was restored when Arp2/3 was added back, indicating that Arp2/3 mediates star formation. Furthermore, the adsorption/dilution experiments had hinted that a decreased amount of factor(s) in the extract were crucial in star formation. Increased levels of a candidate factor, capping protein, antagonized star formation. Decreased levels (for example, at the edge of the coverslip) probably mediate actin-filament elongation.

On the basis of their results, Vignjevic *et al.* attempted reconstitution experiments using WASP-coated beads, actin and Arp2/3, but found that they had to add an actin-bundling protein, fascin, to promote star formation. So, the authors propose three steps — nucleation (by Arp2/3), elongation (by decreased capping protein) and bundling (by fascin) — for star, or filopodia, formation.

Katrin Bussell

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PROTEIN TRANSLOCATION

Doing the three-step

The TIM22 complex — a translocase of the inner mitochondrial membrane — is responsible for inserting precursor proteins that contain several transmembrane segments and internal signal peptides (for example, metabolite carriers) into the inner mitochondrial membrane. To do this, TIM22 uses the membrane potential ($\Delta\psi$) as its only external energy source, but exactly how does this complex fulfil its role? Now, in *Science*, Nikolaus Pfanner and colleagues provide new insights.

A complete, functional TIM22 complex had never before been purified, so the authors first developed a method to isolate the entire, functional, yeast TIM22 complex. Once purified, they reconstituted the complex into liposomes and fused them with a planar lipid bilayer. They then monitored the changes in current recordings that occurred on addition of an internal signal peptide (P2) from the phosphate carrier.

Purified Tim22 — an integral membrane subunit of the TIM22 complex — has previously been shown to form a single voltage-activated pore, which ‘flickers’ on addition of P2 at a $\Delta\psi$ greater than 140 mV. By contrast, Pfanner and colleagues found that the TIM22 complex has two tightly coupled pores. When P2 was added to TIM22, they found that the flickering/gating occurred in only one of the two pores (the other pore was mainly closed), and that this gating was more rapid than for Tim22 alone, and could be induced at a lower $\Delta\psi$ (75 mV).

So, how does this information relate to the natural process *in organello*? Mitochondrial import of carrier proteins occurs in five stages, and stage IV — insertion into the inner membrane — has remained poorly understood. Pfanner and co-workers therefore monitored the effects of lowering $\Delta\psi$, using the protonophore CCCP, on the late

stages of import of both the phosphate carrier and the dicarboxylate carrier.

At high $\Delta\psi$ (no CCCP), both carriers were mainly present in their mature, dimeric form (stage V), whereas, in the absence of $\Delta\psi$ (high CCCP levels), they were present in the lower-molecular-weight stage III form (that is, they are probably associated with small, soluble Tim proteins in the intramembrane space). However, at intermediate $\Delta\psi$ values (<60 mV), the authors could observe a stage IV intermediate — a TIM22–carrier complex. For both carriers, restoring $\Delta\psi$ resulted in stage V being reached, which indicates that this stage IV intermediate represents a productive translocation intermediate.

However, Pfanner and colleagues further noticed that a small, but significant, carrier fraction associated with TIM22 in the absence of $\Delta\psi$, and they proposed that this fraction might represent a ‘tethered’ form of the carrier, rather than the ‘docked’ stage IV intermediate described above. Indeed, they showed that the tethered intermediate was more resistant to ionic-strength increases than the docked intermediate, which indicates that the “...tethered and docked forms of the carrier reside in different molecular environments at the TIM22 complex, the tethered protein probably being in a more hydrophobic environment”.

The twin-pore TIM22 complex therefore inserts precursors into the inner mitochondrial membrane in three steps. First, the precursor is tethered to TIM22 in a $\Delta\psi$ -independent step. Then, in the first voltage-dependent step, the precursor docks in TIM22 (a $\Delta\psi$ below that needed to influence pore activity was sufficient for this step, which indicates that $\Delta\psi$ acts directly on the precursor). Finally, in the second voltage-dependent step, a $\Delta\psi$ greater than ~70 mV and the presence of a signal peptide activate pore gating to complete the membrane-insertion process.

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References and links

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