Checking folding inside and out

The ER is a site of both synthesis and quality control. Rejected, improperly folded proteins are destroyed by a well-characterized machinery (the ER-associated degradation [ERAD] pathway) in the cytosol and ER membrane that extracts and chews up any unfolded proteins. But the mechanisms for recognizing unfolded proteins as targets for destruction are less well characterized.

The few unfolded substrates to be examined fall into a simple pattern: transmembrane proteins are recognized by one system and soluble, secreted proteins by another. Vashist and Ng (page 41) take a closer look and find that the two transmembrane proteins previously examined had defects in their cytoplasmic domains. When the defects are instead in the luminal domains of transmembrane proteins, these proteins are treated in the same way as soluble luminal proteins.

Thus, the two recognition categories depend not on membrane association but on the site of the unfolded domain. Any protein with an unfolded cytoplasmic domain is recognized by one system (ERAD-C), whereas proteins (both transmembrane and soluble) that have unfolded domains in the ER lumen are recognized by a second system (ERAD-L). Proteins subject to ERAD-C are retained in the ER and destroyed rapidly, whereas proteins subject to ERAD-L must travel to the Golgi and return back to the ER before being destroyed. One possible function for the transport step might be the tagging of the unfolded protein with a Golgi-specific sugar modification.

The location of the unfolded domain (star) is what matters at the ER.

If a protein has unfolded domains in both cytoplasm and lumen, the ERAD-C pathway takes precedence, and ERAD-L pathway proteins are not needed. This suggests either that the two pathways constitute an ordered series of checkpoints or that, when the ERAD-C and ER-to-Golgi recognition machineries are head to head, the former is more aggressive in pinning down its substrates.

Don’t digest the messenger

Helping to create the lysosome is a dangerous job—stick with your task for too long and you might end up as dinner. Seaman (page 111) and Arighi et al. (page 123) now describe how the cation-independent mannose 6-phosphate receptor (CI-MPR) escorts lysosomal enzymes toward their future home but then escapes just in time thanks to a complex of proteins called the retromer.

The retromer was first characterized in yeast, where it drags Vps10p from endosomes back to the Golgi. Vps10p and the mammalian CI-MPR have no sequence homology but do perform similar functions. So the researchers tested whether the retromer could also rescue CI-MPR.

They first confirmed that both CI-MPR and the mammalian retromer are located in endosomes, with additional CI-MPR in the trans-Golgi network. After either knockout or siRNA knockdown of the retromer component Vps26, far more of the CI-MPR was found in endosomal compartments, with the rest of it spilling over either to the plasma membrane or to a nasty end at the hands of the lysosome.

This destruction of the CI-MPR apparently compromised its normal function. CI-MPR normally leads lysosomal enzymes out of the Golgi, but with retromer failing to recycle CI-MPR, fewer of these enzymes were able to reach their destination and attain their fully mature lysosomal state.

Arighi et al. found evidence for direct binding between CI-MPR and another retromer component, Vps35, and visualized retromer on endosome-derived tubules that showed microtubule-dependent movements. Retromer may form a coat that promotes formation of these tubules, but it remains unclear just how this tubule formation is timed to occur after arrival of the receptor from the Golgi but before transport into lysosomes.
Foci of decay

Cytoplasmic structures are sites of active mammalian mRNA decay, according to Cougot et al. (page 31). The group had previously identified cytoplasmic foci that included two human mRNA decapping enzymes. They now add subunits of a deadenylase, exonuclease, and possible helicase to the list of proteins found at these sites. After inhibition of a 5'–3' exonuclease, poly(A)+ RNA accumulates at the same sites, further suggesting that these are locations for active degradation of RNA rather than passive storage centers for degradation factors. The foci almost completely disappear after addition of either translational inhibitors that are known to stabilize RNAs or transcriptional inhibitors that deplete the cytoplasm of all mRNA.

Similar structures have been seen in yeast, although these structures were fewer in number and only visible under certain nutrient conditions. Both findings suggest that the cytoplasm is more structured than previously thought. The regulation of foci formation remains a subject for future studies.

Brain construction goes straight

B raincs of mice lacking the cell adhesion molecule L1 are a mess. Failures in neural migration, pathfinding, morphogenesis, and fasciculation result in shortages of cells in various regions and aberrant architecture. But now Itoh et al. (page 145) report that homophilic binding of L1 is not necessary for axonal guidance and neuronal migration in the central nervous system (CNS).

L1 was one of the first neural cell adhesion molecules to be discovered, and its binding partners have been proliferating ever since. Many of those binding partners contact several of L1’s many domains, making individual contributions difficult to tease apart. Itoh et al., however, succeed in ablating only a subset of L1’s binding interactions via a deletion of L1’s sixth Ig domain.

The resulting protein does not bind either to itself or to α5β1 integrin. And yet mice expressing only this variant have brains and spinal cords that look normal. Thus, it seems that L1 homophilic binding, earlier found to mediate neurite outgrowth in vitro, is not needed for this function in the CNS.

When the L1 variant is backcrossed into another mouse strain, the progeny get hydrocephalus. This swelling of the brain results when cerebrospinal fluid is not correctly cleared from the brain ventricles. The link between L1 mutation and hydrocephalus has been noted before, in humans, but its mechanistic basis remains unclear.

Forming a shmoo

M ating selection, yeast style, consists of polarizing and projecting in the right direction. Matheos et al. (page 99) define a new step in this polarization dance—a step that may link the extracellular yeast pheromone signal to the intracellular establishment of polarized actin cables.

The proposed link involves phosphorylation of Bni1p, a cable-generating formin protein, by Fus3p, a MAP kinase. Fus3p got its name because cells lacking this kinase could not fuse during mating. Sure enough, activated Fus3p was long ago found to lead to transcription of fusion genes. Then Fus3p was found to help arrest the cell cycle of mating cells. But replacement of both of these functions did not restore fusion to cells lacking Fus3p.

Now Matheos et al. find that Fus3p is also needed for polarization of the cell and localization of Bni1p to the tips of shmoo, or mating projections. Overexpression of Bni1p overcomes the need for Fus3p, but multiple actin-containing projections replace the normal single shmoo.

All of this activity is set in motion when pheromone binds its receptor, liberating two G protein activities: a βγ complex that turns on both Fus3p and Cdc42-mediated actin polymerization, and an α subunit that helps localize the Fus3p that activates Bni1p. The need for both pathways may be a failsafe mechanism. Or perhaps it is related to the very different types of actin networks that the pathways make. Cdc42 is good at making actin meshworks that push out the cell membrane, but Bni1p is better at making cables that facilitate transport to the site of mating.