In This Issue

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$_2$-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.