Sitting in—not through—a membrane

A putative acyl transferase is lodged in both inner and outer mitochondrial membranes without poking fully through either one, report Claypool et al. (page 379). Point mutations that cause mislocalization of this tafazzin protein within mitochondria or alter its macromolecular interactions are sufficient to cause Barth syndrome.

Barth syndrome is a human disorder in which mitochondrial dysfunction, including disturbed lipid composition, leads to cardiac myopathy and other problems. Previous work showed that the yeast tafazzin protein (Taz1p) is a suitable model for the syndrome and that Taz1p localizes to mitochondria. In the current work, the authors found that Taz1p localized to the inner and outer membranes of the mitochondria, but only to the leaflets that face the intermembrane space. A central loop in the Taz1p protein inserted into the membranes and hung onto them as tenaciously as do transmembrane proteins. But the loop did not extend all of the way through the membrane as is typical for transmembrane proteins. The bulk of Taz1p protruded into the intermembrane space.

Four of the disease-causing point mutations in tafazzin lay in the putative membrane anchor loop. Introduction of each of these mutations into Taz1p altered its association with the membrane. Three of the changes caused Taz1p to localize to the mitochondrial matrix. In the fourth case, the protein remained in the intermembrane space but formed different macromolecular complexes relative to the wild-type protein.

Claypool et al. conclude that tafazzin function is sensitive to both mislocalization and disruption of molecular interactions. The question now is just what that function is. The unusual localization of the protein is consistent with it being an acyltransferase for the mitochondria-specific phospholipid cardiolipin (and perhaps for lyso-phosphatidylcholine), but enzyme assays will be needed to answer the question for sure.

Cell front supports the back

Chemoattractants cause neutrophils to polarize by triggering competing signaling pathways that promote “frontness” and “backness” at opposite poles of the cell. On page 437, Van Keymeulen et al. report that the two responses are not independent. Instead, frontness signals do double duty by reinforcing backness on the cell’s other side.

The chemoattractant fMLP activates receptors that stimulate two different G proteins, Gi and G12/G13. At the front of the neutrophil, Gi increases PIP3 accumulation and turns on Rac and CDC42, causing actin polymers to form a pseudopod. G12/13 stimulates RhoA activity, which causes the back of the cell to contract. The two responses exclude each other locally to produce a single front and a single back, rather than multiple “frontlets” and “backlets” scattered over the cell surface.

Van Keymeulen et al. discovered the key to this tricky balancing act by treating cells with inhibitors of PIP3 synthesis. As expected, the inhibitors impaired frontness responses. They prevented fMLP-dependent activation of Cdc42, reduced activation of Rac, and rendered pseudopods transient, small, and unstable.

Surprisingly, pseudopod multiplicity was found all around the cell periphery because of reduced fMLP-dependent RhoA activity at the back of the cell. Activation of RhoA not only depended on G12/G13, but also required components of the frontness response, including elevated PIP3 and Cdc42 activity.

Thus the polarized neutrophil’s ability to stabilize a single front and a single back depends on long-range augmentation of backness by the frontness program. Van Keymeulen et al. speculate that the long-range effect of frontness is mediated by Cdc42 regulation of microtubules, which extend preferentially to the back of polarized neutrophils.
Mannose receptor regulates motility

If myoblasts are to fuse with nascent myotubes and thus form muscles, they must apparently migrate and chew up extracellular matrix (ECM), according to Jansen and Pavlath (page 403). These activities require expression of the mannose receptor (MR) in differentiating myoblasts.

The pathway is induced when nascent myotubes secrete IL-4, which induced expression of the MR in myoblasts. Although MR-null myoblasts fused normally with other myoblasts, they were retarded in their ability to fuse with nascent myotubes, which is the second stage of myotube formation.

MR expression appears to be required in vivo as well as in vitro for normal myotube formation, as the myofibers in MR-null mice were smaller than those in wild-type animals.

The researchers found that MR-null myoblasts migrated more slowly than wild-type cells in response to chemotactic stimuli. Moreover, the mutant cells internalized less collagen than control cells, indicating that MR, which is an endocytic receptor, is required for efficient clearance of the ECM during cell movement.

Jansen and Pavlath note that MR activity is required for other cell fusion events, but its role in migration and ECM remodeling has not been reported previously. Another member of the MR family, Endo180, is also involved in cell migration and ECM clearance. Therefore, these activities might be a common function for the receptor family. JCB

Early transcription

Early genome remodeling is a black box that somehow helps reset the genome for development. In mice, the zygotic genome is activated early, with a small wave of transcription starting in the one-cell zygote and a much larger wave appearing at the two-cell stage. On page 329, Torres-Padilla and Zernicka-Goetz report that TIF1α helps regulate the initial wave of transcription by controlling genome remodeling.

After fertilization, TIF1α translocated from the cytoplasm to both pronuclei of the one-cell stage embryo, where it localized to discrete regions of the chromatin. TIF1α colocalized with chromatin-remodeling proteins SNF2H and BRG-1 at a subset of active transcription sites.

Blocking TIF1α with RNAi or antibody injection caused mislocalization of the chromatin remodeling proteins and RNA polymerase II, suggesting that TIF1α recruits the proteins to these specific sites in the genome. Many of the treated embryos stopped developing at the two-to-four-cell stage.

Finally, using a modified ChIP procedure, the team found that TIF1α was required for the proper regulation of a specific set of genes. Closer inspection of 10 of the genes indicated that TIF1α increased the transcription of some genes, while decreasing transcription of others. SNF2H RNAi treatment disrupted transcription of some of these genes as well.

The team is currently looking to see how TIF1α leads to chromatin remodeling at this early stage of embryo development. Learning how this first wave of genome activation comes about may be important for understanding what is required for successful genome reprogramming in nuclear transfer experiments. JCB

Contractile ring formation

During cytokinesis the actin–myosin contractile ring forms between two daughter cells, pinching them apart. On page 391, Wu et al. demonstrate that the contractile ring in fission yeast arises from a band of nodes rather than from one single spot, as previously proposed.

Previous experiments yielded two countervailing views of how the actin–myosin ring forms. In one scenario, an actin cable was thought to extend from a single progenitor spot that contained Mid1p/anillin, a large adaptor protein known to be involved in ring formation. In the alternate scenario, Mid1p was thought to accumulate in multiple nodes that then coalesced into a ring.

Using a series of fluorescently tagged proteins, Wu et al. found that myosin-II appeared around the equator of the cell before anaphase and was concentrated in a large number of nodes that form a band around the cell. Subsequently, the nodes melded together to form the actin–myosin ring. In addition to myosin-II these nodes contained several other proteins that participate in contractile ring function, including Cdc12p/formin, Rlc1p/myosin light chain, Rng2p/IQGAP, Cdc15p/PCH, and Mid1p.

Formation of the nodes required Mid1p. In cells lacking Mid1p, Cdc12 and myosin proteins did not accumulate in the neck region. When the team inhibited actin polymerization, the nodes formed but failed to give rise to a contractile ring. The single progenitor spot was not detected under all growth conditions and cells lacking the spot formed normal contractile rings, suggesting that it is not a critical component of the structure. JCB