PROTEIN TRANSLOCATION

Waste-disposal chute

The correct disposal of waste is essential for daily life, and this applies at the cellular level too. Misfolded proteins in the endoplasmic reticulum (ER) are retro-translocated (or dislocated) to the cytosol for degradation, and blocking this pathway causes ER stress. Some components of the pathway are known — for example, the cytosolic ATPase p97 probably 'pulls' misfolded proteins out of the ER. However, the channel that mediates retro-translocation has been a matter of speculation. Two papers in *Nature*, though, now identify a protein that might form such a channel.

In the first paper, Lilley and Ploegh studied the US11 protein of the human cytomegalovirus, which targets the major histocompatibility class-I heavy chain for retro-translocation. A Q192L mutant of US11 cannot maintain this retro-translocation, and the presence of this glutamine residue in the transmembrane region of US11 indicated that it might interact with host proteins.

The authors therefore developed an affinity-purification approach to examine which cellular proteins bind to US11 versus US11^{Q192L}, and they identified a protein — which was named Derlin-1 — that specifically associated with US11. This transmembrane protein is a homologue of *Saccharomyces cerevisiae* Der1, which is required for the degradation of a subset of misfolded ER proteins.

Using antibodies against Derlin-1 in immunoprecipitation experiments, Lilley and Ploegh confirmed the Derlin-1–US11 association and showed that the class-I heavy chain was also recovered with Derlin-1. This was not the case in cells expressing US11^{Q192L}, which indicates that US11 uses its transmembrane region to recruit the class-I heavy chain into a complex with Derlin-1.

Next, the authors created a Derlin-1 construct, in which green fluorescent protein (GFP) was attached to its cytosolic carboxyl terminus. This construct extended the half-life of the class-I heavy chain from 5 to 30 minutes in US11-expressing cells, which indicates that, without blocking the binding of the class-I heavy chain to US11 or Derlin-1, the GFP moiety delays the exposure of the class-I heavy chain to the cytosol.

In the second paper, Rapoport and co-workers used a different approach and identified the receptor that links p97 (also known as VCP) to the ER membrane. They actually isolated two proteins in near-stoichiometric amounts — Derlin-1 and a protein that was named VIMP (for VCP-interacting membrane protein).

As VIMP has a large cytosolic domain, the authors proposed that this domain interacts with p97. This proved to be correct, and they showed that this domain recruits p97 together with its co-factors. Furthermore, using immunofluorescence microscopy, they showed that VIMP seems to recruit p97 to Derlin-1.

Next, Rapoport and colleagues investigated whether Derlin-1–VIMP is involved in retro-translocation, and they showed that Derlin-1–VIMP interacts with the class-I heavy chain in US11-expressing cells. In agreement with Lilley and Ploegh, they also noted that an interaction between Derlin-1 and US11 is functionally important for this process.

In the final part of their study, Rapoport and co-workers tested whether the Derlin-1–VIMP complex has a general function in the retro-translocation of misfolded proteins and their results indicate that it can associate with a large set of substrates. Furthermore, depleting Derlin-1 levels in *Caenorhabditis elegans* using RNA interference induced ER stress in many cell types.

Together, these groups have therefore identified a putative waste-disposal chute in mammals called Derlin-1. Derlin-1 mediates the retro-translocation of a subset of misfolded proteins from the ER and, if it can be shown that Derlin-1 does form a channel, it seems only a matter of time before other such retro-translocation channels are identified.

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References and links

ORIGINAL RESEARCH PAPERS Lilley, B. N. & Ploegh, H. L. A membrane protein required for dislocation of misfolded proteins from the ER. *Nature* **429**, 834–840 (2004) | Ye, Y. *et al.* A membrane protein complex mediates retro-translocation from the ER lumen into the cytosol. *Nature* **429**, 841–847 (2004)

FURTHER READING Schekman, R. Cell biology: a channel for protein waste. *Nature* **429**, 817–818 (2004)

WEB WATCH

Wired for cycling

- www.mpf.biol.vt.edu/research/budding_yeast_model/cyberyeast.htm

Hands up who's managed to plough through a research paper on mathematical modelling. No? Then you are probably an experimentalist... But, web sites might be more accessible when it comes to mathematical models.

The *Budding Yeast Cell Cycle* web site presents a mathematical model of cell-cycle control in budding yeast and is an enhancement of a recent research paper by the groups of John Tyson, Bela Novak and Frederick Cross.

The 'Overview' section presents a complete wiring diagram for the regulation of cell-cycle progression based on published data. The diagram is clickable, allowing the user to review the literature sources.

The regulatory network has been formulated into a set of differential equations (see 'Mathematical model' section) and analysed by computer simulation. The researchers compared the physiology of wild-type cells and >100 mutant strains with the simulated results that were derived from the wiring diagram. The simulation successfully predicted the mutant phenotype in most cases, but some inconsistencies were found (see 'Problems').

The 'Conclusions' section, under 'Overview', takes you to the 'Simulate the model' page, which allows users to change the value of any parameter and re-compute the behaviour of the model. There is a short description of each parameter, its default value and the range that allows a viable cell-cycle simulation.

The web site has just been updated and is now even more user-friendly. Browsing the site should give experimentalists a feel for the potential of mathematical modelling and how its predictive power can be useful to design new experiments.

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