Mitochondria move in on calcium

Mitochondria aren’t just ATP factories. They serve as important components in intracellular signaling by modulating Ca\(^{2+}\) and act as a relay system in apoptosis. And they are dynamic organelles, moving about the cell at a rapid pace. On page 661, Yi et al. integrate these observations by demonstrating that local Ca\(^{2+}\) concentration controls mitochondrial movement.

Initially, the team was focused on the local interactions between the endoplasmic reticulum (ER) and mitochondria in myoblast cells in culture, but then noticed that changes in Ca\(^{2+}\) induced massive fluctuations in the rate of mitochondrial movement. To quantify these changes, the team labeled mitochondria with YFP fused to a mitochondrial targeting sequence. Stimulating the cells with vasoressin, a Ca\(^{2+}\) mobilizing hormone, or inducing localized Ca\(^{2+}\) release from the ER using IP\(_3\), they found that the mitochondria move most at resting Ca\(^{2+}\) concentrations. The organelles came to a standstill when they reached a region with a high concentration of Ca\(^{2+}\) (1–2 \(\mu\)M range) and moved again as the Ca\(^{2+}\) levels went down.

The mitochondria appear to move along microtubules, yet neither of the known microtubule motors are Ca\(^{2+}\)-dependent. The team hypothesizes that myosin Va, which binds calmodulin and is probably regulated by Ca\(^{2+}\), acts as a bridge between the microtubule motors and the mitochondria. They are currently testing the idea by down-regulating myosin Va.

Mitochondrial arrest in regions of high Ca\(^{2+}\) makes biological sense. The organelles would enhance the cell’s local Ca\(^{2+}\) buffering ability by soaking up the cation. In addition, Ca\(^{2+}\) stimulates ATP production in the mitochondria, so the Ca\(^{2+}\) influx would induce a local rise in ATP that could be used to drive ATP-dependent Ca\(^{2+}\) pumps in the ER and the plasma membrane. Together, the system would help speed the clearance of Ca\(^{2+}\), allowing for rapid, short signaling cascades. JCB

ES cells without teratomas

Therapeutic use of embryonic stem cells may be hampered by their proclivity to form pluripotent tumors called teratomas. On page 723, Bieberich et al. describe the use of a ceramide analogue, S18, to induce apoptosis in a subpopulation of embryoid body–derived stem cells (EBCs). Cells that survive the treatment express the neural marker nestin, a Ca\(^{2+}\) mobilizing hormone, or inducing localized Ca\(^{2+}\) release from the ER using IP\(_3\), they found that the mitochondria move most at resting Ca\(^{2+}\) concentrations. The organelles came to a standstill when they reached a region with a high concentration of Ca\(^{2+}\) (1–2 \(\mu\)M range) and moved again as the Ca\(^{2+}\) levels went down.

The S18 selectively affects those EBCs that express prostate apoptosis response-4 (PAR-4) protein, an endogenous inhibitor of atypical PKC\(_{\text{z}}\). Significantly, the majority of the PAR-4–expressing cells also express Oct-4, a marker for pluripotency. Almost all Oct-4–expressing cells were positive for PAR-4, suggesting that teratoma formation might be prevented via elimination of PAR-4–expressing cells with ceramide-induced apoptosis before injection into animals. Sure enough, after injection into the brains of mice, treated cells differentiated into neural cells and some benign tumors, but no invasive tumors. Untreated cells gave rise to a significant number of teratomas in the same animals.

Bieberich et al. hypothesize that coexpression of Oct-4 and PAR-4 may indicate that the expression of PAR-4 is specifically up-regulated at particular stages during ES cell differentiation. Discovering the function and mechanism behind this up-regulation is the next task the team plans to tackle. JCB

Transport if loaded

Nuclear transporters can be viewed as taxis that move cargo across the nuclear envelope. On page 649, Pfäffer et al. report that importin-11, a nuclear transport receptor, may be an especially selective cabbie. Importin-11 transports UbcM2, a ubiquitin (Ub)-conjugating enzyme, but it does so only when the enzyme is charged with a Ub at its active site.

