**Research Roundup**

**Tearing down the spindle**

Disassembly of the mitotic spindle takes some effort, according to Kan Cao, Yixian Zheng (Johns Hopkins University, Baltimore, MD), and colleagues, who show that an ATPase and its binding partners pull apart the spindle.

The spindle is put together by assembly factors that are activated by the cyclin-dependent kinase Cdc2. The loss of Cdc2 activity at the end of mitosis has thus been the conventional explanation for spindle disassembly. “It was thought that dephosphorylation of mitotic spindle assembly factors changed microtubule dynamics directly and led to the transition into the interphase array,” says Zheng. But her group finds that Cdc2 inactivation is not enough—another pathway is required to dismantle the spindle.

This pathway is organized by a complex that includes the p97 AAA-ATPase. The authors suspected p97 involvement because loss of its yeast homologue, Cdc48, causes cell cycle arrest and leaves spindles partly assembled. The group now shows that p97/Cdc48 and its adaptor proteins, Ufd1 and Npl4, take apart the spindle by removing proteins that promote its assembly.

At the end of mitosis, p97 was found to bind to XMAP215 and TPX2—two frog spindle assembly factors—and the spindle-associated kinase, Plx1. The binding prevented much of XMAP215 and TPX2 from accumulating on microtubules. In yeast cells, Cdc48 targeted similar assembly factors for degradation by the proteasome. Without p97/Cdc48 ATPase activity, yeast cells or frog oocytes that had interphase levels of Cdc2 and cyclin B were unable to dismantle mitotic spindles. Microtubules remained dynamic rather than switching to the stable growth needed for interphase array formation.

ATPases related to p97/Cdc48 are chaperones involved in protein unfolding. The p97/Cdc48 complex, however, probably uses its ATPase activity to extract proteins from the spindle and target them for degradation, as it is known to do to membrane-bound proteins at the ER.


**Receptors make a quick exit**

When neurons sense a long-term need for more glutamate receptors, they make a form that speeds through the trafficking network to get to the synapse quickly, according to results from Yuanyue Mu, Michael Ehlers, and colleagues (Duke University, Durham, NC).

Regulating the number of synapse-localized glutamate receptors, including those of the NMDAR variety, is one major strategy for controlling signaling from that synapse. In the short term, decreased synaptic activity promotes the loss of NMDARs and vice versa. But over the long haul, the number of NMDARs is up-regulated at quiet synapses.

Ehlers’ group now demonstrates that this enduring modification is a result of selective mRNA splicing to produce a fast-moving NMDAR variety. Preventing neuronal firing (with sodium channel blockers) increased synaptic NMDARs by increasing the proportion of newly synthesized NMDAR mRNA (and protein) with a C2’—rather than C2—tail. Inclusion of the C2’ tail increased the number of these receptors at synapses because they quickly exited the ER, the bottleneck for most secreted proteins, thanks to a novel COPII-recruiting signal in C2’. In contrast, continuous neuronal firing favored production of the slow-moving C2 variant and thus decreased the number of synaptic NMDARs.

NMDAR activity is needed for this splicing adjustment. As NMDARs are calcium permeable, Ehlers postulates that calcium-regulated splicing factors might process certain neuronal mRNAs. The feedback regulation probably prevents a synapse from reaching an absolute maximum or minimum activity, so that later changes in activity can be detected. “It’s a countering activity,” says Ehlers. “If a synapse keeps getting potentiated, it gets to a maximum rate, and further strengthening would do no good.”

Immune cells take in unknown enemies

MHC class I molecules normally present endogenously synthesized antigens and thus activate cytotoxic T lymphocytes (CTLs). In antigen-presenting cells (APCs), however, some seemingly extracellular antigens are cross-presented: they are loaded onto the class I pathway in addition to their usual presentation by MHC class I. For example, APCs in mice were recently shown to activate antipolio virus CTLs, despite lacking a receptor for the virus, presumably by cross-presenting polio virus antigens on class I. But now Stefan Freigang, Rolf Zinkernagel, and colleagues (University of Zurich, Zurich, Switzerland) show that the missing receptor does not stop viral uptake in APCs, suggesting that cross-presentation is not needed as an explanation. “Even in a situation where everybody would suspect that cross-presentation is going on, we see that APCs are taking in the virus,” says Freigang. His group finds viral RNA in APCs of the receptorless mice. Further, the RNA had to be translated to elicit CTL responses. Thus, even if cross-presentation—which does not require translation—might occur, it is too inefficient under physiological infection conditions to cause an immune response.

Not all cells are susceptible to viral infection, since only transgenic mice expressing the polio virus receptor develop disease symptoms. So what is special about APCs is not clear. “We can only speculate on how the virus gets into the cell,” says Freigang. Perhaps a subset of APCs has the ability to take in a variety of pathogens with a nonspecific receptor.


Fold me or leave me

New results from Markus Eser and Michael Ehrmann (Cardiff University, Cardiff, UK) indicate that the protein that sends secreted proteins through the translocon also prevents cytoplasmic proteins from meeting the same fate.

SecA helps proteins fold (right), unless they have a signal sequence (left). SecA bound to unfolded proteins even if they did not contain signal sequences. For proteins lacking an export signal, SecA promoted their folding to an active state. Once folded, SecA no longer binds, so secretion is thwarted. SecA did not have chaperone activity with signal sequence–containing proteins. What accounts for this difference is not yet known, but perhaps strong binding of SecA to the signal sequence disrupts its ability to promote folding. Yeast and human cells do not have a SecA homologue, but a different translocon-associated chaperone may perform an analogous function.


Fusion gets in the groove

The only confirmed mechanism for protein-mediated membrane fusion involves the formation of helix bundles, in which helices attached to two membranes pack against one another to draw together the membranes. In the final fused state, six-helix bundles are formed by HIV Env and paramyxovirus F proteins, members of the class I group of viral fusion proteins. Although the class I founding member, influenza HA, also forms a six-helix bundle, Heather Park, Jennifer Gruenke, and Judith White (University of Virginia, Charlottesville, VA) now show that this bundle is not sufficient for fusion.

For HA, interactions between a nonhelical region and a trimer of helices cause fusion. Using mutational analyses, the group shows that fusion requires contacts between a long chain, which they call the leash, near the viral membrane with the helices near the target membrane (usually a host endosome as the virus escapes into the cytoplasm). They suggest that packing of the leash into the grooves of the helices condenses HA, thus bringing together viral and host membranes.

“There’s nothing holy about helix–helix interactions as a means to pull membranes together,” says White. “You can do it with other types of interactions.” This may help explain why class II viral fusion proteins work although they do not have a lot of helical structure.