Myo6 gets choosy

Stimulated neurons that lack myosin VI (Myo6) fail to endocytose a subtype of glutamate receptors called AMPARs, report Osterweil et al. on page 329. However, the neurons do not have a general endocytosis defect, suggesting a role for Myo6 in specific endocytic events.

Unlike other myosins, Myo6 moves toward the minus ends of actin filaments. Thus, in polarized cells or cell regions, such as the dendritic spines of neurons, Myo6 moves toward the inside of the cell. Dominant-negative mutants of Myo6 have a generalized defect in endocytosis, but this may be explained by Myo6’s interaction with AP2, a clathrin adaptor protein, rather than Myo6’s normal function.

Osterweil et al. turned instead to Myo6 mutant mice (sv/sv animals). Neurons in the hippocampi of these mice made fewer synaptic connections and had shorter dendritic spines than normal. Additionally, when the researchers stimulated sv/sv neurons in culture with either AMPA or insulin, both of which cause endocytosis of AMPARs, the cells showed less AMPAR internalization than control cells. The sv/sv cells were normal for transferrin endocytosis, suggesting that only specific endocytic targets are affected.

In ongoing work the group finds that only some endocytic events are blocked in other organs of sv/sv animals, suggesting Myo6 may regulate specific endocytosis in a variety of cell types.

A GTP signal to the nucleolus

Nucleolar size and cell growth rate are positively correlated, perhaps based on ribosome biogenesis being localized to the nucleolus, but little is known about how nucleolar size is controlled. One possible mechanism is reversible localization of key regulators. On page 179, Tsai and McKay identify the mechanism controlling one such localization system. They find that nucleostemin, a nucleolar protein found preferentially in stem cell and cancer cells and required for them to remain in the cell cycle, localizes to the nucleolus when it is GTP bound.

Based on mutants, three regions of nucleostemin affect nucleolar localization. An NH2-terminal basic region conferred short-lived nucleolar binding, whereas an internal domain appeared to inhibit entry into the nucleolus. However, this inhibitory function was turned off when GTP was bound to the third region, a GTP-binding domain. Furthermore, mutations that blocked GTP binding reduced nucleolar localization, as did the addition of an inhibitor of GTP biosynthesis.

Together, the data suggest that the inhibitory domain and the GTP-binding domain work together as a gating mechanism to control nucleostemin’s entry into the nucleolus, and that GTP is the switch that opens the gate. The use of GTP to control nucleolar localization enables the cell to transmit information regarding the surrounding environment to the nucleolus via cell-signaling pathways, and may provide a mechanism to link growth signals with the size and activity of the nucleolus.

Growth and trafficking

The lipid phosphatase Sac1p inhibits transport out of the Golgi and, based on its expected target, should increase transport out of the ER. Now, Faulhammer et al. find that Sac1p switches from its ER role to the Golgi role in response to lower nutrient levels, thus reducing trafficking and providing an initial link between growth and secretion (page 185).

Faulhammer et al. found that Sac1p localized to the ER during exponential growth and was retained there by a direct interaction with an integral ER membrane protein called dolichol phosphate mannose synthase, or Dpm1p. When the researchers transferred these fast growing cells to nutrient-poor media, Sac1p moved to the Golgi within minutes.

Sac1p was physiologically active in both compartments, a point that has previously been missed by researchers focusing on fast-growing cells. In the ER, the protein stimulated trafficking, but in the Golgi, it reduced the amount of phosphatidylinositol-4-phosphate and slowed vesicle traffic.

It is not yet clear what alteration in Dpm1p or Sac1p facilitates relocation of Sac1p to the Golgi during nutrient deprivation, but the rapid response suggests that it is likely to be some sort of protein modification event. Two major signaling pathways, TOR and RAS, are already known to sense nutrient availability, making them likely candidates for upstream regulators of Sac1p shuttling.
Actin-myosin tears it up

Caspase cleavage of nuclear lamin proteins was thought to be sufficient to cause disintegration of the nucleus during apoptosis. Now, Croft et al. (page 245) show that the actin-myosin cytoskeleton generates a pulling force required to tear apart the structure.

When studying the role of actin and myosin in plasma membrane blebbing and cell contraction during apoptosis, the team noticed that if they inhibited ROCK I, a kinase that increases actin contractility and is activated by caspase cleavage, DNA was efficiently fragmented but did not end up in the expected biochemical fractions. The team hypothesized that ROCK I and the actin cytoskeleton might be involved in apoptotic nuclear breakdown.

In the current study, Croft et al. found that after blocking ROCK I activity and thus myosin light chain (MLC) phosphorylation, the nuclear envelope remained intact. Thus, the actin-myosin cytoskeleton is required for apoptotic nuclear breakdown, even though it is microtubules that do the similar job of nuclear envelope breakdown during mitosis. Furthermore, transfecting cells with a mutant form of MLC that prevents actin bundling and contraction prevented both plasma membrane blebbing—as expected from previous results—and nuclear envelope breakdown. The team also found that myosin ATPase activity, which catalyzes shortening of actin filaments, was required for nuclear breakdown.

When the team blocked caspase cleavage of nuclear lamin proteins, ROCK I activity altered nuclear morphology but wasn’t sufficient to cause nuclear breakdown. Conversely, in cells lacking intact lamin proteins, ROCK I activation was enough to break apart the nucleus.

The team concludes that nuclear breakdown requires two cellular processes: contraction of the actin-myosin cytoskeleton, which results from ROCK I activation, and caspase cleavage of lamin proteins. What isn’t yet clear is how the actin cytoskeleton links to the nuclear membrane. JCB

Modeling distinct compartments

Einhrich and Rapoport (page 271) have used mathematical modeling to generate the first explanation of how a bidirectional transport system can generate unique compartments, such as the ER and Golgi, despite constant vesicle movement between them. The model provides testable predictions about the vesicle transport system in cells.

In modeling a two-organelle system, the team found that they only needed to include two molecular components of the vesicle transport system: the coat proteins for budding; and the SNARE proteins for fusion. Coat proteins regulate budding from distinct compartments: COPI from the Golgi (to the ER); and COPII from the ER (to the Golgi). Meanwhile, SNARE proteins work in pairs, with a v-SNARE localized in the vesicle membrane and the t-SNARE in the target membrane. Different SNAREs direct vesicle fusion to specific organelles.

The new model only worked when it incorporated differential affinity of one coat protein for one set of SNARE proteins versus the other. If both coat proteins bound all SNAREs with equal affinity, then bidirectional vesicle transport would result in two uniform organelles. If the model assumed that one coat protein bound one pair of SNAREs with at least a 10-fold preference over the other SNARE pair, then the model accurately maintained two unique compartments.

One nonintuitive aspect of the model is that it predicts that both members of a SNARE pair, the v- and t-SNAREs, accumulate in the target compartment, a prediction borne out by experimental observations from other groups.

COPII is known to have high affinity for SNAREs that target the Golgi, and the model predicts that COPI should preferentially bind to ER SNAREs. If, however, the coat protein’s binding affinity for a SNARE pair is too strong, then all of those SNARE proteins would accumulate in the target compartment, leaving vesicles to bud from the other compartment without any SNAREs that would allow it to fuse to the target membrane. The model also predicts that if one SNARE pair is overexpressed, then the size of its target compartment would increase. JCB