MEMBRANE DYNAMICS

Family ties

Membrane fusion is mainly controlled by proteins from two large families — SNAREs, which are thought to drive fusion, and Rabs, which act as regulators. But tethering factors, which attach the membranes to each other before tight docking and fusion can occur, had so far resisted classification as a protein family. New data in *Developmental Cell* from Sean Munro's laboratory now show that they might be distantly related to each other after all.

Whyte and Munro carried out a screen for *Saccharomyces cerevisiae* mutants that would be synthetically lethal with a mutation in *RIC1*, a gene that encodes a subunit of the exchange factor for the Golgi Rab GTPase Ypt6. Several genes fitted the bill, among which was the previously uncharacterized *DOR1*. On further investigation, the authors found that Dor1 is part of a complex that also contains Sec34 and Sec35, two proteins known to be part of a tethering factor complex that functions in the Golgi. The other five subunits of the complex were identified as previously uncharacterized proteins, and were renamed Cod1, Cod2, Cod3, Cod4 and Cod5. So, in one go, Whyte and Munro identified six new proteins and characterized the composition of the Sec34/Sec35 tethering complex.

But although the eight proteins form a complex, the deletion mutants had different phenotypes. Whereas deletion of *COD1* was lethal, mutants lacking *COD3*, *SEC34* and *SEC35* grew very slowly and had defects in the organization of their internal membranes and in Golgi processing. By contrast, mutants lacking *DOR1*, *COD2*, *COD4* and *COD5* were viable and had no defect in these processes. However, they did have a defect in recycling from endosomes to the Golgi. So, the Sec34/Sec35 complex probably acts at more than one transport step in the Golgi.

Iterative PSI-BLAST searches showed that several members of the Sec34/Sec35 complex are related to other tethering factors. Dor1 is distantly related to Sec5, a component of the exocyst — a complex that functions in tethering exocytic vesicles to specific sites on the plasma membrane. Cod4, on the other hand, is the yeast homologue of mammalian GTC-90 — the only identified component of the so-called Golgi transport complex (GTC). The obvious question as to whether GTC and the Sec34/Sec35 complex are one and the same remains to be addressed.

The authors noticed a short region of homology in the amino terminus of all eight members of the complex, as well as in the amino termini of several other tethering factors, including all eight components of the exocyst, and Vps53 and Vps54 — members of the Vps52/Vps53/Vps54 complex, which functions in prevacuole-to-Golgi transport. This region consists of two amphipathic helices separated by an extended loop, which probably form a coiled coil. Moreover, the homology between Sec34 and Exo70 (a component of the exocyst), and between Sec3 (another component of the exocyst) and Vps52 (a component of the Vps52/Vps53/Vps54 complex) extend well beyond this region, indicating a closer relationship between these factors.

So, it seems that tethering factors might be a family after all — admittedly, one with loose ties. This new finding should make it easier to study their function, if you believe in the motto 'you know one, you know them all'.

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PROTEIN FOLDING

Boxing day

The GroEL–GroES chaperonin system, which is responsible for the folding of a subset of newly synthesized proteins, resembles a cylinder with two cavities (GroEL) and a lid (GroES). Substrates enter this box, are folded and then released. But do all proteins that use chaperonins really need to cram themselves entirely into the box; and, if so, what could be happening to them while they are inside?

The enzymology of the chaperonin system is very well understood. In the classic *cis* cycle, the folding substrate binds to one cavity of GroEL — operationally defined as the *cis* cavity — and is shortly followed by ATP and GroES. ATP hydrolysis in the *cis* cavity causes ATP to bind to the *trans* cavity, which in turn induces the release of GroES and the substrate from the *cis* cavity.

What is less understood is the principle underlying the chaperonin-mediated folding process. In particular, there is much debate about whether the GroEL–GroES system acts as a passive cage that simply protects the substrate from aggregation, which would occur if the protein were to attempt folding in solution, or whether it actively unfolds misfolded proteins and guides them in their efforts towards correct folding. New data reported in *Cell* by the Hartl and Horwich groups indicate that, as so often happens, the truth might lie somewhere in the middle.

To directly test whether encapsulation in the chaperonin cage is essential for the folding reaction, Brinker and colleagues inhibited the rebinding of substrate to GroEL — and hence, its encapsulation — by binding streptavidin to biotinylated GroEL. Under conditions that favour their aggregation in solution, RuBisCo and rhodanese — two obligate chaperonin substrates — could fold only in the presence of ATP, GroES and nonbiotinylated GroEL. This confirmed that one function of encapsulation is to protect the substrate from aggregation.

To their surprise, the authors found that encapsulation also has a second function: it actively assists folding. Indeed, under conditions in which it could fold freely in solution, RuBisCo folded four times faster if a fully functional chaperonin system was present. But the accelerated folding was substrate specific, as it was not observed for rhodanese. The authors propose that confinement in the narrow space of the cage "smooths the energy landscape" of the folding reaction, either by preventing the formation of trapped intermediates or by facilitating progression towards the folded state. The observed difference might depend on the size of the substrate — RuBisCo is ~50 kDa, whereas rhodanese is only ~33 kDa — or on whether the substrate has a tendency to form kinetically trapped intermediates, as RuBisCo has.

But, if substrate size is an issue, what happens to substrates that are too large to be encapsulated? Chaudhuri and colleagues studied folding of mitochondrial aconitase, an 82-kDa monomeric enzyme that is known to aggregate in chaperonin-deficient mitochondria. They found that both GroEL and GroES are required for aconitase folding *in vivo* and *in vitro*.