UbcM2 is an E2 enzyme, which works with E1 and E3 proteins to polyubiquitinate and tag proteins for degradation in the proteasome. To test whether importin-11 preferentially transports the Ub-charged UbcM2 or the unloaded enzyme, Pfäffer et al. performed coimmunoprecipitation assays with wild-type UbcM2, a mutant enzyme that is constitutively loaded with Ub, or a mutant that cannot be loaded. Importin-11 selectively bound the Ub-charged forms of UbcM2. Furthermore, in vitro pull-down assays showed that, if ATP or the E1 enzyme that loads Ub onto the UbcM2 active site were depleted, importin-11 did not bind UbcM2. In cell assays, catalytically inactive UbcM2 failed to localize to the nucleus. Only a subset of E2 enzymes bound to importin-11. This specific interaction may control the enzyme’s access to potential substrates, including some involved in cell cycle progression. Plus, if importin-11 gobbles up all the Ub-charged UbcM2, then the enzyme cannot ubiquitinate cytoplasmic proteins. JCB
Making a raft with oligomers

Epithelial cells differentially segregate proteins to their apical and basolateral surfaces. For some apical localization events, association of glycosylphosphatidylinositol (GPI)-anchored proteins with detergent-resistant rafts is necessary but not sufficient. Now, on page 699, Paladino et al. show that the key for proper protein sorting may be the formation of high molecular weight aggregates of apically targeted GPI-anchored proteins as they segregate into rafts in the Golgi.

The researchers separated rafts on sucrose gradients. The rafts containing apical proteins, like PLAP and GFP-GPI, had a high concentration of protein when compared with rafts with basolaterally targeted proteins. Mutations of GFP-GPI that impaired formation of high molecular weight complexes caused mis-targeting of the protein to the basolateral surface. Using pulse-chase experiments, the researchers found that oligomerization occurred as the protein-laden rafts made their way through the trans-Golgi network.

The team hypothesizes that oligomerization of the apically targeted proteins in the Golgi helps them to form large rafts that then pinch off on their way to the apical surface. The new hypothesis contradicts work by Polishchuk et al. (Nat. Cell Bio. 2004. 6:297–307). In their paper, Polishchuk et al. proposed that apical and basolateral proteins exit the trans-Golgi network together in vesicles targeted to the basolateral membrane, and that the apical proteins are subsequently resorted and transcytosed to the apical membrane. Paladino et al. didn’t see any trafficking of apical proteins to the basolateral surface and suggest that the difference in the results might result from differing culture conditions.

If oligomerization is driving apical segregation, the question remains as to what induces oligomerization. Paladino et al. are currently testing one possibility: that glycosylation or other protein modifications are used to gather together the protein. JCB

Invadolysin opens many doors

In a search for proteins involved in chromosome condensation, McHugh et al. (page 673) have identified a novel metalloprotease, IX-14/invadolysin, that is required for both mitosis and cell motility in Drosophila, and probably also in human cells.

The mutation, identified in a chemical mutagenesis screen, blocks cell proliferation as evidenced by underdeveloped brains and imaginal discs in larvae. It disrupts compaction of both interphase and mitotic chromosomes, such that mitotic chromosomes are hypercondensed in length but with a loose peripheral halo of chromatin. The mutation also results in aberrant spindle assembly and increased levels of some nuclear envelope proteins. Possible substrates for the metalloprotease that might explain these phenotypes are unknown, but enzyme assays did suggest that invadolysin can cleave the nuclear envelope protein lamin Dm0. By contrast, previous studies of lamin changes during mitosis had focused on phosphorylation.

In human cells, the majority of the protein was in ring-shaped formations that resemble invadopodia. These structures help degrade extracellular matrix and thus aid metastasis. Consistent with a possible role in cell migration, invadolysin localizes to the leading edge of motile macrophages. Returning to Drosophila, the team found that germ cells failed to migrate normally in mutant embryos, indicating that the protein is also involved in cell migration in flies.

Given that invadolysin seems to have its fingers in diverse processes, McHugh et al. hypothesize there are multiple forms of the protein that may be differentially regulated and localized. These forms may link the related processes of cell growth and migration. JCB