Reconstituting a chloroplast transport system

In the first successful reconstitution of a chloroplast transport system, only three proteins are required for GTP-dependent translocation of preproteins into proteoliposomes, according to Enrico Schleiff, Marko Jelic, and Jürgen Soll (Maximilian Universität München, Munich, Germany). Based on this in vitro system, the authors propose that the preprotein first binds to Toc34 in a GTP-dependent manner and is then passed off to Toc159, which pushes the polypeptide through the Toc75 channel itself, also in a GTP-dependent process.

“We were rather surprised that such a minimal system could actually translocate a protein,” says Schleiff. It was previously thought that molecular chaperones associated with the Tic complex generated the force to drive the protein through the channel.

Combining the new results with structural information, which shows the ratio of Toc34, Toc75, and Toc159 in an assembled channel is 4:4:1, Schleiff and colleagues hypothesize that Toc159 acts as a dynamic component of the system. They envision that it drives the preprotein through the narrow channel with repeated up and down strokes, in a motion that resembles a sewing machine needle. The movement required for such a stroke is not very large, says Schleiff; with a channel height of 10 nm, it would only require the movement of ~60 amino acids, assuming a helical region. “The receptor Toc159 has 1,500 amino acids, so a movement of 60 amino acids is easy to achieve without disturbing the entire structure.”

References: Schleiff, E., et al. 2003. Proc. Natl. Acad. Sci. USA. 10.1073/pnas.0730860100.
Schleiff, E., et al. 2003. J. Cell Biol. 160:541–551.

Calcium waves in membrane fusion

Phagolysosome fusion is a calcium dependent process. Now, Randall Worth and colleagues (University of Pennsylvania School of Medicine, Philadelphia, PA), and Howard Petty and colleagues (University of Michigan School of Medicine, Ann Arbor, MI) find that a three amino acid motif in the IgG receptor FcγRIIA causes a calcium wave traveling along the plasma membrane to split in two, with one part sweeping over the phagosome surface. Mutation of the LTL sequence, prevents wave bifurcation and inhibits phagolysosome fusion.

Using high speed imaging techniques, the authors can watch the calcium wave move around the plasma membrane after phagocytosis. As the wave approaches the phagosome, it splits in two with part of the wave crossing a bridge formed by a thread of ER that connects the plasma membrane and the phagosome.

This ER thread has been seen before in electron micrographs, but its function was unclear. “We’ve seen the signal moving between the two organelles and the thread would account for it,” says Petty. He speculates that the LTL sequence forms part of a super-molecular aggregate between the plasma membrane, the ER, and the phagosome. When it is mutated the complex fails to form, the bridge is not built, and the calcium wave continues along the plasma membrane, ignoring the phagosome. And since calcium is a major trigger for membrane fusion, it fits with the model that phagolysosome fusion is impaired in the mutants.

Reference: Worth, R.G., et al. PNAS. 10.1073/pnas.0836650100.

Toxoplasma uses tough love

Parasites are known for outwitting their hosts in unexpected ways, and Toxoplasma gondii is no exception. To ensure that it does not kill its host prematurely, T. gondii secretes a protein that induces a strong host cellular immune response. This response slows the infection—and enables the parasite to complete its life cycle. Now, Julio Aliberti, Jesus Valenzuela, Alan Sher, and colleagues (NIH, Bethesda, MD) have identified the T. gondii protein that triggers the immune response and found that it mimics a chemokine.

The T. gondii protein, cyclophilin-18 (C-18), binds to the CCR5 receptor on the surface of dendritic cells and induces the expression of high levels of IL-12, a positive regulator of cell-mediated immunity. The fact that C-18 works through a chemokine receptor is unusual. “In general this sort of [IL-12] induction has been thought of as working solely through Toll-like receptors,” says Sher.

Although C-18 alone is already a more effective IL-12 activator than other known triggers like CPG or LPS, the team has evidence that there is a second immune-stimulating factor produced by T. gondii that boosts the levels even further. This second factor appears to work via a Toll receptor, but the ligand has not yet been identified.

Reference: Aliberti, J., et al. 2003. Nat. Immunol. 10.1038/ni915.