Targeting the inner nuclear membrane

Proteins destined for the inner nuclear membrane (INM) start out in the peripheral ER. Diffusion from the ER will get them to the contiguous outer nuclear membrane (ONM), but the next step could involve either vesicular transport, short-lived fusions between INM and ONM, or movement along the lipid bilayers surrounding nuclear pores. On page 1051, Ohba et al. suggest that the last explanation applies. Moreover, their data suggest that the process can only occur because the nuclear pore complex, which was thought to be a static structure, is constitutively remodeled.

Previous models suggested that INM proteins move through the nuclear pore membrane, but researchers needed a dynamic assay to test the model. By adapting a protein trapping system (Chen et al. 1995. Proc. Natl. Acad. Sci. USA. 92: 4947–4951), Ohba et al. were able to watch fluorescently-labeled FRB reporter proteins move between the peripheral ER and the INM. Under normal conditions, the reporter moved between membranes without restriction. However, when the team added rapamycin to the cells, the FRB reporter accumulated in the INM as the reporter bound to an FK binding protein (FKBP) associated with the nuclear lamina. Thus, the rapamycin-mediated interaction trapped the reporter protein in the INM. The team thinks that native INM proteins become similarly trapped when they associate with nuclear structures.

Only proteins whose luminal and cytosolic domains were under 60 kD gained access to the INM, a limitation noted previously. INM localization was dependent on both energy and temperature, which would be consistent with membrane fusion events. However, because the addition of inhibitors of membrane fusion had no effect on localization in the INM, the team hypothesizes that the energy is required for nuclear pore remodeling. Indeed, addition of antibodies against the nuclear pore protein gp210 blocked localization to the INM. While the pore structure is busy with the remodeling, small integral membrane proteins may slip by, passing into the INM. The team thinks pore remodeling is constitutive because reporter proteins that lacked any native nuclear protein sequence accumulated in the INM in the presence of rapamycin, suggesting that a signal is not required.

Stick hard, signal fast

Cell type specification by Notch (N) in one cell and Delta (Dl) in another must happen quickly, before morphogenesis further changes the cellular landscape. On page 1217 Ahimou et al. find that the adhesion force between N and Dl peaks and drops in just 10 min, symptomatic of a peak and drop in intercellular signaling. Pulling on Dl can accelerate this process.

To measure the in vivo adhesion force between N and Dl, Ahimou et al. attached a Drosophila cell expressing one or the other protein to a cantilever tip of an atomic force microscope (AFM) and placed cells expressing the other protein in a culture dish. When they brought the AFM tip with the cell near those in the culture dish, they found that, unlike many biologically active molecular pairs, there was no attractive force detected between N and Dl. After pushing the cells together, however, it took up to 14 nN of force to detach them, which is 50 to 250 times the force it takes to dislodge streptavidin from biotin.

Binding of Dl to N is known to cause cleavage of the intracellular, signaling domain of N by presenilin. This proceeded at the highest rate when the adhesion forces between the proteins was high. The force required to separate N and Dl expressing cells dropped off after presenilin cleavage and fell to nearly zero in just 10 min, suggesting that the proteins complete their signaling reaction quickly.

Both the brief and rapid signaling, as well as the increased signaling rate induced by Dl pulling, fits with the role of the N-Dl signaling during development, when there are morphogenetic movements and rapid changes in cell identity. Previous work has identified numerous factors that regulate N-Dl signaling. The team plans to use their new AFM system to decipher the biochemical and biophysical impact of these regulators. JCB