A reversal of Tat

Nearly 15 years of in vitro experiments showed that the Tat transport system, which spans the thylakoid membrane in chloroplasts, sends proteins unidirectionally inwards, from stroma to lumen. On page 281, Di Cola and Robinson report in vivo evidence from protoplasts showing that a large fraction of the proteins that move part way through the channel end up back in the stroma.

The Tat system is required for transporting several protein components of a photosynthetic complex to the lumen of the chloroplast. For example, the 23K protein starts out in the cytoplasm as a 33-kD precursor, containing two NH\textsubscript{2}-terminal signal sequences. The first signal sequence is cleaved as the protein moves across the outer chloroplast membrane into the stroma, and the second as the protein passes through the Tat transport complex into the lumen.

When tobacco protoplasts were engineered to express a GFP-labeled pre-23K protein, both signal sequences were cleaved normally. However, only a small proportion of the mature protein ended up in the lumen, even when the researchers expressed minimal amounts of the construct.

The peptidase for the second signal sequence faces the lumen. Di Cola and Robinson conclude that the fully processed 23K proteins detected in the stroma had to have reached the lumen, or at least proceeded part way through the Tat transport system, before they were sent back to the stroma.

When they blocked interaction between the GFP-labeled protein and the Tat system by mutating the recognition domain in the substrate protein, all of the GFP-labeled protein remained in the stroma as expected. But in this case the protein retained its second signal sequence, indicating that a stromal protease cannot cleave off the second signal sequence.

In vivo transport is likely to be more rather than less efficient than in vitro systems. The lack of the reexport ability in the in vitro system may reflect missing components—a theory that the researchers are now testing with add-back experiments. The reexport ability may represent a previously undetected quality control mechanism for thylakoid transport.

A novel export pathway

The lectin galectin-1 is exported from cells via a poorly characterized secretory pathway. On page 373, Seelenmeyer et al. demonstrate that the cell surface receptors to which the lectin binds on the exterior of the cell are also required for export of the glycoprotein from the interior.

Galectin-1 interacts with β-galactoside–containing sugar moieties on extracellular matrix and cell surface receptors. Based on previous work, researchers think galectin-1 is translated and folded in the cytoplasm before localizing to the inside of the plasma membrane and being exported.

Seelenmeyer et al. found that galectin-1 mutants deficient for binding to β-galactoside get stuck in the cytoplasm and are not exported. Moreover, cells that cannot produce the cell surface receptors that bind galectin-1 failed to export wild-type galectin-1. The fungal lectin CGL2, which resembles galectin-1 in its folded shape, was also trapped in the cytoplasm in these mutant cells, though wild-type cells exported it efficiently.

So how is a cell surface receptor working to export a protein from the cytoplasm? The researchers currently have two hypotheses. The receptor could act as a sink, skewing the intracellular–extracellular equilibrium in favor of the extracellular space. In a variant of this model, receptors may reach through a protein-conducting channel to contact the lectin and pull it through the membrane. Alternatively, some β-galactoside–containing glycolipids may be flipped from their normal extracellular orientation to pick up intracellular galectin-1 before being flipped back again.

Galectin-1 and CGL2 may not be the only proteins using a receptor-based export system. Another prime candidate is FGF2. It is a lectin that binds heparan sulfate moieties on proteoglycans and, like galectin-1, it appears to be completely folded before export.
Asymmetry in cytokinesis

Cytokinesis proceeds asymmetrically in *C. elegans* with the furrow invaginating first on one side and then shifting to the other side of the cell, report Audhya et al. on page 267. This taking of turns, and the structural mechanism that enforces it, may ensure that the furrow is localized in a single plane.

The asymmetry came to light when the authors studied CAR-1, which was previously identified in an RNAi study as being required for embryonic cytokinesis. Audhya et al. show that CAR-1 helps regulate maternally supplied RNAs in the germline and early embryo.

These RNAs are apparently required to assemble and maintain functional interzonal microtubules, which form between the separating chromosomes after anaphase. To get a picture of the resulting cytokinesis defect, the team expressed a GFP construct that specifically binds to the lipids of the plasma membrane.

In wild-type worms, the furrow invaginated from one side and then, as progress slowed on that side, it started in from the other side. Simultaneous imaging of microtubules and the plasma membrane marker indicated that the primary furrow proceeded until it bumped into the interzonal microtubule bundles. Because constriction continues at a constant rate, when one side encounters an obstacle, the force shifts to the far side of the cell.

In CAR-1–depleted embryos, interzonal microtubule bundles are missing, so there was no longer a structural transition in which the first furrow stops, and the furrow from the opposite side begins to ingress. JCB

Kinesin at both ends

Two minus end-directed motors, Ncd (Kinesin-14) and dynein, have complementary capture-and-transport roles that shape the spindle during mitosis, report Goshima et al. on page 229.

Spindle microtubules emanate from the centrosomes or, as long thick ropes called K-fibers, from kinetochores. Previous work showed that two minus end-directed molecular motors—dynein and Ncd—are required to intertwine the two types of microtubules and bring them into the familiar diamond shape. In cells lacking either motor, the microtubules splay apart, failing to gather their minus ends near the centrosomes.

Using video microscopy with GFP-labeled components, FRAP, and RNAi, the team found that the motors have some functional redundancy but also have a preference for jobs. Loss of Ncd results primarily in a loss of K-fiber focusing, whereas loss of dynein compromises the pulling of K-fibers toward centrosomes.

Ncd apparently acts as a dynamic cross-linking protein lashing together individual microtubules into K-fibers. But it also accumulates at the plus ends of the centrosomal microtubules, relying on the plus end tracking protein EB1 for attachment. Clustering at ends probably helps the nonprocessive Ncd to grab onto K-fibers with a robust grip. Then dynein takes over, pulling K-fibers along the centrosomal microtubules toward the centrosome. JCB

Stretching and twisting

Cells exposed to repeated stretching along a unidirectional axis reinforce their actin stress fibers and reorient them to run perpendicular to the direction of strain. The two aspects of the response are regulated independently, report Yoshigi et al. (page 209).

Numerous tissues are exposed to repeated mechanical strain and respond by reorganizing their cytoskeletons. Researchers hypothesize that stretch-responsive channels or cell adhesion sites may detect the mechanical force and control downstream changes.

When cells were exposed to mechanical stress, zyxin, a prominent component of focal adhesions, relocalized to actin stress fibers. Zyxin redistribution occurred only when cells were grown on a matrix that allowed integrin engagement, but blocking stretch-activated channels had no effect on zyxin mobilization. Zyxin movement also caused a similar relocalization from focal adhesions to stress fibers of vasodilator-stimulated protein (VASP), which promotes actin polymerization.

In mouse fibroblasts lacking a functional zyxin gene, VASP localization did not shift in response to cyclic stretch, nor did the actin fibers become thickened, as compared with wild-type cells. Significantly, the fibers in the mutant cells were reoriented normally in response to stretch, illustrating that reorientation and reinforcement are mechanistically distinct processes.

It is not yet clear whether zyxin is a mechanosensor itself or is an early protein in the response pathway, but the results do show that focal adhesions are a key factor for detecting and responding to mechanical stress. JCB

Kinesin at both ends

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