In This Issue

Two motors, one transmission

Kinesin II binds to a variety of organelles and transports them toward the plus ends of microtubules—but how are the organelles linked to the motor? On page 297, Deacon et al. provide the surprising answer that kinesin II uses the dynactin complex, an attachment system that is also used by the minus end–directed motor protein dynein. The results provide the first direct physical evidence that anterograde and retrograde organelle transport are coordinated and identify dynactin as a possible master integrator for both systems.

Using Xenopus melanophores, the authors studied the bidirectional transport of melanosomes, pigment-containing organelles that are loaded with both kinesin II and dynein motors. Biochemical analysis of this system shows that the XKAP subunit of kinesin II interacts with the p150Glued subunit of dynactin. p150Glued cannot bind to both dynein and kinesin II at the same time, suggesting that the two motors compete for attachment to the organelle. This competition might be a novel mechanism ensuring that each melanosome recruits equal amounts of each motor, and differential phosphorylation of these motors could then determine whether anterograde or retrograde movement is dominant. Dynactin could therefore mediate organelle capture while coordinating motor selection, binding, and processivity.

A model of missegregation

Most human tumors have an abnormal number of chromosomes, implying that something has gone wrong with their mitotic checkpoint systems, but the genes encoding checkpoint proteins are rarely mutated in tumor cells. On page 341, Babu et al. provide a possible explanation for this apparent contradiction and describe new mouse models that should be useful in studying cancer progression.

The authors disrupted two highly homologous mouse genes, encoding the nuclear transport factor Rae1 and the checkpoint protein Bub3. Homozygous null mutations in either Rae1 or Bub3 are embryonic lethal. Heterozygous mice with only one copy of Rae1 survive, but exhibit mitotic checkpoint defects and chromosome missegregation, and are predisposed to carcinogen-induced lung cancer. Heterozygous Bub3 knockout mice have a strikingly similar phenotype. Surprisingly, overexpressing Rae1 in the mice compensates for haploinsufficiency of either Rae1 or Bub3, indicating significant overlap in the two proteins’ functions.

The results show that the mammalian mitotic checkpoint system is extremely sensitive to underexpression of its components. Epigenetic effects in tumor cells might start a vicious cycle, in which down-regulation of a checkpoint protein causes chromosome loss, leading to the loss of other checkpoint proteins and further chromosome missegregation. The new strains can now be crossed with existing mouse cancer models to study aneuploidy in tumor pathogenesis.

Multimembrane fusion

Yeast mitochondria undergo both fission and fusion events, leading to the formation of branched, reticular structures within cells. Although mitochondrial fission has been characterized relatively well, studies on fusion have been considerably more difficult. In an elegant genetic and biochemical analysis, Wong et al. (page 303) now address some previously conflicting data and establish a new model for the coordination of this complex process.

Previous studies established that the integral mitochondrial outer membrane proteins Fzo1p and Ugo1p are essential for fusion and that the dynamin-related GTPase Mgm1p might be indirectly involved in the process. The new work demonstrates that Mgm1p is in fact an essential component of a fusion complex that also includes Fzo1p and Ugo1p and that Mgm1p functions as a self-assembling GTPase in fusion. Using a novel protease-based technique, the authors also resolve a controversy about the localization of Mgm1p, showing that the protein localizes to the mitochondrial intermembrane space.

The data support a model in which Mgm1p in the inner membrane and Fzo1p and Ugo1p in the outer membrane coordinate the reorganization of both membranes to lay the groundwork for fusion. The authors are now identifying additional regulatory molecules that appear to target Mgm1p.
Urge to merge erects filopodia

Crawling cells often produce both broad, flat lamellipodia and long, thin filopodia. Once formed, both structures appear to use an actin-treadmilling mechanism to extend themselves. During treadmilling, lamellipodia continuously nucleate new filaments, but filopodia continue to use the same filaments. So how does a cell form a filopodium in the first place? Svitkina et al., page 409, address this question with a detailed kinetic and structural analysis, presenting a novel model that should help to guide future work in the field.

Using GFP-tagged versions of the Arp2/3, VASP, and fascin proteins and innovative electron microscopy techniques, the authors examined the molecular dynamics of filopodium initiation in mouse melanoma cells. Rather than forming a distinct nucleation complex, Arp2/3 seems to lay the groundwork for filopodium birth by producing a normal lamellipodial actin array. Conical structures, termed $\Lambda$-precursors, then rise up from this array. VASP gradually coalesces from a broad distribution along the lamellipodial edge into defined spots at the tips of the $\Lambda$-precursors. Fascin then abruptly appears at the $\Lambda$-precursor vertex before filopodia grow from these structures.

The data suggest that, in a normal dendritic actin network in the lamellipodium, the elongating barbed ends of certain actin filaments bind to a set of molecules that protect them from capping and mediate their association with other elongating barbed ends. When these privileged filaments collide, they merge to form the conical $\Lambda$-precursors, and their tip complexes then recruit or activate the bundling protein fascin, allowing filament cross-linking and the growth of long, strong actin bundles characteristic of filopodia. This model also implies that microspikes, and retraction fibers—filopodium-like structures seen in some migrating cells—arise by a similar mechanism and are interconvertible with filopodia.

In separate work, the authors have developed an in vitro system that mimics this convergent elongation process. They are now trying to identify proteins in the filopodial tip complex that regulate initiation.

Anthrax toxin takes charge

Bacterial toxin proteins are generally thought to enter eukaryotic cells by passive hitchhiking, binding to a convenient receptor that is constitutively internalized by endocytosis to ensure their delivery to the cytoplasm. But on page 321, Abrami et al. show that, at least in the case of anthrax, the toxin precisely choreographs its uptake by hijacking a specific endocytic pathway. The work suggests a possible strategy for treating anthrax infections and also highlights a connection between raft-mediated and clathrin-dependent endocytosis, two pathways that were once considered to be mutually exclusive.

*Bacillus anthracis* produces a toxin with three subunits. After binding to the cellular anthrax toxin receptor, a transmembrane protein of unknown function, the protective antigen subunit undergoes cleavage and heptamerization and associates with lipid rafts, whereas the uncleaved protein does not. Artificially clustering the anthrax toxin receptor with an antibody sandwich also targets it to rafts and stimulates its endocytosis. Uptake of the receptor is both raft mediated and clathrin dependent. This latter finding dovetails with another recent report, which also demonstrated a connection between rafts and clathrin during endocytosis of the B cell receptor.

The results indicate that protective antigen, far from being a passive hitchhiker, actively initiates the clustering of a cell surface receptor, sending a signal that induces its uptake. This spatial reorganization of the receptor on the cell surface could be a promising target for novel therapies.