References and links

ORIGINAL RESEARCH PAPER Whyte, J. R. C. & Munro, S. The Sec34/35 Golgi transport complex is related to the exocyst, defining a family of complexes involved in multiple steps of membrane traffic. *Dev. Cell* **1**, 527–537 (2001)

FURTHER READING Pfeffer, S. R. Transport-vesicle targeting: tethers before SNAREs. *Nature Cell Biol.* **1**, E17–E22 (1999)

However, this does not involve encapsulation by the chaperonin system, as aconitase did not become resistant to protease digestion during folding. So, how could GroES — the lid of the box — assist protein folding if not by encapsulation? Using single-ring and mixed-ring GroEL mutants that cannot bind GroES in *trans*, the authors deduced that a novel '*trans* cycle' assists folding of larger substrates. In this alternative pathway, instead of binding to the *cis* ring, GroES binds to the *trans* ring and is required, in addition to ATP, to release the non-native substrate from the *cis* ring.

So, it seems that there is more than one way in which the chaperonin system can assist folding. Depending on their size and propensity to form aggregates, folding substrates are either encapsulated by GroEL–GroES — simply to protect them from aggregation or, in some cases, also to facilitate the progression towards the folded state — or they bind only to GroEL and undergo a *trans* cycle, which somehow assists folding. The fine details of these different folding reactions still elude us but these two studies should bring us a step closer to the light.

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SIGNAL TRANSDUCTION

Staying alive

The cytokine tumour necrosis factor-α (TNFα) is a double-edged sword. On the one hand, it signals survival through nuclear factor κB (NF-κB), but on the other hand it lures cells to their death. TNFα can activate c-Jun amino-terminal kinase (JNK), which can contribute to apoptosis. JNK's role in TNFα-induced cell death is less clear. Two papers in the 15 November issue of *Nature* now show that the outcome of TNFα-induced cell death depends on crosstalk between the NF-κB and JNK pathways. While investigating how NF-κB signalling protects against apoptosis, both groups found that it can downregulate JNK signalling, but the approaches taken and the candidate molecules identified were both different.

Franzoso and colleagues used 'death trap' screening, in which complementary DNA expression libraries derived from wild-type cells that had been treated with TNFα were transfected into cells that lacked NF-κB or its close relative, RelA. The authors then analysed cDNAs from cells that survived treatment with TNFα, and identified *gadd45β*, a member of the Gadd family, which functions in cell-cycle control and DNA repair.

Transcripts of *gadd45β* were strongly induced by TNFα in wild-type, but not in *RelA*$^{-/-}$, cells. And expression of a Gadd45β fusion protein in *RelA*$^{-/-}$ cells protected cells against TNFα-mediated apoptosis. Furthermore, cells that expressed Gadd45β and contained a mutant protein — called IκBαM — that prevents the activation of NF-κB, were resistant to TNFα-mediated apoptosis. In the absence of NF-κB, apoptosis prevails, mitochondria release death-promoting factors and caspases are activated. The expression of Gadd45β averts this, indicating that *gadd45β* is targeted by NF-κB in response to TNFα and functions to prevent apoptosis.

As TNFα also activates JNK, the authors next investigated whether JNK activation is altered in NF-κB-null cells. JNK activity was sustained in response to TNFα, compared with wild-type cells, in which JNK activation was downregulated after 40 minutes of treatment. Cycloheximide — an inhibitor of protein synthesis — prolonged the activation of JNK by TNFα in wild-type cells, indicating that *de novo* synthesis is needed for NF-κB to suppress JNK activation.

So, where does Gadd45β fit into the picture? In IκBαM cells, TNFα-induced JNK activation could still be downregulated if Gadd45β was present. Conversely, targeting wild-type cells with *gadd45β* antisense RNA extended JNK activation in response to TNFα. So the TNFα-mediated downregulation of JNK by NF-κB involves the induction of *gadd45β*, but exactly how Gadd45β inhibits JNK activation remains to be determined.

In the second study, Lin and colleagues used *IKKβ*$^{-/-}$ cells that were impaired in their ability to activate NF-κB signalling, and were therefore sensitive to TNFα-induced cell death. They looked directly at the activity of JNK in these cells in response to TNFα and found that it was sustained, but that the activation could be restored to its normal, transient duration when IKKβ was re-expressed. The same result was found using *RelA*$^{-/-}$ cells and when RelA was re-expressed. Furthermore, re-expressing RelA in *RelA*$^{-/-}$ cells inhibited c-Jun-mediated gene transcription in response to TNFα.

Like Franzoso's group, Lin and co-workers saw that cycloheximide prolonged the activation of JNK by TNFα. So they first investigated whether JNK was inactivated by phosphatases that are induced by NF-κB signalling. After ruling this out — because phosphatase inhibitors had no effect on TNFα-induced JNK activation — they examined whether other known targets of NF-κB could inhibit JNK activation. And indeed, they found that transient expression of X-chromosome-linked inhibitor of apoptosis (XIAP) could do so. Similar to *gadd45β*, *XIAP* was targeted by NF-κB in response to TNFα, and was not induced in *RelA*$^{-/-}$ cells.

Interestingly, the crosstalk between NF-κB and JNK was specific for TNFα, as NF-κB didn't suppress JNK activity in response to interleukin-1 (IL-1) — which also activates NF-κB and JNK. So why is the inhibition of TNFα-mediated JNK activation so important? Both groups showed that persistent activation of the JNK pathway by TNFα is pro-apoptotic, so by inhibiting this, NF-κB might stop TNFα acting as a death cytokine. As IL-1 doesn't induce apoptosis, this might also explain the lack of crosstalk between NF-κB and JNK in response to IL-1.

Although the findings ultimately differ with respect to the identified targets, both groups have found that one way in which NF-κB offers protection against apoptosis is by downregulating the JNK pathway. But how Gadd45β and XIAP exert their influence over JNK signalling currently remains elusive.

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