound**

Skin cell types enter wounds at different times to first seal and then heal. This ordering is directed by the antimigration effect of TGFβ3, according to Bandyopadhyay et al., on page 1093.

The first cells to enter an injured site are epidermal keratinocytes, which migrate across to resurface and close the wound. Later come the deeper dermal cells, including fibroblasts and endothelial cells. Keratinocytes must migrate ahead of dermal cells, or the more aggressive fibroblasts will push them aside, causing scarring or even preventing healing.

The new results show that dermal cells, but not keratinocytes, are temporarily held at bay by the action of a serum-derived factor called TGFβ3. Intact skin is nourished by plasma. But when skin is wounded, plasma converts to serum—a complex mixture of plasma proteins plus newly released cytokines and proteinases.

Unlike plasma, serum is known to contain TGFβ. The TGFβ3 isoform, the group found, halted dermal cell migration. This response depended on high levels of the TβRII receptor on dermal cells. Keratinocytes, on the other hand, had low levels of TβRII and thus were able to migrate in the serum environment.

During healing, serum is replaced by plasma, which the group’s study suggested stimulated dermal cell migration to repair and remodel the wound interior. Chronic problems with wound healing might stem from a disorderly entry of fibroblasts or from the sluggish migration of keratinocytes or dermal cells.

**Neurons survive with NCS-1**

Calcium is a favorite messenger of neurons—it regulates neurotransmission, signal transduction, and synapse plasticity. The calcium-binding protein NCS-1 translates increased intracellular calcium levels into many of these downstream outcomes. The new results show that NCS-1 also activates antiapoptotic pathways in neurons.

Neuronal apoptosis can result from injury or infection. Neurons often survive these insults, however, thanks to activation of the Akt survival pathway by neurotrophic factors such as GDNF. The authors show that this effect requires NCS-1, which is induced by GDNF. When activated by calcium (whose cytoplasmic levels probably increase upon injury), NCS-1 is known to activate phosphoinositide kinases that produce Akt substrates, thus jumpstarting the survival pathway.

Loss of NCS-1 hastened cell death in injured rat brains. It also hampered the long-term survival of neurons under normal culture conditions, suggesting that low levels are required even in the absence of injury. As injury strongly increases the levels of NCS-1, its overexpression did not improve survival further. But in diseases in which neuronal survival and regeneration is impaired, boosting NCS-1 activity might have beneficial effects.